Mechanisms of T Cell Dysfunction in Chronic Lymphocytic Leukemia and Viral Associated Carcinoma

by

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Abstract

T cell exhaustion compromises anti-tumor immunity, and a sustained elevation of co-inhibitory receptors is a hallmark of T cell exhaustion in solid tumors. Similarly, upregulation of co-inhibitory receptors has been reported in T cells in hematological cancers such as chronic lymphocytic leukemia (CLL). $CD8^+$ T cells play an essential role against tumors, but mechanisms associated with their exhaustion in various cancers are diverse and yet to be elucidated. This research study evaluates the mechanism(s) associated with $CD8^+$ T cell phenotype and function in CLL and immunotherapy-related immune signatures in HPV-associated (Human Papilloma Virus) carcinoma.

First, I examined the expression of different co-inhibitory receptors in $CD8^+$ T cells obtained from peripheral blood/bone marrow of CLL patients by flow cytometry. I found CD160 as the most dominant co-inhibitory receptor in these patients. Its expression was associated with an exhausted T cell phenotype, and $CD160^+CD8^+$ T cells were highly antigen-experienced T cells. Furthermore, we proposed chronic stimulation, CD160-containing EVs, and elevated IL-16 levels as mechanisms linked to the expansion of CD160-expressing $CD8^+$T cells in CLL.

Moreover, I found a significant decline in CD26 expressing $CD8^+$ T cells in CLL compared to healthy subjects. My findings demonstrated that $CD26^{high}$ cells were enriched with Mucosal Associated Invariant T (MAIT) cells co-expressing CD161, TVα7.2, and IL-18Rα. Also, I observed that $CD26^{high}$ cells have a rich chemokine receptor profile (e.g., CCR5 and CCR6), profound cytokine (TNF-α, IFN-γ, and IL-2),

and cytolytic molecules (Granzyme B, K, and perforin) expression upon stimulation. Overall, my results demonstrate that $CD26^{+}$ T cells possess a natural polyfunctionality to traffic, exhibit effector functions, and resist exhaustion. In turn, Galectin-9 (Gal-9) and the inflammatory milieu (IL-18, IL-12, and IL-15) in CLL patients contribute to the depletion of $CD26^{high}$ T cells. Hence, depletion of MAIT cells may predispose CLL patients to immune dysfunction and susceptibility to infections.

Furthermore, I conducted bulk RNA sequencing analysis of peripheral immune cells to gain insight into immune checkpoint blockade therapy in advanced HPV-associated carcinoma patients. I discovered that the immune cell signature in responders to immunotherapy is entirely different from non-responders at the baseline and following treatment. The genes and pathways related to myeloid immune responses were more prominent in the non-responding group, which supports their suppressive role in this group. In agreement, non-responders had higher levels of IL-8 and IL-18 at the baseline than responders. In contrast, responders had higher $CD8^{+}$ T cells at the endpoint.

These observations highlight potential mechanisms of T cell exhaustion in hematologic malignancies such as CLL versus solid tumors. My studies have provided a novel insight into the underlying mechanism(s) of immune dysfunction with potential clinical implications for precision medicine.

Preface

Research Ethics Approval

This thesis is an original work by Najmeh Bozorgmehr. This research project, of which this thesis is a part, received the ethics approvals indicated below by the Institutional Health Research Ethics Board of Alberta:

1. Inhibitory receptors and erythroid suppressor cells in lymphoma, Myelodysplastic syndromes (MDS) and Chronic lymphocytic Leukaemia, protocol HREBA#CC-17-0307, valid until March 4, 2024.
2. Developing novel cancer Immunotherapy drugs, protocol #Pro00063463, valid until August 31, 2023.
3. Biology of CD71 and host defense, protocol AUP0001021, valid until August 23, 2023.
4. LATENT: Avelumab with Valproic acid in Virus-associated Cancer, protocol HREBA#CC-17-0374, approved at the University of Calgary, valid until August 24, 2023.

Contribution of others in this study

Two chapters (Chapter 2, and Chapter 3) of the research included in this thesis have already been published and the chapter 4 is in the process of publication.

A version of Chapter 2 is published in the *Journal for Immunotherapy of Cancer* (**Bozorgmehr N**, Okoye I, Oyegbami O, Xu L, Fontaine A, Cox-Kennett N, Larratt LM, Hnatiuk M, Fagarasanu A, Brandwein J, Peters CA, Elahi S. Expanded antigen-experienced $CD160^{+}CD8^{+}$effector T cells exhibit impaired effector functions in chronic lymphocytic leukemia. J Immunother Cancer. 2021 Apr;9(4): e002189. doi: 10.1136/jitc-2020-002189). I was involved in all aspects of the research, from design, data collection, analysis, interpretation, and writing. However, I would like to acknowledge the contribution of others to this project. Dr. Isobel Okoye helped me with the EV isolation. Lai Xu assisted me with the image stream. Dr. Olaide Oyegbami helped me with western blotting. Dr. Anthea Peters, Dr. Amelie Fontaine, Nanette Cox-Kennett, Dr. Loree M Larratt, Dr. Mark Hnatiuk, and Dr. Andrei Fagarasanu contributed to CLL patients' recruitment.

A version of Chapter 3 is published in the Journal of *Experimental Hematology and Oncology* (**Bozorgmehr N**, Hnatiuk M, Peters AC, Elahi S. Depletion of polyfunctional $CD26^{high}CD8^{+}$ T cells repertoire in chronic lymphocytic leukemia. Exp Hematol Oncol. 2023 Jan 27;12(1):13. doi: 10.1186/s40164-023-00375-5). I performed all the data collection, experiments, analyses, interpretation, and writing. Dr. Anthea Peters and Dr. Mark Hnatiuk contributed to CLL patients' recruitment.

A version of Chapter 4 has been submitted to the journal of Molecular oncology and is under review (**Bozorgmehr N**, Syed H, Mashhouri S, Walker J, and Elahi S. Transcriptomic profiling of peripheral blood cells in HPV-associated carcinoma patients receiving the combined Valproic acid and Avelumab therapy. Molecular oncology. Under review). I performed all the data collection, experiments, analyses, interpretation, and writing. Dr. John Walker, director of the LATENT Clinical Trial study, contributed to patient recruitment and provided clinical data. Dr. Juan Antonio Jovel helped me to analyze RNAseq data. Hussain Syed helped me in preparing scripts for RNAseq data analysis. Siavash Mashhouri enabled me to collect and prepare blood samples for the study. Most importantly, a special thank you to cancer patients and healthy controls that generously agreed to donate blood for research, as this project would have been impossible without their blood donation.

To my mom and dad
For their unconditional love, support, and encouragement

To my loving husband
For his endless love, support, and patience

To Sarvenaz and Arian
The forever loves of my life

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1 Chapter 1: Introduction

Finding new and efficient treatments for cancer is crucial as it remains a major global health problem. Conventional treatments available for cancers are surgery, radiation therapy, or chemotherapy alone or in combination. A very novel and promising area of cancer therapy is immunotherapy. This type of treatment harnesses the host immune system to fight cancer. Several immunotherapy approaches are available, including developing tumor vaccines, engineered T cells (CAR-T), Immune checkpoint blockers, and gene therapies [1]. However, developing immunotherapy approaches requires a better understanding of tumor biology and immune responses in different cancers. For example, immune responses in blood cancers are quite different from solid tumors as they could be affected by the tumoral immune cells circulating throughout the body. These properties impair the immune responses to the tumor and pathogens, at the same time, exposed tumor cells are more accessible to immunotherapy or targeted therapy than solid tumors. Even among solid tumors, the immunogenicity of the tumor can affect the response to immunotherapy [2]. Given the clear differences between liquid and solid tumors, for a deep mechanistic understanding, I compared these two types of cancers for this Ph.D. project. Chronic Lymphocytic Leukemia (CLL) is a blood cancer, and Human Papilloma Virus-associated carcinoma (HPV-associated carcinoma) is a solid tumor. My main goal was to analyze $CD8^+$ T cells or cytotoxic T lymphocytes (CTLs) effector functions as the significant anti-tumor defense system and factors that impact their operation.

1.1. Chronic Lymphocytic Leukemia

1.1.1 Epidemiology

CLL is the most common leukemia in adults in Western countries [3]. Based on the Global Burden of Disease (GBD) study, the incidence rate of CLL was 0.76 in 1990 and has surged to 1.34 per 100,000 in 2019 [4]. Regarding geographical distribution, North America, Western, and Central Europe had the highest rate of age-adjusted CLL in 2019, while rapid growth has been reported in East Asia, Central Europe, and Latin America [4]. The American Cancer Society has estimated 18,740 new CLL cases and 4490 related deaths in 2023 [5]. The US National Cancer Institute Surveillance Epidemiology and End Results (SEER) Program has reported age-adjusted new cases of 4.7 per 100,000 individuals per year (obtained from 2015-2019 data) [3]. The approximate lifetime risk of developing CLL is 0.6 percent, and CLL accounted for 1.1 percent of all new cancer cases and 0.7 percent of all cancer deaths in 2022 in the United States [3]. Men have a two-times higher risk of developing CLL than women, and the risk increases with age [5]. The average age of most CLL patients is 70 years old, and 10% of patients are less than 55 years old [3].

1.1.2 Origin and Nature of malignant cells

CLL is a chronic lymphoproliferative disorder (lymphoid malignancy) defined by clonal expansion of functionally incompetent, mature, malignant $CD5^+$ B cells in peripheral blood, bone marrow, and secondary lymphoid tissues [6]. CLL is very similar to Small lymphocytic Lymphoma (SLL), with different manifestations. The CLL term is used when the malignant B cells appear in the blood, whereas the SLL term is only used

when the disease involvement is nodal [7]. Based on the mutation status of IgHV (immunoglobulin heavy chain variable region) genes, CLL has been stratified into two subsets, different in the clinical course. Malignant B cells in the Un-mutated variant (U-CLL) originate from pre-germinal center B cells ($CD5^+CD27^-$), whereas the mutated variant (M-CLL) derives from post-germinal center B cells ($CD5^+IgM^+CD27^+$)[8]. Approximately half of the cases of CLL carry un-mutated IgHV genes (U-CLL) [8]. The characteristic features of malignant B cells in humans are very similar to naive and $CD5^+$ B cells [8].

1.1.3 Risk factors

Genetic and environmental factors have been identified in susceptibility to CLL [9]. An 8.5-fold increased risk of developing CLL has been reported in first-degree relatives [10]. Moreover, the concordance of CLL is higher among monozygotic than dizygotic twins [11]. Exposure to Agent Orange [12] and insecticides [13] might be a risk factor for CLL development.

1.1.4 Pathogenesis

The process of stepwise malignant transformation starting from a hematopoietic stem cell (HSC) has been understood in CLL [14]. The development of CLL is often launched by the gain or loss of large chromosomal materials [15]. During the development of CLL, further mutations might occur that affect the course of the disease and prognosis. Eleven recurrent somatic copy number variations and 44

mutated genes have been spotted [15]. These mutations are involved in signaling related to MYC activity, mitogen-activated protein kinase (MAPK), RNA processing and export, DNA damage signaling and repair [15,16]. Moreover, epigenetic alterations such as DNA methylation changes known as epimutations are identified [17]. Survival and maintenance of malignant B cells depend on a permissive tumor microenvironment that will be discussed later [18].

1.1.5 Clinical presentations

Most CLL patients are asymptomatic, and the disease is diagnosed after the detection of increased lymphocyte counts in the peripheral blood [6]. The extent of clinical manifestations is different from feeling well to disease-related symptoms. The common symptoms are fatigue, weight loss, night sweats, anemia, and increased frequency of infections associated with hypogammaglobulinemia [6]. Common infections are respiratory and urinary tract infections, predominantly due to bacteria (67%), viruses (25%,) and Fungi (7%) [19]. The risk of viral reactivation increases during the disease. Varicella zoster virus reactivation is expected after the initiation of treatment [20]. Sometimes, patients present with symptoms related to autoimmune cytopenia, such as hemolytic anemia or purpura. Patients can also develop enlarged lymph nodes, spleen, and liver [9].

1.1.6 Diagnosis

Diagnosis of CLL is based on the complete blood cell counts (CBC), peripheral blood smear, and flow cytometry. For some situations, requesting FISH (Fluorescence in

situ hybridization), cytogenetic analysis, and bone marrow or lymph node biopsy is required [21].

• CBC and Peripheral blood smear

The initial diagnosis starts with detecting circulating B lymphocytes ≥5,000 cells per µl in CBC, maintained for at least three months [6]. Morphologic examination of the peripheral blood smear shows a monotonous population of bland looking lymphoid cells with scant cytoplasm, small round nuclei, condensed chromatin, and inconspicuous nucleoli. The presence of smudge cells representing disrupted leukemic cells are common [6].

• Immuno-phenotyping

Flow cytometry is required to evaluate the immunophenotype profile of leukemic cells [22]. Typical malignant B cells are positive for CD19, CD5, CD23, and low levels of CD20 [23]. They lack the expression of CD10, which differentiates them from Follicular lymphoma. They express CD200, which helps to distinguish them from mantle cell lymphoma. Some atypical cells are CD11cdim and SIgdim (Surface immunoglobulin) with restricted light chain expression [23].

• FISH Cytogenetic analysis

FISH analysis for del (17p) and TP53 mutation is requested for predictive purposes only in patients who require treatment [23]. Similarly, IgHV mutation testing should be requested for the patients who require treatment only once and should not be repeated at a later time point as the results will not change over time [23].

• Bone marrow aspiration and lymph node biopsy

Marrow aspiration/biopsy is not required for the initial diagnosis but is often performed to assess the bone marrow involvement pattern [7]. The bone marrow is infiltrated by monochromic small B cells in four patterns: nodular, interstitial, mixed (nodular and interstitial), and diffuse. The diffuse pattern is typically correlated with more advanced diseases [7]. A lymph node biopsy might be carried out in patients with enlarged lymph nodes to evaluate for lymphoma. The hallmark of CLL is the pseudo follicles or proliferation centers in lymph nodes, as it is not present in other types of lymphoma [21].

1.1.7 Clinical course and prognosis

The outcome of CLL is highly variable. The five-year survival is around 80% in men and 85% in women [24]. Although some patients have progressive diseases and require therapy relatively soon, most have indolent diseases and don't need treatment for many years [24]. However, most patients are in between these two ends. Several prognostic factors and predictive models are defined to identify patients who will require treatment. Specific genetic, molecular, biochemical, and clinical features are used for this purpose [9].

- *Staging*

There are two widely used clinical staging systems to predict the prognosis. The modified Rai staging system (Table 1) is more commonly used in Canada and the United States [9, 25], whereas in Europe the Binet staging system (Table 2) is more popular [26]. The presence of anemia or thrombocytopenia predicts high-risk disease with less median survival.

Stage (Rai)	Description	Risk Status (Modified Rai)	Median Survival(yrs)
0	Lymphocytosis, lymphoid cells >30% in blood or bone marrow	Low	11.7
I	Stage 0 with enlarged node (s)	Intermediate	8.3
II	Stage 0-I with splenomegaly, hepatomegaly, or both	Intermediate	5.8
III	Stage 0-II with hemoglobin < 110 g/L	High	2.0-4.0
IV	Stage 0-III with platelets < 100 x 10^9/L	High	2.0-4.0

Table 1. Modified Rai staging system in CLL [9]

Stage	Description	Median survival (yrs)
A	Hemoglobin ≥ 100 g/L and platelets ≥ 100 x 10^9/L and < 3 involved nodal areas	>10
B	Hemoglobin ≥ 100 g/L and platelets ≥ 100 x 10^9/L and ≥ 3 involved nodal areas	5
C	Hemoglobin < 100 g/L and platelets < 100 x 10^9/L, and any number of involved nodal areas	2.0-4.0

Table 2. Binet staging system in CLL [26]

- *Cytogenetic testing*

FISH is used to find cytogenetic abnormalities [27]. Around 80% of patients are positive. Most common abnormalities are [27]:

- del (13q): 14-20%
- Deletions or trisomy in chromosome 12: 11-18%
- del (11q): 10-32%
- del (6q): 2-9%
- del (17p): 3-27%

Patients carrying normal karyotype or isolated del (13q) are low-risk, long-term survivors [28]. However, patients with del (17p) or del (11q) have poor prognosis [28]. These patients are less responsive to chemotherapy but better respond to BCR (B Cell Receptor) or BCL-2 inhibitors. These patients might be candidates for allogeneic stem cell transplantation [21]. Other cytogenetic abnormalities do not impact decisions for treatment options [21].

- *IgHV mutation status*

Hypermutation in the IgHV genes is called a Mutated-CLL variant and is present in 50% of patients [27]. These patients have improved survival, whereas Un-mutated CLL patients have a fast progressive disease [27].In addition, the VH3.21 gene is an unfavorable prognostic marker regardless of IgHV mutation status [23]. IgHV mutation status should be requested in all patients for whom FCR (Fludarabine + Cyclophosphamide + Rituximab) therapy is considered for treatment [27]. The value of routine testing of IgHV in older patients is not yet established [27].

- *Molecular markers*

The presence of ZAP70, CD49d, or CD38 is a poor prognostic marker [9].

- *Serum markers*

Some serum markers have been identified for predictive applications. Serum Thymidine kinase (TK) in early stages correlates with tumor mass and proliferative activity of CLL [23]. High levels of CD23 are associated with diffuse bone marrow infiltration and rapid lymphocyte doubling time [23]. Serum TK and CD23 are not routinely available in Canada. Serum B2M (B2-microglobulin) is available in Canada and correlates with clinical stage and overall survival [27]. The value of prognostic markers in elderly patients is questionable [29].

- *Clinical features*

Common factors that are associated with worse prognosis are male sex, age >65 years, poor fitness due to medical comorbidities, high-stage disease at initial evaluation, and high absolute lymphocyte count (> 50,000 cells per μl) [9].

1.1.8 Treatment

Patients with early-stage CLL are managed with watch and wait [23]. The decision to initiate treatment depends on disease activity and progression based on clinical staging, presence of symptoms, or lymphocyte doubling time [23]. Treatment options include chemotherapy, immunotherapy, targeted therapy, or more commonly used combination therapies [21].

- *Chemotherapy*

Chemotherapy has been used for decades as the mainstay of treatment. Commonly used drugs are purine analogs (Fludarabine, Cladribine, Pentostatin) and alkylating agents (Chlorambucil, Cyclophosphamide, Bendamustine) [21]. The notable side effects of chemotherapy, such as the increased risk of infection and development of myelodysplasia, or secondary cancer, have urged the development and implication of new therapeutics in CLL [9].

- *Immunotherapy*
    - Monoclonal antibodies

The most widely used immunotherapy in CLL is the application of monoclonal antibodies targeting tumor-specific antigens such as CD20, CD19, CD37, and CD52 [30]. CD20 is a glycosylated transmembrane protein present on the surface of mature B cells [21]. Rituximab and Obinutuzumab are anti-CD20 monoclonal antibodies and are more commonly used as single or combination therapy in CLL [21]. Long-term follow-up is a survival benefit for combined rituximab and chemotherapy [31].

    - Immunomodulatory drugs

Lenalidomide, a second-generation thalidomide analog known as an Immuno-modulatory drug, has had favorable responses in high-risk cytogenetic patients such as del (17q) and un-mutated IgHV patients [32]. It restores T cell function by improving synapse signaling and downregulating PD-1 on T cells [33].

    - Immune checkpoint inhibitors: Anti-PD-1/PD-L1 antibodies

PD-1/PD-L1 axis has been identified as essential to maintain malignant cell survival by inducing T cell exhaustion [34]. Evidence of T cell exhaustion [35] and upregulation of PD-L1 on malignant B cells [34] have proposed anti-PD-1 or anti-PD-L1 therapy as promising in relapsed or transformed CLL patients when used in combination with other drugs [36–38]. Clinical trials are ongoing to evaluate the response of CLL patients to such therapy (NCT04781855) (NCT04271956).

- CAR-T cell therapy

Chimeric antigen receptor (CAR), with specificity for the B-cell antigen CD19, has been used and shown promising long-term effects in treating CLL patients [39]. Longer overall and progression-free survival in relapsed or refractory CLL patients has been shown [40,41]. A clinical trial study is ongoing to evaluate efficacy and cytotoxicity (NCT03331198) [42].

- *Allogenic hematopoietic stem cell transplantation (HSCT)*

Allogenic stem cell transplantation is a cellular immunotherapy for younger, fitter patients who are resistant to fludarabine or carry high-risk cytogenetics [43]. Despite improvements in procedure and supportive care, HSCT is associated with significant risk and mortality in the first two years due to infections or GVHD (Graft versus host disease) [44]. However, it remains the treatment of choice in some high-risk patients [44].

- *Targeted therapy*

    - Inhibitors of BCR signaling

B-cell receptor (BCR) signaling seems crucial in the growth and survival of malignant B cells in CLL [45]. The BCR signaling is propped up by various tyrosine kinases such as phosphatidylinositol 3-kinase (PI3K), Bruton tyrosine kinase (BTK), spleen tyrosine kinase (Syk), ZAP70, as well as Src family kinases [46]. Targeting these kinases using specific inhibitors has transformed the therapy of lymphoid malignancies into a new era [46].

    - BCL-2 inhibitors

Venetoclax is a small molecule that inhibits BCL-2 and induces apoptosis in malignant B cells [47]. The benefits have been shown in relapsed or refractory disease [47]. Tumor lysis syndrome is a possible side effect that needs appropriate dosing and monitoring [47].

- *Combination therapy*

Using drug combinations with synergistic effects and non-additive toxicity in CLL treatment is very beneficial. Chemotherapy combinations or chemoimmunotherapy are commonly used as more efficient therapeutic options [21].

1.1.9 Complications

- *Infections*

CLL patients are prone to frequent infections due to their compromised immune systems due to the disease or the side effects of treatments. As a result of immune deficiency, live vaccines are not recommended [23].

- *Autoimmune cytopenia*

Approximately 11% of CLL patients develop Autoimmune Hemolytic Anemia (AIHA) [23]. Auto-antibodies against red blood cells in CLL are responsible for AIHA development [48].

- *Richter syndrome*

Richter syndrome is the conversion of CLL to a high-grade lymphoma, mainly diffuse large B cell lymphoma (DLBCL). Around 2-10% of CLL patients develop Richter syndrome [23].

- *Tumor lysis syndrome*

Tumor lysis syndrome is caused by the rapid destruction of tumor cells and the release of their cellular contents into circulation [49]. This life-threatening syndrome is uncommon in CLL and might happen in treatment with Venetoclax, idelalisib, kinase inhibitors, and ibrutinib [49].

- *Secondary malignancies*

Developing second malignancies in CLL patients is an essential complication following targeted therapies and accounts for 1.7 fold increase risk of cancer [50]. Skin cancers

are the most common type of malignancy. Statistics have shown that CLL patients have an inherent tendency to develop cancer that increases with treatment [50,51].

1.1.10 Immune Responses in CLL

Immune dysregulation is a significant feature of CLL from its early stages; it worsens during clinical observation, even in the absence of progression [19]. Immune dysregulation is more prominent in cases of advanced, relapsed, or refractory disease [19]. A dysfunctional immune system in CLL increases the incidence of secondary malignancies [52] and infections [53], the significant causes of mortality for CLL patients. CLL involves both innate and adaptive immune responses, leading to immune suppression from the early stages [19]. Mechanisms remain unclear; however, the complex crosstalk between the CLL clones and the immune environment has been suggested [19].

- *Tumor microenvironment*

Malignant B cells persistently accumulate *in vivo* but face spontaneous apoptosis *in vitro* [54]. So, this implies that external signals from a permissive microenvironment protect them from apoptosis rather than being an intrinsic feature of leukemic cells [54]. Survival of transformed B cells relies on interaction with cellular and soluble mediators in a permissive microenvironment supported by stromal cells such as monocyte-derived nurse-like cells [18]. In CLL infiltrating tissues, such as bone marrow and secondary lymphoid organs, the hallmark is the presence of pseudo follicular structures named proliferation centers composed of malignant B cells, antigen-presenting cells (APCs), and numerous $CD4^+$ T cells [54,55]. Malignant B

cells are active players in creating a supportive microenvironment for their growth and survival. Nurse-like cells constitutively secret CXCL13, a potent chemoattractant for malignant cells [56]. After exposure to nurse-like cells or stimulation, malignant B cells generate chemokines such as CCL22 [55], CCL3, and CCL4 [57] to attract other tumor-supportive cells. So, these indicate the possibility that the creation and maintenance of the CLL microenvironment depend on a dynamic evolution and crosstalk between leukemic and other supportive cells.

- *Innate immune response*

The innate immune response is the frontline in inflammation and infections. Multiple elements of the innate immune response are affected in CLL patients.

  - Neutrophils: are defective in C5a-induced chemotaxis and phagocytic killing of non-opsonized bacteria [58].

  - Monocytes: The most evident defect in innate response in CLL is the increased populations of non-classical, M2 phenotype of monocytes with gene expression associated with immunosuppression [59]. These cells show dysfunctionalities related to deficiencies in β -Glucoronidase, Lysozyme, and myeloperoxidase [19].

- NK (Natural Killer) cells: are increased in CLL but are dysfunctional and show impaired cytotoxic activity and defective expression of NKG2D coreceptor [60].

- Complement proteins: Complement proteins coat pathogens and eliminate them [61]. C1-C4 proteins are reduced in 40% of CLL patients, which increases the risk of infection [62].

- *Adaptive immune response*

  - Humoral response

CLL is the malignancy of B lymphocytes and is intrinsically characterized by humoral immune response dysfunction. Immune suppression in CLL results from hypogammaglobulinemia due to B cell defects [19]. The severity increases with the progression of the disease and finally progresses to involve all immunoglobulin classes with a particularly marked decline in IgG3 and IgG4 subclasses [63]. Low IgA and IgG levels are correlated with morbidity and mortality [64]. IgM declines in the early phases of the disease and does not affect the increased risk of infection [65].CLL patients manifest a poor response to vaccines [66]. Antibody responses to primary and secondary challenges with antigens are often insufficient from the early stages. It has been suggested that direct suppression of B-cell function and the impairment of cognate T helper cells contribute to poor vaccine responses [66]. Malignant B cell clones often have features of the regulatory B cells producing immunosuppressive IL-10 [67], and expression of CD5, CD27, CD24, prototypes of regulatory B cells

[67]. Malignant B cells resemble anergic B cells operating in a T-cell-independent fashion [68], more prominent in the mutant variant of CLL [69].

- Cellular response

Absolute T cell counts are increased in early-stage disease [70], with a higher increase in $CD8^+$ T cells, leading to a reduction in CD4/CD8 ratio [71]. Increased mobilization from secondary lymphoid tissues or CLL-specific immune response has been suggested [72]. Chronic viral infections such as cytomegalovirus (CMV) trigger an increase in peripheral T cells in healthy donors and may explain the T cell increase in CLL. However, increased $CD4^+$ T cells in the lymph node of CLL patients, co-localizing with $CD38^+$ tumor cells, suggests that local activation increases them [73,74]. Functional impairment of T cells in CLL has been investigated, such as defects in cytotoxic pathways [75] and impaired immunologic synapse formation with antigen-presenting cells (APCs) [76].

- **$CD4^+$ T cells**

$CD4^+$ T cells (T helper cells) are the foremost players in adaptive immune responses [61]. They recognize the antigens on the surface of APCs through HLA class II, and then they get activated, release cytokines, and manage the activation of other immune cells, including B cells, $CD8^+$ T cells, monocytes, and macrophages [61]. $CD4^+$ T cells are heterogenous populations with different functions. These cells are increased in CLL and mainly belong to memory and effector subsets accompanied by a relative loss in naïve populations [77]. $CD4^+$ T cells in CLL are dysfunctional [78]. T regulatory cells (Tregs), a subset of $CD4^+$ T cells that possess immunoregulatory

functions, have been increased in the late stages of CLL [79]. T follicular helper (T_{FH}) cells, mainly located in secondary lymphoid organs, are increased in CLL and support malignant B cell survival and proliferation [80].

- **$CD8^+$ T cells**

$CD8^+$ T cells or Cytotoxic T Lymphocytes (CTLs) are the primary type of T cells that can eliminate tumor cells [81]. T cell receptors (TCR) on $CD8^+$ T cells recognize specific antigens expressed by APCs through HLA class I, which causes $CD8^+$ activation [81]. Activated $CD8^+$ T cells utilize several mechanisms to kill target cells that are mainly exerted by the secretion of cytotoxic molecules such as perforins and granzymes [82]. Granzymes are found in cytotoxic granules of $CD8^+$ T cells and NK cells [82]. Granzyme B (GzmB) is the most expressed granzyme out of five types of granzymes in humans (A, B, H, K, and M) [82]. GzmB utilizes multiple mechanisms to induce apoptosis in the target cell: DNA fragmentation, releasing reactive oxygen species (ROS), and manipulating pro-apoptotic signaling [83,84]. GzmB induces apoptosis through the caspase-dependent or independent pathways [87]. Caspases 3, 7, 8, and 10 are triggered by granzymes. However, caspase 3 exerts the most crucial role in $CD8^+$ T cell-mediated cytotoxicity [85,86]. The caspase-independent mechanism through Bid activation permeabilizes the outer membrane of mitochondria and releases pro-apoptotic proteins [85,87]. Perforin is a glycoprotein that creates membranous pores and leads to small molecules, such as calcium, entering the target cells [88]. Following calcium influx, a repair cascade in the membrane starts. Then intracellular perforin creates pores in the large endosome, allowing the granzyme integration into the cytosol to direct its' apoptotic function

[89]. Thus granzymes and perforin should be co-endocytosed to exert their function [90].

1.1.11 T cell exhaustion

T cell exhaustion defines hyporesponsive T cells to chronic antigenic stimulation, such as chronic viral infections and cancers [91,92].In this scenario, $CD8^+$ T cells gradually lose their effector functions, such as cytotoxicity, cytokine production, and proliferation. Steady upregulation of multiple co-inhibitory receptors is the hallmark of T cell exhaustion [93,94]. Co-inhibitory receptors are various types of molecules that have evolved to prevent the overactivation of immune cells and regulate immune responses [95]. However, during antigen persistence in chronic conditions, the expression of co-inhibitory receptors is elevated and sustained. Ever since various types of co-inhibitory receptors have been identified in solid and hematologic malignancies. Programmed cell death protein 1 (PD-1), cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4), T cell immunoreceptor with Ig and ITIM domains (TIGIT), T- cell immunoglobulin and mucin-domain containing-3 (TIM-3), 2B4, CD160, Galectin-9 (Gal-9), and LAG-3 are on the list [96], and the list is growing. Co-inhibitory receptors expressed on the surface of T cells upon interaction with their cognate ligands on the tumor cells or antigen-presenting cells induce an inhibitory signal, resulting in effector CTL function impairment. This interaction site is called the immune checkpoint [97,98]. The receptor-ligand binding of immune checkpoints can be blocked by monoclonal antibodies. So far, monoclonal antibodies against CTLA-4 (Ipilimumab), PD-1 (Nivolumab, Pembrolizumab, Cemiplimab), and PDL-1 (Avelumab, Durvalumab, Atezolizumab) have received US FDA approval with

promising durable clinical responses in some cancer [99]. Some pieces of evidence suggests co-inhibitory receptors take part in T cell exhaustion. For example, the PD-1 inhibitory receptor blockade reinvigorates exhausted $CD8^+$ T cells and improves immune response to chronic infections and cancer [97]. Also, as exhaustion gets more severe, autoimmunity decreases [100]. The underlying mechanism(s) associated with the upregulation of co-inhibitory receptors and, subsequently, the induction of CTL exhaustion is diverse. Several factors in the TME include tumor antigen load and potency, soluble mediators (cytokines, chemokines, exosomes, and metabolomics), immunoregulatory cells, and cell-intrinsic changes in transcriptional profiles recognized as associating factors [101].

Reports have shown that $CD8^+$ T cells in CLL are exhausted/dysfunctional in human and mouse models [35,77,102]. Upregulation of multiple co-inhibitory receptors on T cells in CLL, such as PD-1, CD160, 2B4, and TIGIT, has been demonstrated [77]. However, some reports have shown that in hematologic malignancies such as CLL, T cells are defective in priming rather than being exhausted [103]. Previously, PD-1/PD-L1 axis contribution to T cell dysfunction in CLL has been reported [104]. However, PD-1 blockade, as tested in clinical trials for treating CLL, has not been effective. It seems that the PD-1/PD-L1 axis is not adequate to revitalize dysfunctional T cells in CLL patients and emphasizes the role of other co-inhibitory receptors. Recently the involvement of a metabolic immune checkpoint, IL4I1, has been related to resistance to immune checkpoint blockade therapy in mouse models [105]. The role of epigenetic transformations in inducing T-cell exhaustion in CLL has been debated [106]. Therefore, ample evidence parallels $CD8^+$ T cell dysfunction in CLL, and the mechanism has yet to be elucidated.

1.1.12 CD160

CD160 (also known as BY55 or NK1) is a 27 KD glycosylphosphatidylinositol (GPI)-anchored glycoprotein of the immunoglobulin superfamily [107]. In humans, this molecule is mainly expressed on the surface of immune cells, including NK cells, lymphocytes [108], mast cells [109], and non-immune cells, such as activated endothelial cells [110]. It is highly expressed in malignant B cells in hematologic malignancies including CLL [111]. Three isoforms of this molecule are GPI-anchored, transmembrane (TM), and soluble forms. T cells express mainly GPI-anchored and some TM isoforms [108]. Soluble CD160 is formed by shedding this molecule with metalloproteases from the cell membrane [112].

Various functions of the CD160 molecule have been reported, and it seems that it is related to the cell types and the cognate interacting ligands. For example, CD160 in NK cells is mainly stimulatory [113,114]. In contrast, in $CD4^+$ T cells, CD160 binds to the extracellular domain of HVEM (herpes virus entry mediator) [115] and transcends inhibitory signals to $CD4^+$ T cells [116]. The role of CD160 on $CD8^+$ T cells is more complex and controversial. Soluble CD160 interacts with the MHC-I complex prohibits binding $CD8^+$ T cells to MHC-I and impairs MHC-I-dependent $CD8^+$ T cells cytotoxicity [112]. CD160 on $CD8^+$ T cells shows a co-inhibitory function in HIV [117], whereas in Listeria monocytogenes [118] and allograft skin rejections [119] a co-stimulatory role has been offered. These discrepancies seem related to the interaction with its cognate ligands, as binding to MHC-I or HVEM transduces stimulatory or inhibitory signals, respectively [116,118]. Also, the activation of CD160 on malignant B cells in CLL prolongs their survival through PI3-kinase/AKT signaling pathways [120]. CD160 expression in HTLV-1 infection has been attributed to T cell exhaustion [121].

Dysfunctional $CD160^+$ $CD8^+$ T cells in pancreatic cancers have been reported [122]. The Soluble form of CD160 mediates immune evasion in melanoma [123]. In CLL, upregulation of CD160 on $CD8^+$ T cells and co-expression with multiple co-inhibitory receptors such as 2B4 and PD-1 that lead to T cell exhaustion has been demonstrated [33,35].

1.1.13 IL-16

IL-16 (Interleukin-16) is identified as a pro-inflammatory cytokine [124]. It is generated as pro-IL-16 that lacks a signal peptide [124]. Caspase-3 in $CD8^+$ T cells converts pro-IL-16 to active IL-16 [125]. The main ligand for IL-16 is CD4 and CD9 on $CD4^+$ T cells [124]. IL-16 is chemotactic for $CD4^+$ T cells, monocytes, and eosinophils [126]. The main sources of IL-16 are non-immune and immune cells including epithelial cells, synovial fibroblasts, lymphocytes, macrophages, mast cells, and eosinophils [127–130]. $CD4^+$ and $CD8^+$ T cells constitutively express IL-16 mRNA. However, the protein is synthesized upon activation of T cells [124] and subsequently activates $CD4^+$ T cells [131]. Reports have shown that IL-16 activates the production of pro-inflammatory cytokines by human monocytes [132]. IL-16 expression has been increased in inflammatory processes such as rheumatoid arthritis, asthma, colitis, atopic dermatitis, systemic lupus erythematosus, and multiple sclerosis [124]. Of note, it has a role in inhibiting HIV-1 replication [133]. Elevated Serum levels in lymphoid malignancies have been reported [134]. It has been shown that malignant plasma cells in multiple myeloma produce IL-16 [135] which is involved in the disease progression [136]. So, in multiple myeloma, serum IL-16 has been suggested as a diagnostic marker and therapeutic target [137].

Likewise, serum IL-16 levels in gastric cancer have been suggested as a prognostic/diagnostic marker [138].

1.1.14 CD26

CD26 or DPP4 (dipeptidyl peptidase 4) is a transmembrane glycoprotein with extracellular, transmembrane, and intracytoplasmic domains [139]. The extracellular domain has three regions and is designated for multiple functions. The glycosylated and cysteine-rich regions bind to different molecules [139].

The catalytic region has enzymatic activity and cleaves off the amino-terminal dipeptides from chemokines and peptide hormones [140]. For example, CD26 inactivates glucagon-peptide-1 (GLP) and gastric-inhibitory protein (GIP) to stabilize glucose levels [141]. CD26 is involved in T cell trafficking by catalyzing chemokines and binding to the extracellular matrix [142,143]. CD26 regulates diverse chemokines, such as RANTES (CCL5), Eotaxin, Stromal-derived Factor-1α (SDF-1α/ CXCL12), and macrophage-derived chemokine (MDC/CCL22) [142]. Also, the CD26 molecule binds to fibronectin and collagen, which is involved in T cell trans-endothelial migration and homing [143].

CD26 is expressed on various non-immune cells (fibroblasts, endothelial, epithelial, mesothelial cells) and immune cells (B and T lymphocytes, NK cells, dendritic cells, macrophages, and erythroid precursors/progenitors) [144–148]. T lymphocytes are the principal CD26-expressing cells in the immune system [149].

CD26 has a significant role in T cell development and differentiation and is considered a thymus maturation marker, and is highly expressed in single-positive $CD4^+$ and $CD8^+$ T cells [149]. CD26 knock-out mice showed decreased $CD4^+$ T cell numbers

and impaired functionality [150]. Similarly, diabetic patients who receive CD26 inhibitors as glucose-regulating medications showed expansion of Tregs and paradox suppression in Th1, Th2, and Th17 cells [151].

The role of CD26 in the adenosine pathway to regulate T cell activation and proliferation has been demonstrated. CD26 is a docking site for ADA (Adenosine Deaminase). ADA converts immunosuppressive adenosine to Inosine [152]. Moreover, ADA: CD26 interaction sends stimulatory signals to T cells [153]. Furthermore, CD26 binding to Caveolin-1 on APCs transduces stimulatory signals via CARMA-1 and enhances T-cell activation [154].

$CD26^{high}$-expressing $CD4^+$ T cells have demonstrated several anti-tumor properties in different cancer models [155]. $CD26^{high}$ cells are good candidates for adoptive T-cell transfer therapies due to their enhanced migration and persistence [155]. The role of CD26 expressing $CD8^+$ T cells is not entirely determined. Their role in forming memory $CD8^+$ T cells in viral infections and transducing co-stimulatory signals has been recognized [156].

1.1.15 MAIT cells

Recently, it has been shown that MAIT (Mucosal Associated Invariant T) cells express a $CD26^{high}$ marker that can be used as a surrogate marker to identify MAIT cells [157].In humans, MAIT cells are unconventional T cells with innate-like properties and are determined by expressing a semi-invariable T-cell receptor- α chain composed of TVα7.2 [158]. In addition, MAIT cells express high levels of IL-18Rα, CD161, and CD26. These cells are present in tissues and blood circulation. MAIT cells

recognize riboflavin (Vitamin B12) metabolites released from microbial pathogens in the context of MHC class I related protein 1 (MR-1) [159].

1.1.16 Galectin-9 (Gal-9)

Gal-9 is a β-galactoside-binding lectin [160] and possesses two carbohydrate recognition domains (CRDs) attached by a linker peptide [161]. Gal-9 is constitutively expressed in different non-immune and immune cells, including B cells, T cells, monocytes, and mast cells [162], present on the membrane, cytoplasm, and nucleus of cells. Also, a considerable amount of Gal-9 is in extracellular spaces [163]. Gal-9 is synthesized in the free ribosome and is secreted via non-conventional pathways with no signal sequence [160]. It has also been shown that it is a part of extracellular vesicles (exosomes) [164]. Reports have shown that extracellular Gal-9 has an overall immunosuppressive role, tested in experimental mouse models of autoimmune and viral infections [165,166]. At cellular levels, the effect of extracellular Gal-9 is quite diverse. Various roles of Gal-9, such as chemoattraction, migration, cell adhesion, and apoptosis [172,175], are due to binding to multiple receptors. These receptors are CD137, CD44, TIM-3, PDI (protein disulfide isomerase), and IgE [163,167–169]. The inhibitory effect of Gal-9 on T cells and the pro-inflammatory effect on NK cells and monocytes have been shown [170,171]. Gal-9 modulates immune responses by suppressing Th1 and Th17 and induces Tregs [162].Gal-9 interaction with the TIM-3 ligand suppresses antigen-specific CTL effector functions and is involved in $CD8^+$ T cell exhaustion [172]. However, Gal-9 can regulate T-cell functions without TIM-3 [173]. Our group has done extensive work on the role of Gal-9 in HIV, COVID-19, and cancer patients. In HIV patients, we

showed that Gal-9 is expressed on terminally exhausted T cells [174], and NK cells expressing Gal-9 define a subpopulation with impaired cytotoxic activity [175]. Moreover, we have reported that neutrophils release Gal-9 in HIV patients, which promotes T cell activation [176]. Furthermore, in SARS-COV-2 infection, we have noted that plasma soluble Gal-9 is highly elevated and was positively correlated with pro-inflammatory cytokines/chemokines [177]. We showed that recombinant Gal-9 *in vitro* increased secretion of IL-6 and TNF-α from monocytes and NK cells [177]. Besides, significant downregulation of Gal-9 on neutrophils in COVID-19 patients was in line with Gal-9 shedding in COVID-19 patients [177]. These observations suggest that soluble Gal-9 plays a role in cytokine release syndrome in COVID-19 [177]. The Gal-9:TIM-3 interaction is involved in the immune evasion of human acute myeloid leukemia cells [178]. Our group has shown that plasma Gal-9 is elevated in viral-associated solid tumor patients, and Gal-9-expressing T and NK cells are exhausted in the periphery and tumor microenvironment [179]. Reports have shown that soluble Gal-9 affects T cells by two distinct pathways, one via TCR (T cell receptor) activation and transducing signal through LCK, and the other one by the induction of apoptosis [180]. Taken together, some studies suggest that antibodies neutralizing Gal-9 might be considered as a potential target of immunotherapy [181].

1.2 HPV-associated carcinoma

1.2.1 Virology

HPV-associated carcinomas are caused by persistent HPV infections [182]. HPV is non-enveloped, double-stranded DNA belonging to *papillomaviridae* [183]. HPV

infects humans and is transmitted through sexual and skin-to-skin contact [183]. About 80% of individuals get infected with HPV at some point. However, in most people immune system clears the infection [184]. Based on genomic sequence, more than 200 HPV variants have been identified; 14 of them are high-risk variants and can cause cancer [183]. HPV types 16 and 18 are the major high-risk variants inducing cancers [183]. HPV infects the epithelial cells and causes squamous cell carcinoma in anogenital (cervix, vulva, vagina, penis, anal), and head and neck (oropharynx, tonsils, larynx, paranasal sinuses) areas [183].

1.2.2 Epidemiology

About 12% of all cancers are caused by viruses [182]. So far, seven oncogenic viruses have been identified, including Human Papillomavirus (HPV), Hepatitis C virus (HCV), Hepatitis B virus (HBV), Human Herpes virus type-8 (HHV-8), Human T cell lymphotropic virus -1 (HTLV-1), Epstein-Barr virus (EBV), and Merkel cell polyomavirus (MCV) [182]. HPV is responsible for most viral-associated cancer cases worldwide [182]. Overall new cases of HPV-associated carcinoma in the United States were 13.68 and 11 per 100,000 women and men in 2017 [185]. About 4% of all types of cancer are caused by HPV globally [186]. Approximately 2% are in high-income countries, and 8% are in low-income countries [186]. Most HPV-associated carcinoma in women is cervical cancer. However, in men, they are more located in oropharyngeal areas [187]. In detail, more than 90% of cervical and anal cancers, about 70% of vulvar and vaginal cancers, and more than 60% of penile cancers are caused by HPV [187]. Tobacco and alcohol have accounted for most cases of oropharyngeal cancers in the past, but recent statistics revealed that about 70% of

oropharyngeal carcinoma are linked to HPV [187]. Recently the incidence of cervical carcinoma has dropped dramatically due to early diagnosis and prevention by vaccines [187]. However, the rate of HPV-related laryngeal cancer in men has surged [187]. Considering age, cervical cancers are frequently diagnosed in younger generations (50 years old) compared to other anatomical locations. Anal and oropharyngeal carcinoma are diagnosed at younger ages in men rather than women [187].

1.2.3 Pathogenesis

Prolonged HPV infection induces multistep oncogenesis in epithelial cells. The first changes are pre-malignant intraepithelial squamous or glandular lesions [188]. In the cervix, it is called Cervical Intraepithelial Neoplasia (CIN), further classified as CIN-1, CIN-2, and CIN-3, representing mild, moderate, and severe dysplastic changes [189]. Most CIN lesions subside voluntarily; however, some untreated precancerous lesions will become invasive cancers over the years. Infection with high-risk HPV variants is a risk factor for carcinogenesis. The integration of HPV DNA into the host genome has been proposed as a mechanism for carcinogenesis [190,191]. Subsequently, E1/E2 open reading frames of HPV DNA will be disrupted, leading to loss of E2 control on E6/E7 regulation [192]. E6 and E7 are HPV viral oncoproteins causing decreased expression of p53 and inactivation of retinoblastoma protein (pRb) [193]. As p53 and pRb are tumor suppressor proteins and the checkpoints of cell cycles, the loss of their function leads to uncontrolled cell proliferation [194]. Interestingly, only high-risk variants of HPV oncoproteins (E6/E7) can bind to p53 and pRb [195]. Degraded p53 inactivates cyclin-dependent kinase

inhibitor p21 [196]. As a result, in response to many stimuli, cells enter the S phase, the proliferating phase, and cannot enter the cell cycle arrest, the G1 phase [196]. Likewise, the E7 viral protein releases E2F transcription factors that impulsively push the cells through the S phase [194,197].

1.2.4 Clinical presentation

Cervical cancer is often asymptomatic at early stages, while advanced disease causes symptoms such as pelvic pain, discharge, dyspareunia, and abnormal vaginal bleeding [198]. Head and neck carcinoma symptoms vary depending on the site of the tumor. Most patients have a prolonged sore throat, difficulty swallowing, and voice changes; some have neck pain, headaches, difficulty breathing, and recurrent sinus infections [199].

1.2.5 Diagnosis

Histopathologic examination of tumor biopsy is necessary to diagnose squamous cell carcinoma [198,199]. HPV infection should be screened by measuring p16 protein expression in tumor tissues (Immunohistochemistry), HPV DNA in tumor cell nuclei (*in situ* hybridization or PCR), or E6/E7 mRNA (PCR) [199].

1.2.6 Clinical course and prognosis

Staging remains the most critical prognosis determinant in head, neck, and cervical carcinoma [198,199]. Recently HPV status has been considered an essential prognostic criterion [199]. Head and neck HPV^+ tumors have a better survival rate and better response to treatments than their HPV^- counterparts, and there is more

interest in applying less rigorous treatment approaches [200]. However, as most cervical cancers are HPV^+, it seems that HPV status is less practical as a prognostic determinant in these cancers. Multiple analyses have shown that HPV negativity is linked to tumor progression [201].In addition, the HPV genotype may be applicable as a prognostic marker in cervical cancer, as HPV-18 positivity showed a poorer prognosis from the early stages of cancer [202]. Likely, it has been demonstrated that non-European variants of HPV-16 have more substantial oncogenic properties to progress invasive tumors [203]. Like the HPV genotype, viral load has also been associated with prognosis in cervical and anal carcinoma [204,205].

Reports have shown an increased incidence of HPV-associated carcinoma in immunodeficient patients such as HIV/AIDS [206] and patients who received organ transplants [207].

1.2.7 Treatment

- *Conventional treatments*

HPV infection is not curable. Pap test as a screening test has had the advantage of early cervical cancer diagnosis [198]. The treatment options varied depending on the tumor's grade, stage, and location. Treatments include surgery, radiotherapy, chemotherapy, targeted therapy, or combination therapies [208]. Of note, most patients with HPV^+ cancer receive similar treatment as HPV^- cancers if they are at the exact anatomical location [199].

- Immunotherapy

    - *Targeting PD-1/PD-L1 axis*

High expression of PD-1 on CTLs is the main reason for T cell exhaustion in various tumors. Multiple studies have shown the independent role of the PD-1/PD-L1 pathway in cancer progression in cervical, head, and neck cancers [209].PD-1 binds to its ligands, PD-L1/PD-L2, present on tumor cells or APCs and induces T cell exhaustion through various mechanisms. The intracytoplasmic domain of PD-1 owns an immunoreceptor tyrosine-based switch motif (ITSM) that activates SHP1/SHP2 phosphatases. SHP1 and SHP2 prevent further activation of downstream signaling pathways in T cells. Moreover, PD-1/PD-L1 binding upregulates inhibitory genes such as BATF [210] and inhibits the function of the co-stimulatory molecule CD28 on T cells [211]. In 2018, FDA approved pembrolizumab, an anti-PD-1 monoclonal antibody as a first-line treatment alternative used as mono or combined with chemotherapy, for PD-L1 expressing head and neck carcinoma, regardless of their HPV status [212]. However, some patients fail to respond to treatments that necessitate combination therapies [199].

    - *Valproic acid (VA)*

Valproic acid (VA) is a histone deacetylase (HDAC) inhibitor used for the treatment of patients with neurologic disorders for decades. Recently, a novel effect in eradication of cancer cells has been revealed [213]. Increased CTL response following VA treatment in HPV-associated cervical cancers has been reported [214]. Histone acetylation affects gene transcription by posttranslational

modification of histones [215]. HDACs repress gene transcription as they open the chromatin frame [216]. These gene transcriptional changes impact various functions such as DNA repair, apoptosis, cell cycle control, differentiation, and metabolism. Almost all cancer cells show increased HDAC activity that affects gene expression and cell differentiation in many aspects [217].

HDAC inhibitors (HDACi) are a new class of therapeutic agents as anti-cancer drugs that alter the transcription of histone and non-histone proteins [218]. FDA has approved several HDACis for some cancers in combination with other therapies [216]. Immune checkpoint blockade therapy combined with HDACi has shown promising results *in vitro* and *in vivo.* VA's impact on immune cells is very diverse. Reports have shown that VA modulates innate and adaptive immune responses [219]. Thus, these reports support the potential efficacy of VA in cancer treatment.

1.2.8 Immune responses in HPV-associated carcinoma

Antigen presentation by tumor cells or APCs is essential for T cell activation. Tumors often use strategies to impair antigen presentation pathways involving HLA class I and II [220]. HPV hides its antigens from the immune system by inducing mutations in the HLA loci of the host genome or uses its own E5 gene to impair endosomal-cell surface transit of peptide-bound HLA complexes [221]. These changes occur in early infection and control the persistence, replication, and distribution to support the malignant transformation of the host cells [221].

Host cells prompt anti-viral responses by activating the cGAS-STING pathway, which increases the expression of genes encoding IFN type I and pro-inflammatory

cytokines [222]. HPV oncoproteins dampen the cGAS-STING pathway through multiple mechanisms, yet to be determined [223].

The TME of squamous cell carcinoma consists of immune and non-immune cells [224]. Non-immune cells include cancer cells and stromal cells such as cancer-associated fibroblasts and endothelial cells. Immune cells consist of Tumor-infiltrating lymphocytes (TILs: B cells, T cells, NK cells) and myeloid cells (Macrophages, neutrophils, dendritic cells, and myeloid-derived suppressor cells (MDSCs) [225]. The TME is highly infiltrated by immune cells. Of note, HPV^+ tumors have higher tumor intraepithelial lymphocytes than HPV^- counterparts [226]. Reports have shown that TME in HPV-associated carcinoma is highly suppressive. The balance of anti-tumor immunity and immunosuppressive immunity is associated with tumor evasion and outcome [225]. Anti-tumor immunity is mainly mediated by CTLs and NK cells, whereas immunosuppressive activity is mediated by Treg cells, MDSCs, and M2 macrophages [199].

Several evasion mechanisms have been identified in HPV-associated carcinoma. One is the secretion of inhibitory cytokines and chemokines by tumor and stromal cells that recruits and creates immunosuppressive cells in TME. IL-6, TGF-β, VEGF, and IL-10 are essential inhibitory mediators [224]. The second mechanism is the upregulation of PD-L1, which dampens the cytotoxic activity of CTLs [227]. Intra-tumoral lymphocytes express higher PD-L1 than HPV^- tumors. PD-L1 upregulation results from the high IFN-γ in HPV^+ tumors rather than the direct effect of the virus [228]. Likewise, MDSCs and Treg cells in TME express PD-L1. Not to mention that viral oncoproteins (E5, E6, E7) also induce changes in tumor cells in favor of immune

escape [229]. Also, the loss of TRAF3 that codes anti-viral proteins incorporates immune evasion [230].

1.2.9 Myeloid-derived suppressor cells (MDSCs)

Myeloid cells constitute most peripheral blood immune cells and include a diverse cell population with distinct functions [231]. Three populations of mature myeloid cells in the blood are Granulocytes, Monocytes, and Dendritic cells. These cells are very well known as innate immune cells that are indispensable to orchestrating functions of innate and adaptive immune responses. The persistent stimulation in cancer generates myeloid cells, including neutrophils and monocytes, that have immunosuppressive functions [232,233]. They are grouped into three subsets: PMN-MDSC resembling neutrophils, M-MDSC resembling monocytes, and a minimal subset including progenitors of myeloid cells [234]. Some Markers are defined for immunophenotyping MDSCs, such as PMN-MDSCs, as $CD11b^{+}CD14^{-}CD15^{+}$ or $CD11b^{+}CD14^{-}CD66b^{+}$, M-MDSCs as $CD11b^{+}CD14^{+}HLA\text{-}DR^{-/low}$ $CD15^{-}$, and early stage MDSCs as $Lin^{-}CD33^{+}HLA\text{-}DR^{-}$ [234].M-MDSCs are recognized from monocytes by low HLA-DR expression. Recent research has shown LOX-1 (Lectin-like Oxidized Low-density Lipoprotein-1) as a marker to identify MDSCs from mature myeloid cells [235].

MDSCs suppress immune cells, mainly T cells, through several mechanisms. One is via producing soluble inhibitory mediators such as Reactive Oxygen Species (ROS), Arginase-1 (ARG-1), iNOS (inducible nitric oxide synthase), TGF- β , IL-10, cyclooxygenase-2 (COX-2), and IDO (Indoleamine 2,3-dioxygenase) [236]. Also,

they can suppress T cells through cell-cell interaction by binding to co-inhibitory receptors. MDSCs express ligands such as PD-L1 to attach to the cognate receptor on T cells [237]. In addition, MDSCs promote angiogenesis by generating VEGF, FGF, and MMP9 and enhance metastasis [238]. Recently, MDSCs as a resistant mechanism to immune checkpoint blockade therapy has been suggested [239].

1.2.10 IL-8

IL-8 (CXCL8) is an ELR^+ CXC chemokine generated from tumor cells, stromal cells, and myeloid cells and interacts with its receptors CXCR1 and CXCR2 on the surface of tumor cells and myeloid cells. CXCR1 and CXCR2 are both transmembrane G protein-coupled receptors highly expressed on neutrophils. IL-8 specifically recruits neutrophils to inflamed tissues [240]. CXCR1 is dominant in IL-8-mediated chemotaxis, whereas CXCR2 is more specifically involved in angiogenesis and keratinocyte proliferation [241]. IL-8 role in both angiogenesis and tumor progression has been widely studied. Elevated serum levels have also been shown in metastatic melanoma [242]. IL-8 is elevated in head and neck squamous cell carcinoma, correlates with poor prognosis, and promotes tumor progression through the inactivation PTEN/activated STAT3 pathway. The phosphorylation of STAT3 is the critical downstream signaling of IL-8 and has a role in epithelial-to-mesenchymal transition, and inactivation of PTEN enhances IL-8 secretion [243].

IL-8 produced by melanoma cells are involved in the expansion and recruitment of MDSCs and poor outcome [244].

1.2.11 NETosis

Netosis is a distinct form of cell death in neutrophils that involves the expulsion of nuclear DNA content along with lytic granular proteins extracellularly, called NETs [245]. This mechanism of death plays a crucial role in clearing pathogens such as fungi and bacteria [245]. Despite the essential role of NETosis in anti-bacterial processes, recently the role of NETosis in cancer formation and progression has been suggested. So far, NETosis has been described in diffuse large B cell lymphoma, lung, breast, and ovarian cancer [246]. Of note, NETosis has been correlated with poor prognosis and is mainly observed in advance stages of cancers [247]. Some studies have shown the correlation of plasma IL-8 with circulating NETs [248]. In addition, the role of NETS in hindering the effect of immunotherapy has been made clear [249].

1.3 Research hypothesis and objectives

1.3.1 Hypothesis

T cell exhaustion is defined by the upregulation of multiple co-inhibitory receptors leading to diminished effector functions of T cells [93,94]. T cell exhaustion has been distinguished in chronic antigenic stimulations such as cancer [91,92]. CLL as a blood cancer [35,77,102] and HPV-associated carcinoma as a solid tumor show features of T cell exhaustion [250] with some variation that might be related to different underlying mechanisms. These differences are reflected in response to immune checkpoint blockade therapy that targets the co-inhibitory receptors/ligands to reverse T cell exhaustion. In contrast to HPV-associated carcinoma, immune checkpoint blockade therapy in CLL has not been promising [224].

Due to the significance of T cell response in anti-tumor defense, exploring the underlying mechanism that induces T cell dysfunction in CLL is necessary. Moreover,

understanding resistant mechanisms to immunotherapy in HPV-associated carcinoma patients will assist in a better elucidation of T cell responses. Findings will help design future cancer treatments with applications in precision medicine.

We hypothesize that CTLs in the peripheral blood of CLL and HPV-associated cancer patients are exhausted. The following objectives have been defined to test this hypothesis:

1.3.2 Objective 1.

To identify co-inhibitory receptors associated with CTL dysfunction in the blood and bone marrow of CLL patients.

Hypothesis 1: CTLs in CLL patients express higher levels of multiple co-inhibitory receptors than healthy controls, inhibiting their effector functions.

Rationale: Results from several cancers have shown that CTL function is regulated by multiple inhibitory pathways triggered by distinct and non-redundant co-inhibitory receptors [251]. The role of co-inhibitory receptors in CTL exhaustion has been mainly related to PD-1 and CTLA-4 upregulation [252]. However, in CLL, contradictory findings on the effect of the anti-PD-1 blockade have been reported. Participation of other co-inhibitory receptors in the evolution of T cell exhaustion in CLL should be considered. Further investigation of dysfunctional CTLs in CLL patients will assist in identifying other upregulated co-inhibitory receptors. These functional assays will establish whether CTL functions differ in clusters of CTLs that express individual or multiple co-inhibitory receptors.

1.3.3 Objective 2.

To characterize CTL function and phenotype linked to CD26 molecule in the blood of CLL patients.

Hypothesis 2: Chronic inflammation in CLL decreases the CD26-expressing CTLs, impacting their effector function and migration.

Rationale: Decreased $CD26^{+}$ lymphocytes in patients with Non-Hodgkin Lymphoma (NHL) compared to healthy controls have been reported [253]. CD26 is essential in the adenosine pathway in converting suppressive adenosine to inosine [254]. Decreased CD26 expression on CTLs dampens adenosine degradation, and excess adenosine dampens CTL functions [255]. In addition, the CD26 molecule delivers co-stimulatory signals to T cells [156]. CD26 is essential in regulating chemokines due to its enzymatic activity [256]. Collectively, decreased CD26 levels in CTLs may be a marker of CTL exhaustion in CLL patients that requires further investigation. Our preliminary data from the CLL study has shown the downregulation of CD26 on CTLs. I will build on these observations by investigating the role of CD26 on CTL effector functions in CLL patients. As the CD26 expression pattern on CTLs identifies two distinct populations on flow cytometry panels as $CD26^{low}$ and $CD26^{high}$, I will compare the effector functions and characteristics in these two populations. Due to the multiple functions of the CD26 molecule in T cells, I will investigate CD26 downregulation in CLL and the impact on T cell functions. CD26 expressing CTLs in CLL has not been thoroughly investigated. The findings will enable us to determine the expression pattern of CD26 molecule in CTLs and its correlation with other molecules that may help to clarify the decreased CD26 expression and underlying mechanisms in CLL. Thus, we anticipate that the altered expression of CD26 can

impact CTL functions in terms of cytotoxicity, proliferation, and migration. Understanding why and how CD26 is downregulated can provide a novel window of immunotherapy.

1.3.4 Objective 3.

Transcriptional profiling of PBMCs to understand the mechanisms that may explain the response to immunotherapy in HPV-associated carcinoma.

Hypothesis 3: Immunotherapy modulates transcriptome that is different in responders versus non-responders.

Rationale: HPV-associated carcinoma is a good candidate for immune checkpoint blockade therapy. The FDA approved pembrolizumab (anti-PD-1) in 2018 for recurrent or metastatic cervical cancer [257]. However, a favorable response to immunotherapies could be improved by increasing the immunogenicity of the tumors in combination with immune checkpoint inhibition. Studies have shown a better response for immune checkpoint blockade therapy in immunogenic tumors such as Melanoma [2]. Furthermore, it has been shown that the diversity in baseline gene expression might affect the patient's response to immunotherapy. Therefore, I anticipate that the gene alteration might not be the same for all the patients in each group. Comparing the transcript alterations with clinical response to the treatment will help to identify the mechanistic pathways that have a role in response to therapy. Also, according to the concept that some baseline transcript expressions might be associated with a more favorable clinical response, data could be interpreted as predictive markers for response to immunotherapy. Meanwhile, studying pathways

related to immune functions might reveal some clues related to T cell exhaustion and reversion in cancer patients.

In addition, we analyze the impact of VA on the circulating immune cells as a novel treatment in combination with immune checkpoint blockade therapy. VA is a histone-deacetylase (HDAC) inhibitor indicated for treating patients with seizures, but recently it has shown a promising role in the treatment of cancer and HIV [213]. VA has a broad impact on cancer cells as it acetylates histones, opens the chromatin, and modifies the expression of genes associated with apoptosis, cell cycle, and differentiation [258]. The impact of VA on modulating innate and adaptive immune responses has been studied [219]. In particular, VA was recently shown to increase CTL response in HPV-associated cervical cancer cells [214]. Further investigations are required to understand how VA affects the immune system in the presence of immune checkpoint blockade therapy. Findings may provide a novel approach to enhance the efficacy of immunotherapy.

1.3.5 Scope of the Thesis

Three hypotheses and objectives are tested in this study as outlined in three chapters (chapters 2-4), with two additional chapters for the introduction (chapter 1) and a final chapter (chapter 5) for general discussion and conclusion.

Chapter 2 tested the first hypothesis and focused on the expression of different co-inhibitory receptors on $CD4^+$ and $CD8^+$ T cells in CLL patients. We observed elevated levels of CD160 on T cells along with other co-inhibitory receptors. Further studies examined the function of $CD8^+$ T cells regarding the expression of co-inhibitory

receptors, particularly CD160. At last, some mechanisms related to the expansion of CD160-expressing $CD8^+$ T cells in CLL were proposed. The results related to this chapter are already published in the Journal for Immunotherapy of Cancer (Bozorgmehr et al., 2021).

Chapter 3 tested the second hypothesis and focused on the role of CD26 on $CD8^+$ T cells in CLL patients. The expression of CD26-expressing $CD8^+$ T cells and its' subpopulations, $CD26^{low}$ and $CD26^{high}$ was investigated, followed by further characterization. Finally, we examined the function of $CD8^+$ T cells linked to CD26 expression on $CD8^+$ T cells and found the impact of the inflammatory milieu of CLL on $CD26^+$ $CD8^+$ T cell depletion as a proposed mechanism. The results related to this chapter were recently published in the Journal of Experimental Hematology and Oncology (Bozorgmehr et al., 2023).

Chapter 4 tested the third hypothesis and analyzed transcriptional profiles of advanced HPV-associated carcinoma patients receiving combined immune checkpoint blockade therapy and valproic acid. RNAseq was performed on peripheral blood mononuclear cells, and the transcriptional changes were compared in responders versus non-responders at different time points, followed by plasma cytokine profile analysis. This study revealed differential transcription profiles and pathways distinguishing responders from non-responders at the baseline and the end of treatment. The results related to this chapter were recently submitted to the Journal of Molecular Oncology and is under review.

Lastly, Chapter 5 discusses the outcome of these studies, their interrelationship, and their significance in the cancer setting. Study limitations and future directions are highlighted as well.

1.4 References

1. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. Nat Rev Immunol [Internet]. 2020 [cited 2021 Oct 26];20:651–68. Available from: https://www.nature.com/articles/s41577-020-0306-5

2. Bai R, Lv Z, Xu D, Cui J. Predictive biomarkers for cancer immunotherapy with immune checkpoint inhibitors. Biomark Res [Internet]. 2020 [cited 2021 Oct 27];8:34. Available from: https://doi.org/10.1186/s40364-020-00209-0

3. Chronic Lymphocytic Leukemia - Cancer Stat Facts [Internet]. SEER. [cited 2023 Mar 9]. Available from: https://seer.cancer.gov/statfacts/html/clyl.html

4. Yao Y, Lin X, Li F, Jin J, Wang H. The global burden and attributable risk factors of chronic lymphocytic leukemia in 204 countries and territories from 1990 to 2019: analysis based on the global burden of disease study 2019. Biomed Eng OnLine [Internet]. 2022 [cited 2023 Mar 11];21:4. Available from: https://doi.org/10.1186/s12938-021-00973-6

5. Key Statistics for Chronic Lymphocytic Leukemia [Internet]. [cited 2023 Mar 9]. Available from: https://www.cancer.org/cancer/chronic-lymphocytic-leukemia/about/key-statistics.html

6. Chiorazzi N, Rai KR, Ferrarini M. Chronic lymphocytic leukemia. N Engl J Med. 2005;352:804–15.

7. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood [Internet]. 2016 [cited 2021 Apr 13];127:2375–90. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4874220/

8. Seifert M, Sellmann L, Bloehdorn J, Wein F, Stilgenbauer S, Dürig J, et al. Cellular origin and pathophysiology of chronic lymphocytic leukemia. J Exp Med [Internet]. 2012 [cited 2021 Feb 18];209:2183–98. Available from: https://doi.org/10.1084/jem.20120833

9. Kipps TJ, Stevenson FK, Wu CJ, Croce CM, Packham G, Wierda WG, et al. Chronic lymphocytic leukaemia. Nat Rev Dis Primer [Internet]. 2017 [cited 2023 Mar 10];3:16096. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5336551/

10. Cerhan JR, Slager SL. Familial predisposition and genetic risk factors for lymphoma. Blood. 2015;126:2265–73.

11. Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, et al. Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med. 2000;343:78–85.

12. Baumann Kreuziger LM, Tarchand G, Morrison VA. The impact of Agent Orange exposure on presentation and prognosis of patients with chronic lymphocytic leukemia. Leuk Lymphoma. 2014;55:63–6.

13. Schinasi LH, De Roos AJ, Ray RM, Edlefsen KL, Parks CG, Howard BV, et al. Insecticide exposure and farm history in relation to risk of lymphomas and leukemias in the Women's Health Initiative observational study cohort. Ann Epidemiol. 2015;25:803–10.

14. Kikushige Y, Ishikawa F, Miyamoto T, Shima T, Urata S, Yoshimoto G, et al. Self-renewing hematopoietic stem cell is the primary target in pathogenesis of human chronic lymphocytic leukemia. Cancer Cell. 2011;20:246–59.

15. Landau DA, Tausch E, Taylor-Weiner AN, Stewart C, Reiter JG, Bahlo J, et al. Mutations driving CLL and their evolution in progression and relapse. Nature [Internet]. 2015 [cited 2023 Mar 10];526:525–30. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4815041/

16. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature [Internet]. Nature Publishing Group; 2009 [cited 2023 Mar 10];461:1071–8. Available from: https://www.nature.com/articles/nature08467

17. Landau DA, Clement K, Ziller MJ, Boyle P, Fan J, Gu H, et al. Locally Disordered Methylation Forms the Basis of Intratumor Methylome Variation in Chronic Lymphocytic Leukemia. Cancer Cell [Internet]. 2014 [cited 2023 Mar 10];26:813–25. Available from: https://www.sciencedirect.com/science/article/pii/S1535610814004164

18. Burger JA, Ghia P, Rosenwald A, Caligaris-Cappio F. The microenvironment in mature B-cell malignancies: a target for new treatment strategies. Blood [Internet]. 2009 [cited 2023 Mar 10];114:3367–75. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4969052/

19. Morrison VA. Infectious complications in patients with chronic lymphocytic leukemia: pathogenesis, spectrum of infection, and approaches to prophylaxis. Clin Lymphoma Myeloma. 2009;9:365–70.

20. Dearden C. Disease-specific complications of chronic lymphocytic leukemia. Hematol Am Soc Hematol Educ Program. 2008;450–6.

21. Hallek M, Al-Sawaf O. Chronic lymphocytic leukemia: 2022 update on diagnostic and therapeutic procedures. Am J Hematol [Internet]. 2021 [cited 2023 Mar 11];96:1679–705. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/ajh.26367

22. Brugiatelli M, Bandini G, Barosi G, Lauria F, Liso V, Marchetti M, et al. Management of chronic lymphocytic leukemia: practice guidelines from the Italian Society of Hematology, the Italian Society of Experimental Hematology and the Italian Group for Bone Marrow Transplantation. Haematologica. 2006;91:1662–73.

23. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Döhner H, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood. 2008;111:5446–56.

24. Zenz T, Mertens D, Küppers R, Döhner H, Stilgenbauer S. From pathogenesis to treatment of chronic lymphocytic leukaemia. Nat Rev Cancer. 2010;10:37–50.

25. Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS. Clinical staging of chronic lymphocytic leukemia. Blood. 1975;46:219–34.

26. Binet JL, Auquier A, Dighiero G, Chastang C, Piguet H, Goasguen J, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer. 1981;48:198–206.

27. Moreno C, Montserrat E. New prognostic markers in chronic lymphocytic leukemia. Blood Rev. 2008;22:211–9.

28. Zenz T, Eichhorst B, Busch R, Denzel T, Häbe S, Winkler D, et al. TP53 mutation and survival in chronic lymphocytic leukemia. J Clin Oncol Off J Am Soc Clin Oncol. 2010;28:4473–9.

29. Foà R, Del Giudice I, Cuneo A, Del Poeta G, Ciolli S, Di Raimondo F, et al. Chlorambucil plus rituximab with or without maintenance rituximab as first-line treatment for elderly chronic lymphocytic leukemia patients. Am J Hematol. 2014;89:480–6.

30. Freeman CL, Gribben JG. Immunotherapy in Chronic Lymphocytic Leukaemia (CLL). Curr Hematol Malig Rep [Internet]. 2016 [cited 2023 Mar 11];11:29–36. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4796351/

31. Fischer K, Bahlo J, Fink AM, Goede V, Herling CD, Cramer P, et al. Long-term remissions after FCR chemoimmunotherapy in previously untreated patients with CLL: updated results of the CLL8 trial. Blood. 2016;127:208–15.

32. Sher T, Miller KC, Lawrence D, Whitworth A, Hernandez-Ilizaliturri F, Czuczman MS, et al. Efficacy of lenalidomide in patients with chronic lymphocytic leukemia with high-risk cytogenetics. Leuk Lymphoma. 2010;51:85–8.

33. Ramsay AG, Clear AJ, Fatah R, Gribben JG. Multiple inhibitory ligands induce impaired T-cell immunologic synapse function in chronic lymphocytic leukemia that can be blocked with lenalidomide: establishing a reversible immune evasion mechanism in human cancer. Blood. 2012;120:1412–21.

34. Greaves P, Gribben JG. The role of B7 family molecules in hematologic malignancy. Blood. 2013;121:734–44.

35. Riches JC, Davies JK, McClanahan F, Fatah R, Iqbal S, Agrawal S, et al. T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. Blood. 2013;121:1612–21.

36. Rogers KA, Huang Y, Dotson E, Lundberg J, Andritsos LA, Awan FT, et al. Use of PD-1 (PDCD1) inhibitors for the treatment of Richter syndrome: experience at a single academic centre. Br J Haematol. 2019;185:363–6.

37. Sagiv-Barfi I, Kohrt HEK, Czerwinski DK, Ng PP, Chang BY, Levy R. Therapeutic antitumor immunity by checkpoint blockade is enhanced by ibrutinib, an inhibitor of both BTK and ITK. Proc Natl Acad Sci U S A. 2015;112:E966-972.

38. Ding W, LaPlant BR, Call TG, Parikh SA, Leis JF, He R, et al. Pembrolizumab in patients with CLL and Richter transformation or with relapsed CLL. Blood. 2017;129:3419–27.

39. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med. 2011;365:725–33.

40. Turtle CJ, Hay KA, Hanafi L-A, Li D, Cherian S, Chen X, et al. Durable Molecular Remissions in Chronic Lymphocytic Leukemia Treated With CD19-Specific Chimeric Antigen Receptor-Modified T Cells After Failure of Ibrutinib. J Clin Oncol Off J Am Soc Clin Oncol. 2017;35:3010–20.

41. Frey NV, Gill S, Hexner EO, Schuster S, Nasta S, Loren A, et al. Long-Term Outcomes From a Randomized Dose Optimization Study of Chimeric Antigen Receptor Modified T Cells in Relapsed Chronic Lymphocytic Leukemia. J Clin Oncol Off J Am Soc Clin Oncol. 2020;38:2862–71.

42. Siddiqi T. Updated Follow-up of Patients with Relapsed/Refractory Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma Treated with Lisocabtagene Maraleucel in the Phase 1 Monotherapy Cohort of Transcend CLL 004, Including High-Risk and Ibrutinib-Treated Patients. ASH; 2020 [cited 2023 Mar 11]. Available from: https://ash.confex.com/ash/2020/webprogram/Paper140491.html

43. Gribben JG. Stem cell transplantation in chronic lymphocytic leukemia. Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant. 2009;15:53–8.

44. Dreger P, Schetelig J, Andersen N, Corradini P, van Gelder M, Gribben J, et al. Managing high-risk CLL during transition to a new treatment era: stem cell transplantation or novel agents? Blood. 2014;124:3841–9.

45. Petlickovski A, Laurenti L, Li X, Marietti S, Chiusolo P, Sica S, et al. Sustained signaling through the B-cell receptor induces Mcl-1 and promotes survival of chronic lymphocytic leukemia B cells. Blood [Internet]. 2005 [cited 2023 Mar 11];105:4820–7. Available from: https://www.sciencedirect.com/science/article/pii/S0006497120534198

46. Wiestner A. Emerging role of kinase-targeted strategies in chronic lymphocytic leukemia. Hematol Am Soc Hematol Educ Program. 2012;2012:88–96.

47. Roberts AW, Davids MS, Pagel JM, Kahl BS, Puvvada SD, Gerecitano JF, et al. Targeting BCL2 with Venetoclax in Relapsed Chronic Lymphocytic Leukemia. N Engl J Med [Internet]. Massachusetts Medical Society; 2016 [cited 2023 Mar 11];374:311–22. Available from: https://doi.org/10.1056/NEJMoa1513257

48. Sthoeger ZM, Sthoeger D, Shtalrid M, Sigler E, Geltner D, Berrebi A. Mechanism of autoimmune hemolytic anemia in chronic lymphocytic leukemia. Am J Hematol. 1993;43:259–64.

49. Cheson BD, Heitner Enschede S, Cerri E, Desai M, Potluri J, Lamanna N, et al. Tumor Lysis Syndrome in Chronic Lymphocytic Leukemia with Novel Targeted Agents. The Oncologist [Internet]. 2017 [cited 2023 Apr 3];22:1283–91. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5679833/

50. Beiggi S, Johnston JB, Seftel MD, Pitz MW, Kumar R, Banerji V, et al. Increased risk of second malignancies in chronic lymphocytic leukaemia patients as compared with follicular lymphoma patients: a Canadian population-based study. Br J Cancer [Internet]. 2013 [cited 2023 Apr 3];109:1287–90. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3778273/

51. Eversman A, Cao S, Boughan KM, Gallogly MM, Malek E, Metheny L III, et al. Risk of Secondary Malignancy in CLL Patients Treated with Novel Targeted Agents. Blood [Internet]. 2020 [cited 2023 Apr 3];136:46–7. Available from: https://doi.org/10.1182/blood-2020-140042

52. Solomon BM, Rabe KG, Slager SL, Brewer JD, Cerhan JR, Shanafelt TD. Overall and cancer-specific survival of patients with breast, colon, kidney, and lung cancers with and without chronic lymphocytic leukemia: a SEER population-based study. J Clin Oncol Off J Am Soc Clin Oncol. 2013;31:930–7.

53. Teh BW, Tam CS, Handunnetti S, Worth LJ, Slavin MA. Infections in patients with chronic lymphocytic leukaemia: Mitigating risk in the era of targeted therapies. Blood Rev. 2018;32:499–507.

54. Caligaris-Cappio F. Role of the microenvironment in chronic lymphocytic leukaemia. Br J Haematol [Internet]. 2003 [cited 2023 Mar 10];123:380–8. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1365-2141.2003.04679.x

55. Ghia P, Strola G, Granziero L, Geuna M, Guida G, Sallusto F, et al. Chronic lymphocytic leukemia B cells are endowed with the capacity to attract CD4+, CD40L+

T cells by producing CCL22. Eur J Immunol [Internet]. 2002 [cited 2023 Mar 10];32:1403–13. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/1521-4141%28200205%2932%3A5%3C1403%3A%3AAID-IMMU1403%3E3.0.CO%3B2-Y

56. Bürkle A, Niedermeier M, Schmitt-Gräff A, Wierda WG, Keating MJ, Burger JA. Overexpression of the CXCR5 chemokine receptor, and its ligand, CXCL13 in B-cell chronic lymphocytic leukemia. Blood [Internet]. 2007 [cited 2023 Mar 10];110:3316–25. Available from: https://doi.org/10.1182/blood-2007-05-089409

57. Burger JA, Quiroga MP, Hartmann E, Bürkle A, Wierda WG, Keating MJ, et al. High-level expression of the T-cell chemokines CCL3 and CCL4 by chronic lymphocytic leukemia B cells in nurselike cell cocultures and after BCR stimulation. Blood [Internet]. 2009 [cited 2023 Mar 10];113:3050–8. Available from: https://doi.org/10.1182/blood-2008-07-170415

58. Kontoyiannis DP, Georgiadou SP, Wierda WG, Wright S, Albert ND, Ferrajoli A, et al. Impaired bactericidal but not fungicidal activity of polymorphonuclear neutrophils in patients with chronic lymphocytic leukemia. Leuk Lymphoma. 2013;54:1730–3.

59. Maffei R, Bulgarelli J, Fiorcari S, Bertoncelli L, Martinelli S, Guarnotta C, et al. The monocytic population in chronic lymphocytic leukemia shows altered composition and deregulation of genes involved in phagocytosis and inflammation. Haematologica [Internet]. 2013 [cited 2021 Feb 27];98:1115–23. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3696616/

60. Huergo-Zapico L, Acebes-Huerta A, Gonzalez-Rodriguez AP, Contesti J, Gonzalez-García E, Payer AR, et al. Expansion of NK cells and reduction of NKG2D expression in chronic lymphocytic leukemia. Correlation with progressive disease. PloS One. 2014;9:e108326.

61. Marshall JS, Warrington R, Watson W, Kim HL. An introduction to immunology and immunopathology. Allergy Asthma Clin Immunol [Internet]. 2018 [cited 2023 Mar 12];14:49. Available from: https://doi.org/10.1186/s13223-018-0278-1

62. Füst G, Miszlay Z, Czink E, Varga L, Pálóczi K, Szegedi G, et al. C1 and C4 abnormalities in chronic lymphocytic leukaemia and their significance. Immunol Lett. 1987;14:255–9.

63. Rozman C, Montserrat E, Viñolas N. Serum immunoglobulins in B-chronic lymphocytic leukemia. Natural history and prognostic significance. Cancer. 1988;61:279–83.

64. Visentin A, Compagno N, Cinetto F, Imbergamo S, Zambello R, Piazza F, et al. Clinical profile associated with infections in patients with chronic lymphocytic leukemia. Protective role of immunoglobulin replacement therapy. Haematologica [Internet]. 2015 [cited 2021 Mar 8];100:e515–8. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4666345/

65. Rizzo D, Chauzeix J, Trimoreau F, Woillard JB, Genevieve F, Bouvier A, et al. IgM peak independently predicts treatment-free survival in chronic lymphocytic leukemia and correlates with accumulation of adverse oncogenetic events. Leukemia [Internet]. Nature Publishing Group; 2015 [cited 2021 Mar 8];29:337–45. Available from: https://www.nature.com/articles/leu2014198

66. Grywalska E, Zaborek M, Łyczba J, Hrynkiewicz R, Bębnowska D, Becht R, et al. Chronic Lymphocytic Leukemia-Induced Humoral Immunosuppression: A Systematic Review. Cells. 2020;9.

67. DiLillo DJ, Weinberg JB, Yoshizaki A, Horikawa M, Bryant JM, Iwata Y, et al. Chronic lymphocytic leukemia and regulatory B cells share IL-10 competence and immunosuppressive function. Leukemia [Internet]. Nature Publishing Group; 2013 [cited 2021 Mar 8];27:170–82. Available from: https://www.nature.com/articles/leu2012165

68. Apollonio B, Scielzo C, Bertilaccio MTS, Ten Hacken E, Scarfò L, Ranghetti P, et al. Targeting B-cell anergy in chronic lymphocytic leukemia. Blood. 2013;121:3879–88, S1-8.

69. Coelho V, Krysov S, Steele A, Sanchez Hidalgo M, Johnson PW, Chana PS, et al. Identification in CLL of circulating intraclonal subgroups with varying B-cell receptor expression and function. Blood [Internet]. 2013 [cited 2021 Mar 8];122:2664–72. Available from: https://doi.org/10.1182/blood-2013-02-485425

70. Christopoulos P, Pfeifer D, Bartholomé K, Follo M, Timmer J, Fisch P, et al. Definition and characterization of the systemic T-cell dysregulation in untreated indolent B-cell lymphoma and very early CLL. Blood. 2011;117:3836–46.

71. Pourgheysari B, Bruton R, Parry H, Billingham L, Fegan C, Murray J, et al. The number of cytomegalovirus-specific CD4+ T cells is markedly expanded in patients with B-cell chronic lymphocytic leukemia and determines the total CD4+ T-cell repertoire. Blood. 2010;116:2968–74.

72. Forconi F, Moss P. Perturbation of the normal immune system in patients with CLL. Blood. 2015;126:573–81.

73. Pizzolo G, Chilosi M, Ambrosetti A, Semenzato G, Fiore-Donati L, Perona G. Immunohistologic study of bone marrow involvement in B-chronic lymphocytic leukemia. Blood. 1983;62:1289–96.

74. Patten PEM, Buggins AGS, Richards J, Wotherspoon A, Salisbury J, Mufti GJ, et al. CD38 expression in chronic lymphocytic leukemia is regulated by the tumor microenvironment. Blood. 2008;111:5173–81.

75. Görgün G, Holderried TAW, Zahrieh D, Neuberg D, Gribben JG. Chronic lymphocytic leukemia cells induce changes in gene expression of CD4 and CD8 T cells. J Clin Invest. 2005;115:1797–805.

76. Ramsay AG, Johnson AJ, Lee AM, Gorgün G, Le Dieu R, Blum W, et al. Chronic lymphocytic leukemia T cells show impaired immunological synapse formation that can be reversed with an immunomodulating drug. J Clin Invest. 2008;118:2427–37.

77. Roessner PM, Seiffert M. T-cells in chronic lymphocytic leukemia: Guardians or drivers of disease? Leukemia [Internet]. Nature Publishing Group; 2020 [cited 2023 Mar 12];34:2012–24. Available from: https://www.nature.com/articles/s41375-020-0873-2

78. Palma M, Gentilcore G, Heimersson K, Mozaffari F, Näsman-Glaser B, Young E, et al. T cells in chronic lymphocytic leukemia display dysregulated expression of immune checkpoints and activation markers. Haematologica [Internet]. 2017 [cited 2023 Mar 12];102:562–72. Available from: https://haematologica.org/article/view/8007

79. Giannopoulos K, Schmitt M, Kowal M, Wlasiuk P, Bojarska-Junak A, Chen J, et al. Characterization of regulatory T cells in patients with B-cell chronic lymphocytic leukemia. Oncol Rep. 2008;20:677–82.

80. Le Saos-Patrinos C, Loizon S, Zouine A, Turpin D, Dilhuydy M-S, Blanco P, et al. Elevated levels of circulatory follicular T helper cells in chronic lymphocytic leukemia contribute to B cell expansion. J Leukoc Biol [Internet]. 2023 [cited 2023 Mar 12];113:305–14. Available from: https://doi.org/10.1093/jleuko/qiad006

81. Zhang N, Bevan MJ. CD8+ T Cells: Foot Soldiers of the Immune System. Immunity [Internet]. Elsevier; 2011 [cited 2023 Mar 12];35:161–8. Available from: https://www.cell.com/immunity/abstract/S1074-7613(11)00303-7

82. Krzewski K, Coligan J. Human NK cell lytic granules and regulation of their exocytosis. Front Immunol [Internet]. 2012 [cited 2023 Mar 12];3. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2012.00335

83. Darmon AJ, Ley TJ, Nicholson DW, Bleackley RC. Cleavage of CPP32 by granzyme B represents a critical role for granzyme B in the induction of target cell DNA fragmentation. J Biol Chem. 1996;271:21709–12.

84. Aguiló JI, Anel A, Catalán E, Sebastián A, Acín-Pérez R, Naval J, et al. Granzyme B of cytotoxic T cells induces extramitochondrial reactive oxygen species production via caspase-dependent NADPH oxidase activation. Immunol Cell Biol. 2010;88:545–54.

85. Lord SJ, Rajotte RV, Korbutt GS, Bleackley RC. Granzyme B: a natural born killer. Immunol Rev. 2003;193:31–8.

86. Atkinson EA, Barry M, Darmon AJ, Shostak I, Turner PC, Moyer RW, et al. Cytotoxic T lymphocyte-assisted suicide. Caspase 3 activation is primarily the result of the direct action of granzyme B. J Biol Chem. 1998;273:21261–6.

87. Barry M, Heibein JA, Pinkoski MJ, Lee SF, Moyer RW, Green DR, et al. Granzyme B short-circuits the need for caspase 8 activity during granule-mediated cytotoxic T-lymphocyte killing by directly cleaving Bid. Mol Cell Biol. 2000;20:3781–94.

88. Voskoboinik I, Whisstock JC, Trapani JA. Perforin and granzymes: function, dysfunction and human pathology. Nat Rev Immunol. 2015;15:388–400.

89. Thiery J, Keefe D, Boulant S, Boucrot E, Walch M, Martinvalet D, et al. Perforin pores in the endosomal membrane trigger release of endocytosed granzyme B to the cytosol of target cells. Nat Immunol [Internet]. 2011 [cited 2023 Mar 13];12:770–7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3140544/

90. Shi L, Keefe D, Durand E, Feng H, Zhang D, Lieberman J. Granzyme B binds to target cells mostly by charge and must be added at the same time as perforin to trigger apoptosis. J Immunol Baltim Md 1950. 2005;174:5456–61.

91. Wherry EJ. T cell exhaustion. Nat Immunol. 2011;12:492–9.

92. Blank CU, Haining WN, Held W, Hogan PG, Kallies A, Lugli E, et al. Defining "T cell exhaustion." Nat Rev Immunol. 2019;19:665–74.

93. Okoye IS, Houghton M, Tyrrell L, Barakat K, Elahi S. Coinhibitory Receptor Expression and Immune Checkpoint Blockade: Maintaining a Balance in CD8+ T Cell Responses to Chronic Viral Infections and Cancer. Front Immunol. 2017;8:1215.

94. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12:252–64.

95. Schnell A, Bod L, Madi A, Kuchroo VK. The yin and yang of co-inhibitory receptors: toward anti-tumor immunity without autoimmunity. Cell Res [Internet]. Nature Publishing Group; 2020 [cited 2023 Apr 3];30:285–99. Available from: https://www.nature.com/articles/s41422-020-0277-x

96. Andrews LP, Yano H, Vignali DAA. Inhibitory receptors and ligands beyond PD-1, PD-L1 and CTLA-4: breakthroughs or backups. Nat Immunol. 2019;20:1425–34.

97. McLane LM, Abdel-Hakeem MS, Wherry EJ. CD8 T Cell Exhaustion During Chronic Viral Infection and Cancer. Annu Rev Immunol. 2019;37:457–95.

98. Philip M, Schietinger A. Heterogeneity and fate choice: T cell exhaustion in cancer and chronic infections. Curr Opin Immunol. 2019;58:98–103.

99. Vaddepally RK, Kharel P, Pandey R, Garje R, Chandra AB. Review of Indications of FDA-Approved Immune Checkpoint Inhibitors per NCCN Guidelines with the Level of Evidence. Cancers. 2020;12:738.

100. McKinney EF, Lee JC, Jayne DRW, Lyons PA, Smith KGC. T cell exhaustion, costimulation and clinical outcome in autoimmunity and infection. Nature [Internet].

2015 [cited 2021 Mar 29];523:612–6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4623162/

101. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. Nat Rev Immunol. 2015;15:486–99.

102. Vlachonikola E, Stamatopoulos K, Chatzidimitriou A. T Cells in Chronic Lymphocytic Leukemia: A Two-Edged Sword. Front Immunol [Internet]. 2021 [cited 2023 Apr 3];11. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2020.612244

103. Curran EK, Godfrey J, Kline J. Mechanisms of Immune Tolerance in Leukemia and Lymphoma. Trends Immunol. 2017;38:513–25.

104. Brusa D, Serra S, Coscia M, Rossi D, D'Arena G, Laurenti L, et al. The PD-1/PD-L1 axis contributes to T-cell dysfunction in chronic lymphocytic leukemia. Haematologica [Internet]. 2013 [cited 2023 Apr 3];98:953–63. Available from: https://haematologica.org/article/view/6698

105. Sadik A, Somarribas Patterson LF, Öztürk S, Mohapatra SR, Panitz V, Secker PF, et al. IL4I1 Is a Metabolic Immune Checkpoint that Activates the AHR and Promotes Tumor Progression. Cell. 2020;182:1252-1270.e34.

106. Peters FS, Strefford JC, Eldering E, Kater AP. T-cell dysfunction in chronic lymphocytic leukemia from an epigenetic perspective. Haematologica [Internet]. 2021 [cited 2023 Apr 3];106:1234–43. Available from: https://haematologica.org/article/view/haematol.2020.267914

107. Maïza H, Leca G, Mansur IG, Schiavon V, Boumsell L, Bensussan A. A novel 80-kD cell surface structure identifies human circulating lymphocytes with natural killer activity. J Exp Med. 1993;178:1121–6.

108. El-Far M, Pellerin C, Pilote L, Fortin J-F, Lessard IAD, Peretz Y, et al. CD160 isoforms and regulation of CD4 and CD8 T-cell responses. J Transl Med. 2014;12:217.

109. Ortonne N, Ram-Wolff C, Giustiniani J, Marie-Cardine A, Bagot M, Mecheri S, et al. Human and mouse mast cells express and secrete the GPI-anchored isoform of CD160. J Invest Dermatol. 2011;131:916–24.

110. Henry A, Boulagnon-Rombi C, Menguy T, Giustiniani J, Garbar C, Mascaux C, et al. CD160 Expression in Retinal Vessels Is Associated With Retinal Neovascular Diseases. Invest Ophthalmol Vis Sci. 2018;59:2679–86.

111. Farren TW, Giustiniani J, Liu F-T, Tsitsikas DA, Macey MG, Cavenagh JD, et al. Differential and tumor-specific expression of CD160 in B-cell malignancies. Blood. 2011;118:2174–83.

112. Giustiniani J, Marie-Cardine A, Bensussan A. A soluble form of the MHC class I-specific CD160 receptor is released from human activated NK lymphocytes and inhibits cell-mediated cytotoxicity. J Immunol Baltim Md 1950. 2007;178:1293–300.

113. Tu TC, Brown NK, Kim T-J, Wroblewska J, Yang X, Guo X, et al. CD160 is essential for NK-mediated IFN-γ production. J Exp Med. 2015;212:415–29.

114. Maeda M, Carpenito C, Russell RC, Dasanjh J, Veinotte LL, Ohta H, et al. Murine CD160, Ig-like receptor on NK cells and NKT cells, recognizes classical and nonclassical MHC class I and regulates NK cell activation. J Immunol Baltim Md 1950. 2005;175:4426–32.

115. Rodriguez-Barbosa JI, Schneider P, Weigert A, Lee K-M, Kim T-J, Perez-Simon J-A, et al. HVEM, a cosignaling molecular switch, and its interactions with BTLA, CD160 and LIGHT. Cell Mol Immunol. 2019;16:679–82.

116. Cai G, Anumanthan A, Brown JA, Greenfield EA, Zhu B, Freeman GJ. CD160 inhibits activation of human CD4+ T cells through interaction with herpesvirus entry mediator. Nat Immunol. 2008;9:176–85.

117. Peretz Y, He Z, Shi Y, Yassine-Diab B, Goulet J-P, Bordi R, et al. CD160 and PD-1 co-expression on HIV-specific CD8 T cells defines a subset with advanced dysfunction. PLoS Pathog. 2012;8:e1002840.

118. Tan CL, Peluso MJ, Drijvers JM, Mera CM, Grande SM, Brown KE, et al. CD160 Stimulates CD8+ T Cell Responses and Is Required for Optimal Protective Immunity to Listeria monocytogenes. ImmunoHorizons. 2018;2:238–50.

119. Del Rio M-L, Nguyen TH, Tesson L, Heslan J-M, Gutierrez-Adan A, Fernandez-Gonzalez R, et al. The impact of CD160 deficiency on alloreactive CD8 T cell responses and allograft rejection. Transl Res J Lab Clin Med. 2022;239:103–23.

120. Liu F-T, Giustiniani J, Farren T, Jia L, Bensussan A, Gribben JG, et al. CD160 signaling mediates PI3K-dependent survival and growth signals in chronic lymphocytic leukemia. Blood [Internet]. 2010 [cited 2023 Mar 23];115:3079–88. Available from: https://doi.org/10.1182/blood-2009-08-239483

121. Chibueze CE, Yoshimitsu M, Arima N. CD160 expression defines a uniquely exhausted subset of T lymphocytes in HTLV-1 infection. Biochem Biophys Res Commun. 2014;453:379–84.

122. Liu S, Zhang W, Liu K, Wang Y. CD160 expression on CD8+ T cells is associated with active effector responses but limited activation potential in pancreatic cancer. Cancer Immunol Immunother CII. 2020;69:789–97.

123. Gauci M-L, Giustiniani J, Lepelletier C, Garbar C, Thonnart N, Dumaz N, et al. The soluble form of CD160 acts as a tumor mediator of immune escape in melanoma. Cancer Immunol Immunother CII. 2022;71:2731–42.

124. Cruikshank WW, Kornfeld H, Center DM. Interleukin-16. J Leukoc Biol. 2000;67:757–66.

125. Zhang Y, Center DM, Wu DM, Cruikshank WW, Yuan J, Andrews DW, et al. Processing and activation of pro-interleukin-16 by caspase-3. J Biol Chem. 1998;273:1144–9.

126. Cheng G, Ueda T, Eda F, Arima M, Yoshida N, Fukuda T. A549 cells can express interleukin-16 and stimulate eosinophil chemotaxis. Am J Respir Cell Mol Biol. 2001;25:212–8.

127. Chupp GL, Wright EA, Wu D, Vallen-Mashikian M, Cruikshank WW, Center DM, et al. Tissue and T cell distribution of precursor and mature IL-16. J Immunol Baltim Md 1950. 1998;161:3114–9.

128. Rumsaeng V, Cruikshank WW, Foster B, Prussin C, Kirshenbaum AS, Davis TA, et al. Human mast cells produce the CD4+ T lymphocyte chemoattractant factor, IL-16. J Immunol Baltim Md 1950. 1997;159:2904–10.

129. Lim KG, Wan HC, Bozza PT, Resnick MB, Wong DT, Cruikshank WW, et al. Human eosinophils elaborate the lymphocyte chemoattractants. IL-16 (lymphocyte chemoattractant factor) and RANTES. J Immunol Baltim Md 1950. 1996;156:2566–70.

130. Bellini A, Yoshimura H, Vittori E, Marini M, Mattoli S. Bronchial epithelial cells of patients with asthma release chemoattractant factors for T lymphocytes. J Allergy Clin Immunol. 1993;92:412–24.

131. Parada NA, Center DM, Kornfeld H, Rodriguez WL, Cook J, Vallen M, et al. Synergistic activation of CD4+ T cells by IL-16 and IL-2. J Immunol Baltim Md 1950. 1998;160:2115–20.

132. Mathy NL, Scheuer W, Lanzendörfer M, Honold K, Ambrosius D, Norley S, et al. Interleukin-16 stimulates the expression and production of pro-inflammatory cytokines by human monocytes. Immunology [Internet]. 2000 [cited 2023 Mar 24];100:63–9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2326980/

133. Baier M, Werner A, Bannert N, Metzner K, Kurth R. HIV suppression by interleukin-16. Nature. 1995;378:563.

134. Bellomo G, Allegra A, Alonci A, Quartarone E, Pizzoleo MA, Cincotta M, et al. Serum levels of interleukin-16 in lymphoid malignancies. Leuk Lymphoma. 2007;48:1225–7.

135. Mahindra A, Anderson KC. Role of Interleukin 16 in Multiple Myeloma Pathogenesis: A Potential Novel Therapeutic Target? JNCI J Natl Cancer Inst [Internet]. 2012 [cited 2023 Mar 24];104:964–5. Available from: https://doi.org/10.1093/jnci/djs274

136. Templin J, Luetkens T, Atanackovic D. Cytokine Interleukin-16 Supports Progression Of Multiple Myeloma Through Different Molecular Mechanisms. Blood [Internet]. 2013 [cited 2023 Mar 24];122:5367. Available from: https://www.sciencedirect.com/science/article/pii/S0006497119686258

137. Atanackovic D, Hildebrandt Y, Luetkens T, Zander AR, Bokemeyer C, Kroeger N. Interleukin-16 Is An Important Growth-Promoting Factor and a Novel Diagnostic and Therapeutic Target for Multiple Myeloma. Blood [Internet]. 2010 [cited 2023 Mar 24];116:4056. Available from: https://doi.org/10.1182/blood.V116.21.4056.4056

138. Yang H, Han Y, Wu L, Wu C. Diagnostic and prognostic value of serum interleukin-16 in patients with gastric cancer. Mol Med Rep [Internet]. Spandidos Publications; 2017 [cited 2023 Mar 24];16:9143–8. Available from: https://www.spandidos-publications.com/10.3892/mmr.2017.7688

139. Engel M, Hoffmann T, Wagner L, Wermann M, Heiser U, Kiefersauer R, et al. The crystal structure of dipeptidyl peptidase IV (CD26) reveals its functional regulation and enzymatic mechanism. Proc Natl Acad Sci U S A. 2003;100:5063–8.

140. Yaron A, Naider F, Scharpe S. Proline-Dependent Structural and Biological Properties of Peptides and Proteins. Crit Rev Biochem Mol Biol [Internet]. Taylor & Francis; 1993 [cited 2022 Jun 6];28:31–81. Available from: https://doi.org/10.3109/10409239309082572

141. Ahrén B, Hughes TE. Inhibition of dipeptidyl peptidase-4 augments insulin secretion in response to exogenously administered glucagon-like peptide-1, glucose-dependent insulinotropic polypeptide, pituitary adenylate cyclase-activating polypeptide, and gastrin-releasing peptide in mice. Endocrinology. 2005;146:2055–9.

142. Mortier A, Gouwy M, Van Damme J, Proost P, Struyf S. CD26/dipeptidylpeptidase IV—chemokine interactions: double-edged regulation of inflammation and tumor biology. J Leukoc Biol [Internet]. 2016 [cited 2022 Jun 29];99:955–69. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7166560/

143. Brezinschek RI, Lipsky PE, Galea P, Vita R, Oppenheimer-Marks N. Phenotypic characterization of CD4+ T cells that exhibit a transendothelial migratory capacity. J Immunol Baltim Md 1950. 1995;154:3062–77.

144. Shahbaz S, Xu L, Osman M, Sligl W, Shields J, Joyce M, et al. Erythroid precursors and progenitors suppress adaptive immunity and get invaded by SARS-CoV-2. Stem Cell Rep. 2021;16:1165–81.

145. Morimoto C, Torimoto Y, Levinson G, Rudd CE, Schrieber M, Dang NH, et al. 1F7, a novel cell surface molecule, involved in helper function of CD4 cells. J Immunol Baltim Md 1950. 1989;143:3430–9.

146. Bühling F, Junker U, Reinhold D, Neubert K, Jäger L, Ansorge S. Functional role of CD26 on human B lymphocytes. Immunol Lett. 1995;45:47–51.

147. Bühling F, Kunz D, Reinhold D, Ulmer AJ, Ernst M, Flad HD, et al. Expression and functional role of dipeptidyl peptidase IV (CD26) on human natural killer cells. Nat Immun. 1994;13:270–9.

148. Gliddon DR, Howard CJ. CD26 is expressed on a restricted subpopulation of dendritic cells in vivo. Eur J Immunol. 2002;32:1472–81.

149. Dang NH, Torimoto Y, Shimamura K, Tanaka T, Daley JF, Schlossman SF, et al. 1F7 (CD26): a marker of thymic maturation involved in the differential regulation of the CD3 and CD2 pathways of human thymocyte activation. J Immunol Baltim Md 1950. 1991;147:2825–32.

150. Yan S, Marguet D, Dobers J, Reutter W, Fan H. Deficiency of CD26 results in a change of cytokine and immunoglobulin secretion after stimulation by pokeweed mitogen. Eur J Immunol. 2003;33:1519–27.

151. Pinheiro MM, Stoppa CL, Valduga CJ, Okuyama CE, Gorjão R, Pereira RMS, et al. Sitagliptin inhibit human lymphocytes proliferation and Th1/Th17 differentiation in vitro. Eur J Pharm Sci Off J Eur Fed Pharm Sci. 2017;100:17–24.

152. Dong RP, Kameoka J, Hegen M, Tanaka T, Xu Y, Schlossman SF, et al. Characterization of adenosine deaminase binding to human CD26 on T cells and its biologic role in immune response. J Immunol [Internet]. American Association of Immunologists; 1996 [cited 2022 Jun 6];156:1349–55. Available from: https://www.jimmunol.org/content/156/4/1349

153. Martín M, Huguet J, Centelles JJ, Franco R. Expression of ecto-adenosine deaminase and CD26 in human T cells triggered by the TCR-CD3 complex. Possible role of adenosine deaminase as costimulatory molecule. J Immunol Baltim Md 1950. 1995;155:4630–43.

154. Ohnuma K, Uchiyama M, Yamochi T, Nishibashi K, Hosono O, Takahashi N, et al. Caveolin-1 triggers T-cell activation via CD26 in association with CARMA1. J Biol Chem. 2007;282:10117–31.

155. Bailey SR, Nelson MH, Majchrzak K, Bowers JS, Wyatt MM, Smith AS, et al. Human CD26high T cells elicit tumor immunity against multiple malignancies via enhanced migration and persistence. Nat Commun [Internet]. Nature Publishing Group; 2017 [cited 2023 Apr 7];8:1961. Available from: https://www.nature.com/articles/s41467-017-01867-9

156. Hatano R, Ohnuma K, Yamamoto J, Dang NH, Morimoto C. CD26-mediated co-stimulation in human CD8(+) T cells provokes effector function via pro-inflammatory cytokine production. Immunology. 2013;138:165–72.

157. Sharma PK, Wong EB, Napier RJ, Bishai WR, Ndung'u T, Kasprowicz VO, et al. High expression of CD26 accurately identifies human bacteria-reactive MR1-restricted MAIT cells. Immunology. 2015;145:443–53.

158. Treiner E, Duban L, Bahram S, Radosavljevic M, Wanner V, Tilloy F, et al. Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. Nature. 2003;422:164–9.

159. Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, Liu L, et al. MR1 presents microbial vitamin B metabolites to MAIT cells. Nature. 2012;491:717–23.

160. John S, Mishra R. Galectin-9: From cell biology to complex disease dynamics. J Biosci. 2016;41:507–34.

161. O'Brien MJ, Shu Q, Stinson WA, Tsou P-S, Ruth JH, Isozaki T, et al. A unique role for galectin-9 in angiogenesis and inflammatory arthritis. Arthritis Res Ther [Internet]. 2018 [cited 2023 Mar 22];20:31. Available from: https://doi.org/10.1186/s13075-018-1519-x

162. Oomizu S, Arikawa T, Niki T, Kadowaki T, Ueno M, Nishi N, et al. Cell surface galectin-9 expressing Th cells regulate Th17 and Foxp3+ Treg development by galectin-9 secretion. PloS One. 2012;7:e48574.

163. Merani S, Chen W, Elahi S. The bitter side of sweet: the role of Galectin-9 in immunopathogenesis of viral infections. Rev Med Virol. 2015;25:175–86.

164. Keryer-Bibens C, Pioche-Durieu C, Villemant C, Souquère S, Nishi N, Hirashima M, et al. Exosomes released by EBV-infected nasopharyngeal carcinoma cells convey the viral latent membrane protein 1 and the immunomodulatory protein galectin 9. BMC Cancer. 2006;6:283.

165. Reddy PBJ, Sehrawat S, Suryawanshi A, Rajasagi NK, Mulik S, Hirashima M, et al. Influence of galectin-9/Tim-3 interaction on herpes simplex virus-1 latency. J Immunol Baltim Md 1950. 2011;187:5745–55.

166. Moritoki M, Kadowaki T, Niki T, Nakano D, Soma G, Mori H, et al. Galectin-9 ameliorates clinical severity of MRL/lpr lupus-prone mice by inducing plasma cell apoptosis independently of Tim-3. PloS One. 2013;8:e60807.

167. Madireddi S, Eun S-Y, Lee S-W, Nemčovičová I, Mehta AK, Zajonc DM, et al. Galectin-9 controls the therapeutic activity of 4-1BB–targeting antibodies. J Exp Med [Internet]. 2014 [cited 2023 Mar 22];211:1433–48. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4076583/

168. Bi S, Hong PW, Lee B, Baum LG. Galectin-9 binding to cell surface protein disulfide isomerase regulates the redox environment to enhance T-cell migration and HIV entry. Proc Natl Acad Sci [Internet]. Proceedings of the National Academy of Sciences; 2011 [cited 2023 Mar 22];108:10650–5. Available from: https://www.pnas.org/doi/abs/10.1073/pnas.1017954108

169. Wu C, Thalhamer T, Franca RF, Xiao S, Wang C, Hotta C, et al. Galectin-9-CD44 interaction enhances stability and function of adaptive regulatory T cells. Immunity. 2014;41:270–82.

170. Anderson AC, Anderson DE, Bregoli L, Hastings WD, Kassam N, Lei C, et al. Promotion of tissue inflammation by the immune receptor Tim-3 expressed on innate immune cells. Science. 2007;318:1141–3.

171. Yang R, Sun L, Li C-F, Wang Y-H, Yao J, Li H, et al. Galectin-9 interacts with PD-1 and TIM-3 to regulate T cell death and is a target for cancer immunotherapy. Nat Commun [Internet]. Nature Publishing Group; 2021 [cited 2023 Mar 23];12:832. Available from: https://www.nature.com/articles/s41467-021-21099-2

172. Elahi S, Dinges WL, Lejarcegui N, Laing KJ, Collier AC, Koelle DM, et al. Protective HIV-specific CD8+ T cells evade Treg cell suppression. Nat Med. 2011;17:989–95.

173. Su EW, Bi S, Kane LP. Galectin-9 regulates T helper cell function independently of Tim-3. Glycobiology. 2011;21:1258–65.

174. Shahbaz S, Dunsmore G, Koleva P, Xu L, Houston S, Elahi S. Galectin-9 and VISTA Expression Define Terminally Exhausted T Cells in HIV-1 Infection. J Immunol Baltim Md 1950. 2020;204:2474–91.

175. Motamedi M, Shahbaz S, Fu L, Dunsmore G, Xu L, Harrington R, et al. Galectin-9 Expression Defines a Subpopulation of NK Cells with Impaired Cytotoxic Effector Molecules but Enhanced IFN-γ Production, Dichotomous to TIGIT, in HIV-1 Infection. ImmunoHorizons. 2019;3:531–46.

176. Dunsmore G, Rosero EP, Shahbaz S, Santer DM, Jovel J, Lacy P, et al. Neutrophils promote T-cell activation through the regulated release of CD44-bound Galectin-9 from the cell surface during HIV infection. PLoS Biol. 2021;19:e3001387.

177. Bozorgmehr N, Mashhouri S, Perez Rosero E, Xu L, Shahbaz S, Sligl W, et al. Galectin-9, a Player in Cytokine Release Syndrome and a Surrogate Diagnostic Biomarker in SARS-CoV-2 Infection. mBio. 2021;12:e00384-21.

178. Gonçalves Silva I, Yasinska IM, Sakhnevych SS, Fiedler W, Wellbrock J, Bardelli M, et al. The Tim-3-galectin-9 Secretory Pathway is Involved in the Immune Escape of Human Acute Myeloid Leukemia Cells. EBioMedicine. 2017;22:44–57.

179. Okoye I, Xu L, Motamedi M, Parashar P, Walker JW, Elahi S. Galectin-9 expression defines exhausted T cells and impaired cytotoxic NK cells in patients with virus-associated solid tumors. J Immunother Cancer [Internet]. 2020 [cited 2023 Mar 23];8:e001849. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7735134/

180. Lhuillier C, Barjon C, Niki T, Gelin A, Praz F, Morales O, et al. Impact of Exogenous Galectin-9 on Human T Cells. J Biol Chem [Internet]. 2015 [cited 2023 Mar 23];290:16797–811. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4505427/

181. Yang R, Sun L, Li C-F, Wang Y-H, Xia W, Liu B, et al. Development and characterization of anti-galectin-9 antibodies that protect T cells from galectin-9-induced cell death. J Biol Chem [Internet]. 2022 [cited 2023 Mar 23];298:101821. Available from: https://www.sciencedirect.com/science/article/pii/S0021925822002617

182. White MK, Pagano JS, Khalili K. Viruses and Human Cancers: a Long Road of Discovery of Molecular Paradigms. Clin Microbiol Rev [Internet]. American Society for Microbiology; 2014 [cited 2023 Mar 26];27:463–81. Available from: https://journals.asm.org/doi/10.1128/cmr.00124-13

183. Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, et al. The biology and life-cycle of human papillomaviruses. Vaccine. 2012;30 Suppl 5:F55-70.

184. Schiffman M, Doorbar J, Wentzensen N, de Sanjosé S, Fakhry C, Monk BJ, et al. Carcinogenic human papillomavirus infection. Nat Rev Dis Primer [Internet]. Nature Publishing Group; 2016 [cited 2023 Mar 26];2:1–20. Available from: https://www.nature.com/articles/nrdp201686

185. Liao C-I, Francoeur AA, Kapp DS, Caesar MAP, Huh WK, Chan JK. Trends in Human Papillomavirus–Associated Cancers, Demographic Characteristics, and Vaccinations in the US, 2001-2017. JAMA Netw Open [Internet]. 2022 [cited 2023 Mar 26];5:e222530. Available from: https://doi.org/10.1001/jamanetworkopen.2022.2530

186. de Martel C, Georges D, Bray F, Ferlay J, Clifford GM. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. Lancet Glob Health. 2020;8:e180–90.

187. Saraiya M, Unger ER, Thompson TD, Lynch CF, Hernandez BY, Lyu CW, et al. US Assessment of HPV Types in Cancers: Implications for Current and 9-Valent HPV Vaccines. JNCI J Natl Cancer Inst [Internet]. 2015 [cited 2023 Mar 26];107:djv086. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4838063/

188. Balasubramaniam SD, Balakrishnan V, Oon CE, Kaur G. Key Molecular Events in Cervical Cancer Development. Med Kaunas Lith. 2019;55:384.

189. Perkins RB, Guido RL, Saraiya M, Sawaya GF, Wentzensen N, Schiffman M, et al. Summary of Current Guidelines for Cervical Cancer Screening and Management of Abnormal Test Results: 2016–2020. J Womens Health [Internet]. 2021 [cited 2023 Mar 26];30:5–13. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8020523/

190. Peitsaro P, Johansson B, Syrjänen S. Integrated human papillomavirus type 16 is frequently found in cervical cancer precursors as demonstrated by a novel quantitative real-time PCR technique. J Clin Microbiol. 2002;40:886–91.

191. Jeon S, Allen-Hoffmann BL, Lambert PF. Integration of human papillomavirus type 16 into the human genome correlates with a selective growth advantage of cells. J Virol. 1995;69:2989–97.

192. Graham SV. The human papillomavirus replication cycle, and its links to cancer progression: a comprehensive review. Clin Sci Lond Engl 1979. 2017;131:2201–21.

193. Scheffner M, Münger K, Byrne JC, Howley PM. The state of the p53 and retinoblastoma genes in human cervical carcinoma cell lines. Proc Natl Acad Sci U S A. 1991;88:5523–7.

194. Pal A, Kundu R. Human Papillomavirus E6 and E7: The Cervical Cancer Hallmarks and Targets for Therapy. Front Microbiol [Internet]. 2020 [cited 2023 Mar 26];10. Available from: https://www.frontiersin.org/articles/10.3389/fmicb.2019.03116

195. Mietz JA, Unger T, Huibregtse JM, Howley PM. The transcriptional transactivation function of wild-type p53 is inhibited by SV40 large T-antigen and by HPV-16 E6 oncoprotein. EMBO J. 1992;11:5013–20.

196. El-Deiry WS. p21(WAF1) Mediates Cell-Cycle Inhibition, Relevant to Cancer Suppression and Therapy. Cancer Res. 2016;76:5189–91.

197. Wang J, Sampath A, Raychaudhuri P, Bagchi S. Both Rb and E7 are regulated by the ubiquitin proteasome pathway in HPV-containing cervical tumor cells. Oncogene [Internet]. Nature Publishing Group; 2001 [cited 2023 Mar 26];20:4740–9. Available from: https://www.nature.com/articles/1204655

198. Marth C, Landoni F, Mahner S, McCormack M, Gonzalez-Martin A, Colombo N. Cervical cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Ann Oncol [Internet]. Elsevier; 2017 [cited 2023 Apr 4];28:iv72–83. Available from: https://www.annalsofoncology.org/article/S0923-7534(19)42148-0/fulltext

199. Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. Nat Rev Dis Primer [Internet]. Nature Publishing Group; 2020 [cited 2023 Apr 4];6:1–22. Available from: https://www.nature.com/articles/s41572-020-00224-3

200. Mirghani H, Amen F, Blanchard P, Moreau F, Guigay J, Hartl D m., et al. Treatment de-escalation in HPV-positive oropharyngeal carcinoma: Ongoing trials, critical issues and perspectives. Int J Cancer [Internet]. 2015 [cited 2023 Mar 26];136:1494–503. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/ijc.28847

201. Rodríguez-Carunchio L, Soveral I, Steenbergen R, Torné A, Martinez S, Fusté P, et al. HPV-negative carcinoma of the uterine cervix: a distinct type of cervical cancer with poor prognosis. BJOG Int J Obstet Gynaecol [Internet]. 2015 [cited 2023 Mar 26];122:119–27. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/1471-0528.13071

202. Onuki M, Matsumoto K, Tenjimbayashi Y, Tasaka N, Akiyama A, Sakurai M, et al. Human papillomavirus genotype and prognosis of cervical cancer: Favorable survival of patients with HPV16-positive tumors. Papillomavirus Res [Internet]. 2018 [cited 2023 Mar 26];6:41–5. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6218653/

203. Rader JS, Tsaih S-W, Fullin D, Murray MW, Iden M, Zimmermann MT, et al. Genetic variations in human papillomavirus and cervical cancer outcomes. Int J Cancer [Internet]. 2019 [cited 2023 Mar 26];144:2206–14. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6450540/

204. Małusecka E, Chmielik E, Suwiński R, Giglok M, Lange D, Rutkowski T, et al. Significance of HPV16 Viral Load Testing in Anal Cancer. Pathol Oncol Res [Internet]. 2020 [cited 2023 Mar 26];26:2191–9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7471158/

205. Liu Y, Xu C, Pan J, Sun C, Zhou H, Meng Y. Significance of the viral load of high-risk HPV in the diagnosis and prediction of cervical lesions: a retrospective study. BMC Womens Health [Internet]. 2021 [cited 2023 Mar 26];21:353. Available from: https://doi.org/10.1186/s12905-021-01493-0

206. Frisch M, Biggar RJ, Engels EA, Goedert JJ, AIDS-Cancer Match Registry Study Group. Association of cancer with AIDS-related immunosuppression in adults. JAMA. 2001;285:1736–45.

207. Vajdic CM, McDonald SP, McCredie MRE, van Leeuwen MT, Stewart JH, Law M, et al. Cancer incidence before and after kidney transplantation. JAMA. 2006;296:2823–31.

208. Burmeister CA, Khan SF, Schäfer G, Mbatani N, Adams T, Moodley J, et al. Cervical cancer therapies: Current challenges and future perspectives. Tumour Virus Res [Internet]. 2022 [cited 2023 Mar 26];13:200238. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9062473/

209. Enwere EK, Kornaga EN, Dean M, Koulis TA, Phan T, Kalantarian M, et al. Expression of PD-L1 and presence of CD8-positive T cells in pre-treatment specimens of locally advanced cervical cancer. Mod Pathol Off J U S Can Acad Pathol Inc. 2017;30:577–86.

210. Quigley M, Pereyra F, Nilsson B, Porichis F, Fonseca C, Eichbaum Q, et al. Transcriptional analysis of HIV-specific CD8+ T cells shows that PD-1 inhibits T cell function by upregulating BATF. Nat Med [Internet]. 2010 [cited 2023 Apr 4];16:1147–51. Available from: https://europepmc.org/articles/PMC3326577

211. Hui E, Cheung J, Zhu J, Su X, Taylor MJ, Wallweber HA, et al. T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. Science. 2017;355:1428–33.

212. Cohen AC, Roane BM, Leath CA. Novel Therapeutics for Recurrent Cervical Cancer: Moving Towards Personalized Therapy. Drugs. 2020;80:217–27.

213. Duenas-Gonzalez A, Candelaria M, Perez-Plascencia C, Perez-Cardenas E, de la Cruz-Hernandez E, Herrera LA. Valproic acid as epigenetic cancer drug: preclinical, clinical and transcriptional effects on solid tumors. Cancer Treat Rev. 2008;34:206–22.

214. Mora-García M de L, Duenas-González A, Hernández-Montes J, De la Cruz-Hernández E, Pérez-Cárdenas E, Weiss-Steider B, et al. Up-regulation of HLA class-I antigen expression and antigen-specific CTL response in cervical cancer cells by the demethylating agent hydralazine and the histone deacetylase inhibitor valproic acid. J Transl Med [Internet]. 2006 [cited 2021 Jun 8];4:55. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1781077/

215. Grunstein M. Histone acetylation in chromatin structure and transcription. Nature. 1997;389:349–52.

216. Suraweera A, O'Byrne KJ, Richard DJ. Combination Therapy With Histone Deacetylase Inhibitors (HDACi) for the Treatment of Cancer: Achieving the Full Therapeutic Potential of HDACi. Front Oncol [Internet]. 2018 [cited 2022 Sep 3];8. Available from: https://www.frontiersin.org/articles/10.3389/fonc.2018.00092

217. Smith KT, Workman JL. Histone deacetylase inhibitors: anticancer compounds. Int J Biochem Cell Biol. 2009;41:21–5.

218. Marks P, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK. Histone deacetylases and cancer: causes and therapies. Nat Rev Cancer. 2001;1:194–202.

219. Soria-Castro R, Schcolnik-Cabrera A, Rodríguez-López G, Campillo-Navarro M, Puebla-Osorio N, Estrada-Parra S, et al. Exploring the Drug Repurposing Versatility of Valproic Acid as a Multifunctional Regulator of Innate and Adaptive Immune Cells. J Immunol Res. 2019;2019:9678098.

220. Reeves E, James E. Antigen processing and immune regulation in the response to tumours. Immunology. 2017;150:16–24.

221. Hemmat N, Baghi HB. Human papillomavirus E5 protein, the undercover culprit of tumorigenesis. Infect Agent Cancer. 2018;13:31.

222. Zitvogel L, Galluzzi L, Kepp O, Smyth MJ, Kroemer G. Type I interferons in anticancer immunity. Nat Rev Immunol. 2015;15:405–14.

223. Lau L, Gray EE, Brunette RL, Stetson DB. DNA tumor virus oncogenes antagonize the cGAS-STING DNA-sensing pathway. Science. 2015;350:568–71.

224. Canning M, Guo G, Yu M, Myint C, Groves MW, Byrd JK, et al. Heterogeneity of the Head and Neck Squamous Cell Carcinoma Immune Landscape and Its Impact on Immunotherapy. Front Cell Dev Biol. 2019;7:52.

225. Peltanova B, Raudenska M, Masarik M. Effect of tumor microenvironment on pathogenesis of the head and neck squamous cell carcinoma: a systematic review. Mol Cancer [Internet]. 2019 [cited 2023 Apr 4];18:63. Available from: https://doi.org/10.1186/s12943-019-0983-5

226. Ward MJ, Thirdborough SM, Mellows T, Riley C, Harris S, Suchak K, et al. Tumour-infiltrating lymphocytes predict for outcome in HPV-positive oropharyngeal cancer. Br J Cancer. 2014;110:489–500.

227. Ferris RL, Blumenschein G, Fayette J, Guigay J, Colevas AD, Licitra L, et al. Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. N Engl J Med. 2016;375:1856–67.

228. Wang J, Sun H, Zeng Q, Guo X-J, Wang H, Liu H-H, et al. HPV-positive status associated with inflamed immune microenvironment and improved response to anti-PD-1 therapy in head and neck squamous cell carcinoma. Sci Rep. 2019;9:13404.

229. Grabowska AK, Riemer AB. The invisible enemy - how human papillomaviruses avoid recognition and clearance by the host immune system. Open Virol J. 2012;6:249–56.

230. Gu Z, Shi W, Zhang L, Hu Z, Xu C. USP19 suppresses cellular type I interferon signaling by targeting TRAF3 for deubiquitination. Future Microbiol. 2017;12:767–79.

231. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol. 2012;12:253–68.

232. Groth C, Hu X, Weber R, Fleming V, Altevogt P, Utikal J, et al. Immunosuppression mediated by myeloid-derived suppressor cells (MDSCs) during tumour progression. Br J Cancer. 2019;120:16–25.

233. Bronte V, Serafini P, Apolloni E, Zanovello P. Tumor-Induced Immune Dysfunctions Caused by Myeloid Suppressor Cells. J Immunother [Internet]. 2001 [cited 2023 Mar 29];24:431. Available from: https://journals.lww.com/immunotherapy-journal/Abstract/2001/11000/Tumor_Induced_Immune_Dysfunctions_Caused_by.1.aspx

234. Bronte V, Brandau S, Chen S-H, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. Nat Commun [Internet]. Nature Publishing Group; 2016 [cited 2023 Mar 29];7:12150. Available from: https://www.nature.com/articles/ncomms12150

235. Condamine T, Dominguez GA, Youn J-I, Kossenkov AV, Mony S, Alicea-Torres K, et al. Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. Sci Immunol. 2016;1:aaf8943.

236. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol [Internet]. Nature Publishing Group; 2009 [cited 2023 Mar 29];9:162–74. Available from: https://www.nature.com/articles/nri2506

237. Li K, Shi H, Zhang B, Ou X, Ma Q, Chen Y, et al. Myeloid-derived suppressor cells as immunosuppressive regulators and therapeutic targets in cancer. Signal Transduct Target Ther [Internet]. Nature Publishing Group; 2021 [cited 2023 Mar 6];6:1–25. Available from: https://www.nature.com/articles/s41392-021-00670-9

238. Tartour E, Pere H, Maillere B, Terme M, Merillon N, Taieb J, et al. Angiogenesis and immunity: a bidirectional link potentially relevant for the monitoring of antiangiogenic therapy and the development of novel therapeutic combination with immunotherapy. Cancer Metastasis Rev. 2011;30:83–95.

239. Hou A, Hou K, Huang Q, Lei Y, Chen W. Targeting Myeloid-Derived Suppressor Cell, a Promising Strategy to Overcome Resistance to Immune Checkpoint Inhibitors. Front Immunol. 2020;11:783.

240. Bizzarri C, Beccari AR, Bertini R, Cavicchia MR, Giorgini S, Allegretti M. ELR+ CXC chemokines and their receptors (CXC chemokine receptor 1 and CXC chemokine receptor 2) as new therapeutic targets. Pharmacol Ther [Internet]. 2006 [cited 2023 Feb 13];112:139–49. Available from: https://www.sciencedirect.com/science/article/pii/S0163725806000556

241. Addison CL, Daniel TO, Burdick MD, Liu H, Ehlert JE, Xue YY, et al. The CXC chemokine receptor 2, CXCR2, is the putative receptor for ELR+ CXC chemokine-induced angiogenic activity. J Immunol Baltim Md 1950. 2000;165:5269–77.

242. Scheibenbogen C, Möhler T, Haefele J, Hunstein W, Keilholz U. Serum interleukin-8 (IL-8) is elevated in patients with metastatic melanoma and correlates with tumour load. Melanoma Res. 1995;5:179–81.

243. Xu Q, Ma H, Chang H, Feng Z, Zhang C, Yang X. The interaction of interleukin-8 and PTEN inactivation promotes the malignant progression of head and neck squamous cell carcinoma via the STAT3 pathway. Cell Death Dis [Internet]. Nature Publishing Group; 2020 [cited 2023 Feb 13];11:1–14. Available from: https://www.nature.com/articles/s41419-020-2627-5

244. Tobin RP, Jordan KR, Kapoor P, Spongberg E, Davis D, Vorwald VM, et al. IL-6 and IL-8 Are Linked With Myeloid-Derived Suppressor Cell Accumulation and Correlate With Poor Clinical Outcomes in Melanoma Patients. Front Oncol [Internet]. 2019 [cited 2023 Feb 13];9:1223. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6857649/

245. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science. 2004;303:1532–5.

246. Teijeira A, Garasa S, Ochoa MC, Villalba M, Olivera I, Cirella A, et al. IL8, Neutrophils, and NETs in a Collusion against Cancer Immunity and Immunotherapy.

Clin Cancer Res [Internet]. 2021 [cited 2023 Apr 4];27:2383–93. Available from: https://doi.org/10.1158/1078-0432.CCR-20-1319

247. Zhang Y, Guo L, Dai Q, Shang B, Xiao T, Di X, et al. A signature for pan-cancer prognosis based on neutrophil extracellular traps. J Immunother Cancer [Internet]. BMJ Specialist Journals; 2022 [cited 2023 Apr 4];10:e004210. Available from: https://jitc.bmj.com/content/10/6/e004210

248. Nie M, Yang L, Bi X, Wang Y, Sun P, Yang H, et al. Neutrophil Extracellular Traps Induced by IL8 Promote Diffuse Large B-cell Lymphoma Progression via the TLR9 Signaling. Clin Cancer Res Off J Am Assoc Cancer Res. 2019;25:1867–79.

249. Zhang Y, Chandra V, Riquelme Sanchez E, Dutta P, Quesada PR, Rakoski A, et al. Interleukin-17-induced neutrophil extracellular traps mediate resistance to checkpoint blockade in pancreatic cancer. J Exp Med. 2020;217:e20190354.

250. Shamseddine AA, Burman B, Lee NY, Zamarin D, Riaz N. Tumor Immunity and Immunotherapy for HPV-Related Cancers. Cancer Discov [Internet]. 2021 [cited 2023 Mar 26];11:1896–912. Available from: https://doi.org/10.1158/2159-8290.CD-20-1760

251. He X, Xu C. Immune checkpoint signaling and cancer immunotherapy. Cell Res [Internet]. 2020 [cited 2021 Oct 27];30:660–9. Available from: https://www.nature.com/articles/s41422-020-0343-4

252. Vaddepally RK, Kharel P, Pandey R, Garje R, Chandra AB. Review of Indications of FDA-Approved Immune Checkpoint Inhibitors per NCCN Guidelines with the Level of Evidence. Cancers. 2020;12.

253. Matić IZ, Đorđević M, Đorđić M, Grozdanić N, Damjanović A, Kolundžija B, et al. Dipeptidyl peptidase IV: serum activity and expression on lymphocytes in different hematological malignancies. Leuk Lymphoma. 2013;54:2701–6.

254. Tan EY, Mujoomdar M, Blay J. Adenosine Down-Regulates the Surface Expression of Dipeptidyl Peptidase IV on HT-29 Human Colorectal Carcinoma Cells. Am J Pathol [Internet]. 2004 [cited 2021 Oct 18];165:319–30. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1618535/

255. Vijayan D, Young A, Teng MWL, Smyth MJ. Targeting immunosuppressive adenosine in cancer. Nat Rev Cancer [Internet]. Nature Publishing Group; 2017 [cited 2021 Jun 3];17:709–24. Available from: https://www.nature.com/articles/nrc.2017.86

256. Metzemaekers M, Van Damme J, Mortier A, Proost P. Regulation of Chemokine Activity – A Focus on the Role of Dipeptidyl Peptidase IV/CD26. Front Immunol [Internet]. 2016 [cited 2023 Apr 4];7. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2016.00483

257. Frenel J-S, Le Tourneau C, O'Neil B, Ott PA, Piha-Paul SA, Gomez-Roca C, et al. Safety and Efficacy of Pembrolizumab in Advanced, Programmed Death Ligand 1-Positive Cervical Cancer: Results From the Phase Ib KEYNOTE-028 Trial. J Clin Oncol Off J Am Soc Clin Oncol. 2017;35:4035–41.

258. Khan O, La Thangue NB. HDAC inhibitors in cancer biology: emerging mechanisms and clinical applications. Immunol Cell Biol. 2012;90:85–94.

2 Chapter 2: Expanded antigen experienced $CD160^+CD8^+$ effector T cells exhibit impaired effector functions in chronic lymphocytic leukemia.

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2.1 Background

Chronic Lymphocytic Leukemia (CLL), the most common leukemia among adults in western countries, is identified by the clonal expansion of $CD5^+$ mature B lymphocytes in the blood, bone marrow, and secondary lymphoid organs [1,2]. The disease fate is highly influenced by the advanced clinical stage (i.e. Rai stage), the broad range of genetic factors and remarkable contributions from the tumor microenvironment [3]. Malignant B cells (B-CLL) impair both innate and adaptive immune responses and thus increase susceptibility to infections [4]. Several quantitative [5], phenotypic [6], and functional [7] T cell alterations are reported in CLL patients. Although $CD8^+$ T cells play an important role against the tumor, the occurrence of T cell exhaustion compromises their effector functions. Exhausted $CD8^+$ T cells are characterized by the loss of effector functions, altered epigenetic and transcriptional profiles, distinct metabolic style, and the inability to transition to memory T cells [8]. Antigen persistence, soluble mediators, cytokines, and immunoregulatory cells modulate the severity and the pace of T cell exhaustion [9]. The sustained upregulation of co-inhibitory receptors (IRs) is the hallmark of $CD4^+$ and $CD8^+$ T cell exhaustion in chronic viral infections and cancer [10]. Likewise, it has been shown that T cells in CLL upregulate the expression of PD-1, CD160, and 2B4 (CD244) [11], which results in their impairment (e.g. proliferation, cytotoxicity, and cytolytic functions) [12,13]. Comparable alterations in the gene and protein expression in T cells of TCL1 transgenic CLL mouse model in which adopted T cells acquire the characteristic of T cell dysfunction upon tumor antigen encounter have been reported [14].

CD160 as one of these IRs belongs to glycosylphosphatidylinositol-anchored (GPI) glycoprotein of the immunoglobulin superfamily [15]. It was first identified on the membrane of mouse Natural Killer (NK) cells [16], mast cells [17], and B-CLL cells but not on normal B cells [18]. CD160 can exist in three different forms such as the GPI-anchored, transmembrane (TM), and soluble forms. T cells mainly express the GPI but occasionally TM isoforms [19]. The soluble isoform composed of the extracellular regions of CD160 that is susceptible to be cleaved from the cell membrane by a metalloprotease as CD160 shedding has been reported from NK [20] and Mast cells [17] upon activation and degranulation, respectively.

Human/mouse CD160 binds weakly to classical and non-classical MHC-I molecules [21], triggering NK cell cytotoxicity [22], and release of proinflammatory cytokines. The interaction of CD160 with classical and non-classical MHC-I complexes inhibits binding of CD8 to α3 subunits of MHC class I, causing defect in MHC class I dependent $CD8^+$ T cell cytotoxicity [20]. CD160 and B- T-lymphocyte attenuator (BTLA) interact with the cysteine-rich region of the extracellular domain of herpesvirus entry mediator (HVEM) [23,24]. The engagement of CD160 with the soluble HVEM has a costimulatory effect on human NK cell function [25]. Paradoxically, CD160 interaction with HVEM receptor delivers an inhibitory signal to activated $CD4^+$ T cells resulting in diminished cytokine production and proliferation [26]. However, the role of CD160 in $CD8^+$ T cells is more complex. For example, CD160 exhibits an inhibitory function in viral infections such as HIV [27] but a stimulatory property in *Listeria Monocytogenes* infection [28] and the allograft skin reaction [29]. The various role of the CD160 molecule could be related to the expression of its corresponding ligands HVEM or MHC-I. For example, the interaction of CD160 with MHC-I induces a stimulatory signal

[28] whereas interaction with HVEM triggers an inhibitory signal [26]. These discrepancies in the field warrant further investigations to better understand the role of CD160 in T cell function in chronic conditions such as cancer. Moreover, it's unclear how the soluble CD160 is transported out of immune cells and there is a possibility that extracellular vesicles (EVs) are involved in this process.

EVs are small endosomal-derived membrane micro vesicles (50-100 nm) that are released extracellularly and act as intracellular communicators [30]. EVs carry a complex of cargo proteins, lipids, and nucleic acids to target cells. EVs express tetraspanins (CD9, CD63, CD81), cytoskeleton proteins, stress proteins, MHC, RNA, DNA, and glycolipids. Besides, EVs can carry co-inhibitory receptors and modulate the effector functions of different immune cells [31].

In this study, we examined the expression of CD160 on the surface of both $CD8^+$ and $CD4^+$ T cells in 56 CLL patients. We found CD160 upregulation was associated with an exhausted T cell phenotype. Notably, $CD160^+$ $CD8^+$ T cells were highly antigen-experienced/effector T cells, while $CD160^+$ $CD4^+$ T cells were more heterogeneous. The plasma cytokine profile of CLL patients exhibited a pro-inflammatory phenotype. IL-16 was highly elevated in the plasma of CLL patients and was correlated with the Rai stage. Finally, we show that isolated EVs from the plasma of CLL patients can be a source of CD160.

2.2 **Materials and methods**

2.2.1 Study population

The peripheral blood and bone marrow (BM) samples were collected from B-CLL patients. We recruited 56 CLL patients for the study (Supplementary Table 1). The

diagnosis was based on clinical, morphology, and immune-phenotyping features. Peripheral blood from age and sex-matched 25 healthy donors were obtained for comparison. The staging was done based on the clinical data using the Rai staging system reported elsewhere [32].

2.2.2 Cell isolation and purification

The peripheral blood mononuclear cells (PBMCs) and bone marrow mononuclear cells were isolated using Ficoll-Paque gradients (GE Healthcare). $CD8^+$ or $CD3^+$ T cells were isolated by negative selection using the Easysep isolation kits (Stem Cell Technologies) with a purity > 90% (Supplementary Fig. 1a, 1b). For effector T cell ($CD8^+CCR7^-$) isolation, $CD8^+$ T cells were stained with PE-conjugated anti-CCR7 antibody, followed by anti-PE conjugated microbeads (Miltenyi) with a purity of > 90% (Supplementary Fig. 1c). B-CLLs were isolated using the human B-CLL Cell Isolation Kit (Miltenyi) with a purity of > 90% (Supplementary Fig. 1d).

2.2.3 Flow cytometry

The fluorochrome-conjugated antibodies were purchased from BD Biosciences, Thermo Fisher Scientific or Biolegend including human anti-CD3 (SK7), anti-CD4 (RPA-T4), anti-CD8 (RPA-T8), anti-CD5 (UCHT2), anti-CD19 (HIB19), anti-CD160 (BY55), anti-HVEM (94801), anti-2B4 (eBioDM244), anti-TIGIT (MBSA43), anti-PD1 (EH12.1), anti-BTLA (J168-540), anti-GAL-9 (9M1-3), anti-TIM-3 (7D3), anti-CD45RA (HL100), anti-CD45RO (UCHL1), anti-CD62L (DREG-56), anti-CCR7 (3D12), anti-CD27 (0323), anti-CD28 (CD28.2), anti-CD57 (NK-1), anti-TCR-$\alpha\beta$ (T10Bg.1A-31), anti-TCR-$\gamma\delta$ (B1), anti-CD16 (B73.1), anti-CD56 (B159), anti-CD25 (M-A251),

anti-CD69 (FN50), anti-CD-38 (HIT2), anti-HLA-DR (LN3), anti-Glut-1 (202915), anti-CD137 (4B4-1), anti-CD154 (TRAp1), anti-CD122 (Mik-B3), anti-CD107a (H4A3), anti-IL-2 (MQ1-17H12), anti-TNF-α (MAB11), anti-IFN-γ (45.B3), anti-Perforin (dG9), anti-Granzyme-B (GB11), anti-FOXP3 (150D/E4), anti-EOMES (WD1928), anti-T-bet (4B10), anti-TCF-1 (7F11A10). The LIVE/DEAD kit (Life Technologies) was used to assess cell viability. The phosphorylation of STAT-5 was performed using a Phosflow kit (BD Biosciences) according to the manufacturer's instruction. Stained cells were fixed in Paraformaldehyde (PFA 4%) and data were acquired on a Fortessa-X20 or LSR Fortessa-SORP flow cytometry (BD Bioscience). Data were analyzed using Flow Jo software (version 10.7,1). A representative gating strategy for $CD160^+$ T cells is provided (Supplementary Fig. 1e).

2.2.4 Cell culture and *ex vivo* cytokine measurement

PBMCs or isolated $CD8^+$ T cells were cultured and stimulated with soluble Purified NA/LE Mouse anti-human CD3 (UCHT1, 3 μg/ml) and anti-human CD28 (CD28.2, 1 μg/ml) or PMA/Ionomycin (Cell stimulation cocktail, Biolegend) in the presence of the Protein transport inhibitor brefeldin A (BD Biosciences) for 5 hours. After fixation and permeabilization (BD Cytofix/Cytoperm), intracellular cytokine staining was performed according to our previous methods [32,33]. In some experiments, PBMCs were stimulated with anti-CD3/CD28 antibodies and treated with different cytokines such as IL-16 (R&D, 500 ng/ml) for 72 hrs. Then $CD8^+$ T cells were subjected to flow cytometry and RT-PCR for the quantification of CD160. In long-term cultures, every 72 hrs fresh media and cytokine/stimulation cocktails were added. Also, isolated B-

CLL cells (1 x 10^6) were cultured for 12 hours then IL-16 levels were measured in the culture supernatant by the Quantikine ELISA kit (R & D).

2.2.5 Proliferation assay

Isolated $CD3^+$ T cells were labeled with the CFSE dye (Life technologies), then stimulated with the Dyna beads Human T-activator CD3/CD28 (Thermo Fisher Scientific) according to the manufacturing protocol and analyzed 72 hours later.

2.2.6 Image Cytometry

After surface or intracytoplasmic staining cells were fixed with PFA 4% and analyzed. More than 5000 images were collected for each panel after the appropriate compensations were applied using the Amnis Image Stream Mark II image cytometer (EMD Millipore) and. Images were analyzed by the IDEAS software as we have reported elsewhere [34,35]. Only high resolution and in focus images were selected for further analysis.

2.2.7 Mesoplex and ELISA assay

The plasma concentrations of IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12p70, IL-13, TNF-α were measured using the V-plex plus Proinflammatory Panel 1 kit from Meso Scale Discovery (MSD). The plasma levels of GM-CSF, IL-1α, IL-5, IL-7, IL-12/23 p40, IL-15, IL-16, IL-17A, TNF-β, and VEGF were measured using the V-Plex plus cytokine Panel 1(MSD). The concentrations of Eotaxin, MIP-1α, Eotaxin-3, TARC, IP-10, MIP-1β, IL-8, MCP-1, MDC, MCP-4 were measured by the V-Plex Plus Chemokine Panel 1kit (MSD). All assays were performed according to the manufacturer's

instructions. Plasma samples were diluted 2-fold for the proinflammatory and cytokine panels and 4 folds for the chemokine panel. A total of 56 CLL samples and 20 healthy controls were analyzed. Data were acquired on the V-plex® Sector Imager 2400 plate reader. Analyte concentrations were extrapolated from a standard curve calculated using a four-parameter logistic fit using MSD Workbench 3.0 software. The concentration of soluble CD160 in the plasma was detected using by ELISA kit (Sino Biological). The optical density was measured using a microplate reader (Synergy H1 Biotek) set to 450nm and analyzed by Gen5 version 2.07 software.

2.2.8 EVs isolation and uptake assay

Plasma samples were thawed and centrifuged at 16000g for 15 min at 4 °C to remove debris. EVs were isolated using the exoEasy Maxi Kit (Qiagen) according to the manufacturer's instruction. The protein concentration of isolated EVs fraction was determined using the Pierce BCA protein assay kit (Thermo Fisher). Isolated EVs were quantified using the EXOCET Exosome Quantitation Assay kit (SBI, System Biosciences). For the uptake assay, EVs from CLL patients were labeled with the CFSE dye ($40\ \mu M$) for 2 hours at 37℃ as described [37].Unbound dye was removed using Exo-spin columns (Invitrogen). Next, PBMCs were co-cultured with the labeled EVs overnight followed by flow cytometry staining using the Image Stream analyzer [34].

2.2.9 Western blotting

Isolated EVs (40 µg) solubilized in SDS-PAGE buffer were separated by electrophoresis on polyacrylamide gels (7% or 17%, depending on the molecular weight of the target protein). Blocking was done by 5% non-fat dry milk before

probing overnight with the primary antibodies (CD9, CD63, CD81, CD160) (Thermo Fisher Scientific). Then blots were labeled with HRP-conjugated secondary antibodies and transferred to polyvinylidene fluoride (PVDF) membranes for chemiluminescent protein detection (Thermo Fisher Scientific). Membranes were re-probed with loading controls (β-actin, GAPDH). Protein bands were quantified using Image Lab Software v6.0.1 (Bio-rad).

2.2.10 Gene expression analysis

The RNA was isolated from $CD8^+$ T cells from healthy controls and CLL patients using the Direct-zol RNA MicroPrep kit (Zymo Research). Normally, 100 ng of RNA was used for cDNA synthesis using the Quantitect Reverse Transcription Kit (Qiagen). RT-PCR was carried out using Quantitect and RT2 RT-PCR Kits (Qiagen) to measure the expression of CD160, NFKB, TCF-7, Perforin, Granzyme-B, EOMES, and T-bet. Each sample was run in duplicate, using the CFX96 Touch Real-Time PCR Detection System (BioRad). Beta-2-microglobulin was used as a reference gene and the relative fold change of the targeted genes was calculated by the $\Delta\Delta$ CT method.

2.2.11 Statistical analysis

Statistical analysis was performed using GraphPad Prism, v6 (GraphPad Software, Inc.). For comparison, nonparametric Mann-Whitney U tests or Wilcoxon signed-rank tests were used for data sets that were nonpaired or paired, respectively. Data were presented as means and standard deviations of means (SD). Flow cytometry analysis and presentation of distributions were performed using SPICE, version 6 [38]

downloaded from http://exon.niaid.nih.gov/spice. A comparison of distributions was performed using a student's t-test and a partial permutation test.

When more than two groups were compared one-way analysis of variance (ANOVA) followed by Turkey's test was used to compare the results. The expression level of targeted genes between groups was analyzed by one-way ANOVA followed by Tukey's test.

2.3 Results

2.3.1 CLL influences the expression of co-inhibitory receptors on T cells.

We found a significant decrease in percentages of both $CD4^+$ and $CD8^+$ T cells in PBMCs of CLL patients compared to HCs (Fig. 1a). This difference was more prominent in $CD4^+$ T cells as the CD4/CD8 ratio was reduced from 2.7 in HCs to 1.15 in CLLs (Fig. 1a). Unfortunately, we were unable to obtain the whole blood cell count from HCs, therefore, there is a possibility that a large population of malignant B cells in CLL patients influences the T cell count in the peripheral blood. We observed significantly higher percentages of CD160 expressing cells among $CD8^+$ and $CD4^+$ T cells in CLL patients compared to HCs (Fig. 1b-e and Supplementary Fig. 1e). Notably, the percentages of $CD8^+$ and $CD4^+$ T cells expressing CD160 were higher in the BM compared to the peripheral blood (Fig. 1b-e). However, we did not observe any difference in the percentages of CD160 expressing T cells in treatment naïve versus treated patients (Supplementary Fig. 1f). We also evaluated the influence of age on CD160 expression in $CD8^+$ T cells in CLL patients and found a higher proportion of CD160 expressing $CD8^+$ T cells in 65-84 compared to the younger age group (Supplementary Fig. 1g). Since the mutation of the immunoglobulin heavy chain

variable region gene (IgHV) status is considered as a prognostic factor in CLL [39], we compared the percentages of CD160 expressing $CD8^+$ T cells in IgHV mutated versus un-mutated patients. However, we did not observe any significant difference between the groups possibly due to a low sample size (Supplementary Fig. 1h). Once the FISH analysis was performed, we found significantly higher percentages of CD160 expressing cells among $CD8^+$ T cells of Trisomy-12 subjects compared to those with Del13q (Supplementary Fig. 1i). Also, we measured the intensity of CD160 expression on T cells of CLL patients and HCs and found significantly higher expression levels of CD160 on both $CD8^+$ and $CD4^+$ T cells of CLL patients compared to HCs (Fig. 1f-h). Then we analyzed the expression of CD160 at the gene level and found a higher expression of CD160 mRNA in isolated $CD8^+$ T cells of CLLs compared to HCs (Fig. 1i). Although percentages of both $CD8^+$ and $CD4^+$ T cells expressing intracytoplasmic CD160 were significantly higher than those expressing the surface CD160 expression in both CLL patients and HCs (Fig. 1j, 1k and 1d, 1e), T cells expressing the intracytoplasmic CD160 had lower frequencies in CLL patients compared to HCs (Fig.1j, 1k). Also, we found that HVEM, the CD160 ligand, was highly expressed on the surface of T cells without any difference between CLLs and HCs (Fig. 1l, 1m). The same pattern was observed for percentages of B cells expressing HVEM (Supplementary Fig. 1j, 1k). Furthermore, we found significant increase in 2B4, TIGIT and PD-1 but the reduction of BTLA expressing $CD8^+$ T cells in CLL patients compared to HCs, however, the frequency of TIM-3 and Galectin-9 (Gal-9) expressing $CD8^+$ T cells remained unchanged (Fig. 1n and Supplementary Fig. 1l). Of note, we did not find any difference in the frequency of $CD8^+$ T cells expressing these co-inhibitory receptors in the peripheral blood versus the BM (Fig.

1n). Similar observations were made for $CD4^+$ T cells expressing 2B4, TIGIT and PD-1 (Fig. 1o and Supplementary Fig. 1m). It is worth mentioning that the frequency of PD-1 expressing $CD4^+$ T cells was significantly higher in the BM compared to the peripheral blood (Fig. 1o) but the frequencies of TIM-3 and Gal-9 expressing $CD4^+$ T cells were low without any significant difference between CLL patients and HCs (Fig. 1o). Overall, percentages of T cells expressing surface CD160 were significantly increased in CLL patients compared to HCs.

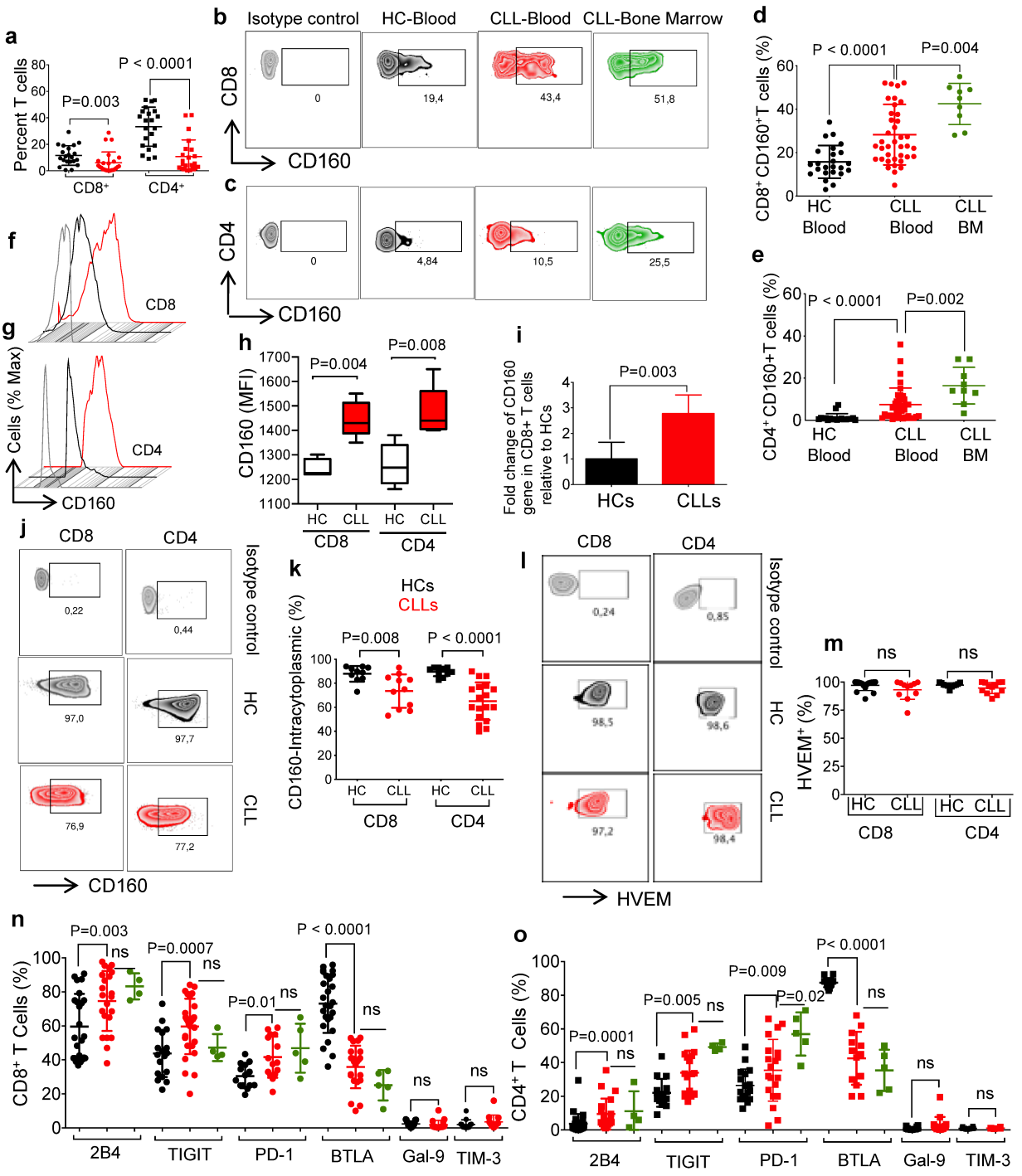


Fig. 1. The expression of co-inhibitory receptors on T cells. (a) Cumulative data showing percentages of $CD8^+$ and $CD4^+$ T cells in PBMCs of CLLs versus HCs. **(b)** Representative flow cytometry plots of $CD160^+CD8^+$ T cells, and **(c)** $CD160^+CD4^+$ T cells in the blood and bone marrow (BM) of CLL patients versus HC blood. **(d)** Cumulative data of percentages of $CD160^+$ among $CD8^+$ T cells, and **(e)** $CD4^+$ T cells in CLLs (blood and BM) versus HC blood. **(f)** Histogram plots of CD160 expression on $CD8^+$, and **(g)** $CD4^+$ T cells measured by the mean fluorescence intensity (MFI), and (**h**) cumulative data in $CD8^+/CD4^+$T cells of CLL patients versus HCs. **(i)** Expression

of CD160 mRNA level in $CD8^+$ T cells of CLL patients (PBMCs) relative to HCs. **(j)** Representative flow cytometry plots, and **(k)** cumulative data of percentages of $CD8^+$ and $CD4^+$ T cells expressing intracytoplasmic (ICS) CD160 in PBMCs of CLL patients versus HCs. **(l)** Representative flow cytometry plots, and **(m)** cumulative data of percentages of $HVEM^+CD8^+$ and $HVEM^+CD4^+$ T cells in CLL patients versus HCs. **(n)** Cumulative data of percentages of $CD8^+$, and **(o)** $CD4^+$ T cells expressing surface expression of 2B4, TIGIT, PD-1, BTLA, GAL-9 and TIM-3 on $CD8^+$ T cells in CLL patients' (blood and BM) versus HC blood. Each dot represents data from a single CLL patient or HC. Fig.1h, I from 6 human subject/group.

2.3.2 $CD160^+$ T cells exhibit impaired effector functions but maintain their proliferative capacity in CLL patients.

We found that $CD160^+$ T cells had impaired production of IL-2, TNF-α and IFN-γ among $CD8^+$ and $CD4^+$ T cells in CLL patients (Fig. 2a-d), which is in agreement with another report [12]. We noted that $CD8^+CD160^+$ T cells compared to their $CD160^-$ counterparts in HCs exhibited the same phenotype as CLLs (Fig. 2e and Supplementary Fig. 2a), however, the frequency of $CD4^+CD160^+$ T cells in HCs was very low to quantify (Supplementary Fig. 2b). Because of the reported differences in the cytokine production capacity of different T cell subsets [40], we measured TNF-α and IFN-γ production in $CD160^+$ and $CD160^-$ in relation to their differentiation status (e.g. naive, central memory, effector memory and effector T cells). We found that $CD160^+$ cells among either $CD8^+$ or $CD4^+$ T cells regardless of their differentiation status expressed significantly lower levels of cytokines compared to their $CD160^-$ counterparts (Fig. 2f-i and Supplementary Fig. 2c). Since the coordinated action of perforin [41] and granzyme-B (GzmB) [42] is essential for the optimal $CD8^+$ T cell-mediated cytotoxicity, we analyzed perforin and GzmB co-expression in $CD8^+$ T cells *ex vivo*. We found that significantly lower percentages of $CD160^+$ compared to $CD160^-$ $CD8^+$ T cells expressed perforin, but this was not the case for GzmB. As such,

significantly lower percentages of perforin/GzmB co-expressing cells were observed in $CD160^+$ compared to $CD160^-$ $CD8^+$ T cells in CLL patients (Fig. 2j, k). In contrast, we detected higher mRNA for GzmB and perforin in $CD8^+$ T cells of CLL patients compared to HCs (Supplementary Fig. 2d, 2e). Higher mRNA for GzmB and perforin but lower protein expression may suggest that the genes are not efficiently translated into protein in $CD8^+CD160^+$ T cells in CLL patients. Moreover, the ability of $CD8^+$ T cells to degranulate in response to stimulation with anti-CD3/CD28 antibodies was assessed by CD107a expression (lysosomal-associated membrane protein I, LAMP-I). We found that although $CD160^+$ and $CD160^-$ $CD8^+$ T cells expressed similar CD107a levels in the absence of stimulation, $CD160^+$ $CD8^+$ T cells exhibited impaired degranulation capacity following stimulation (Fig. 3a-d). Another feature of T cell exhaustion is the gradual loss of proliferation capacity [43]. Thus, we assessed the proliferative capacity of isolated $CD160^+$ versus $CD160^-$ T cells in response to stimulation with anti-CD3/CD28 coated microbeads for 72 hours *in vitro*. However, no significant difference was observed in the percentages of proliferated $CD160^+$ versus $CD160^-$ in both $CD8^+$ and $CD4^+$ T cells (Fig. 3e-h). Because of the difference in proliferative capacities of T cell subsets [34,40], we measured the proliferative capacity of isolated effector T cells, however, both $CD8^+CD160^+$ and $CD8^+CD160^-$ T cells exhibited similar proliferative capacity (Supplementary Fig. 2f-i). Similar observations were made for the Ki67 expression in $CD160^+$ versus $CD160^-$ T cells (Supplementary Fig. 2j, 2k). Thus, our findings show that $CD160^+$ T cells exhibit a dysfunctional phenotype in CLL patients.

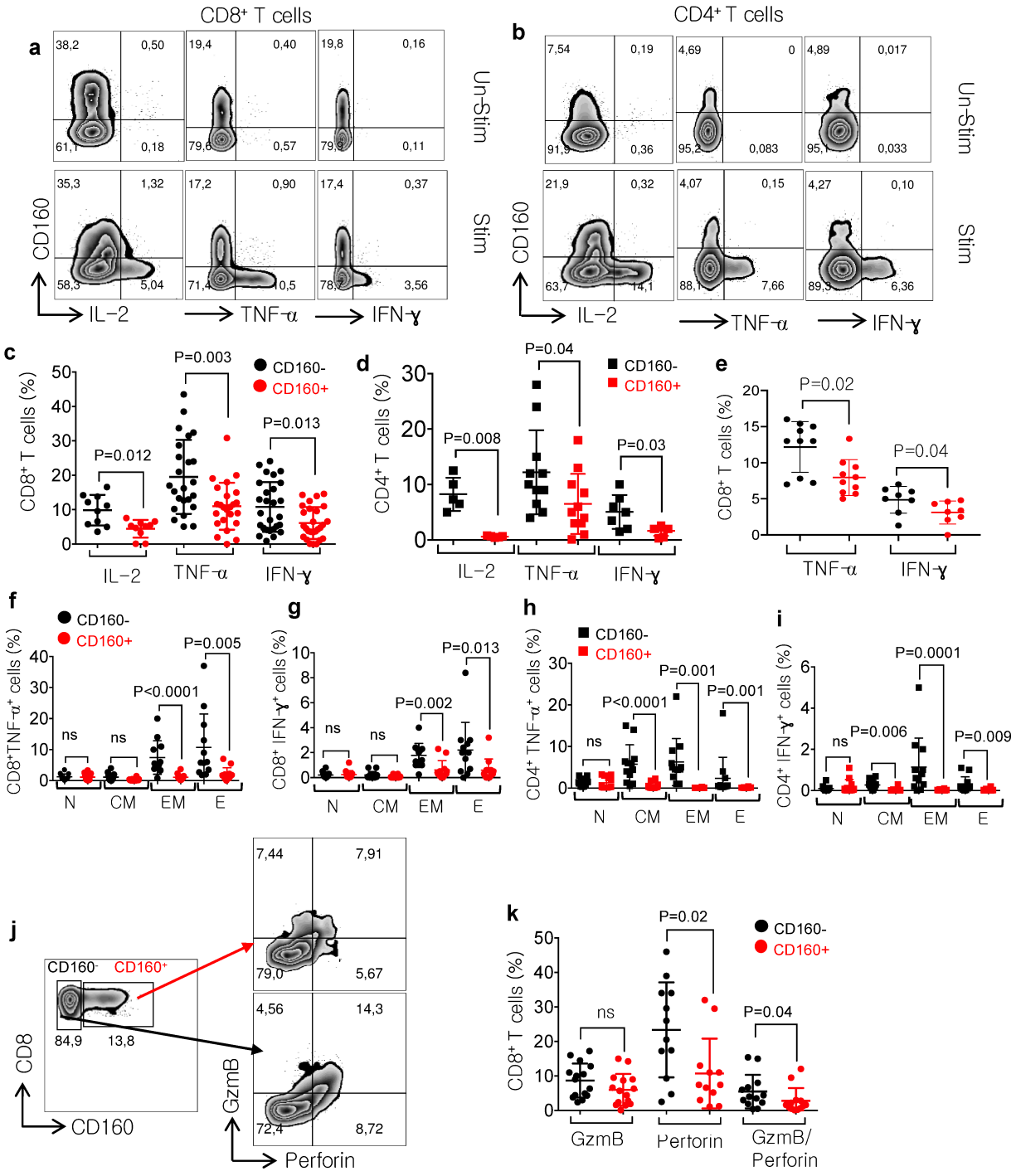


Fig. 2. Impaired cytokine production and cytolytic activity of CD160⁺ T cells in CLL patients.

(a) Representative flow cytometry plots of IL-2, TNF-α and IFN-γ expression in $CD8^+CD160^-$ and $CD8^+CD160^+$, and **(b)** $CD4^+CD160^-$ versus $CD4^+CD160^+$ T cells. **(c)** Cumulative data of percentages of IL-2, TNF-α and IFN-γ expressing cells among $CD8^+CD160^-$ and $CD8^+CD160^+$, and **(d)** $CD4^+CD160^-$ and $CD4^+CD160^+$ T cells after 5 hours of *in vitro* stimulation with anti-CD3/CD28 antibodies. **(e)** Cumulative data

of percentages of TNF-α and IFN-γ expressing cells among $CD8^+CD160^-$ and $CD8^+CD160^+$ T cells in HCs. **(f)** Cumulative data of percentages of TNF-α, and **(g)** IFN-γ expressing cells in $CD8^+CD160^-$ versus $CD8^+CD160^+$ T cells of CLL patients defined as (N: Naive, CM: Central Memory, EM: Effector Memory, E: Effector). **(h)** Cumulative data of percentages of TNF-α, and **(i)** IFN-γ expressing cells in $CD4^+CD160^-$ versus $CD4^+CD160^+$ T cells in different T cell subsets as shown. **(j)** Representative flow cytometry plots, and **(k)** cumulative data of percentages of GzmB and perforin expressing cells in $CD8^+CD160^-$ versus $CD8^+CD160^+$ T cells in CLL patients. For TNF-α and IFN-γ analysis, PBMC's were stimulated with the anti-CD3 (3 $\mu g/ml$) and anti-CD28 (1 $\mu g/ml$) in the presence of protein transporter inhibitor (1:1000) for 5 hours. Each dot represents data from a single CLL patient.

2.3.3 Differential effects of 2B4 and TIGIT expression on cytokine production and cytolytic activity of T cells in CLL patients.

We also evaluated the effect of other highly expressed co-inhibitory receptors such as 2B4 and TIGIT on T cell effector functions in CLL patients. In contrast to CD160 expression, 2B4 expression was associated with a greater cytokine production ability of both $CD8^+$ and $CD4^+$ T cells in CLL patients (Fig. 3i, 3j, and Supplementary Fig. 2l), which is consistent with a previous report [34]. While the expression of TIGIT had no significant impact on TNF-α, and IFN-γ production in $CD8^+$ T cells, it dampened TNF-α, and IFN-γ production in $CD4^+$ T cells (Fig. 3k, 3l, Supplementary Fig. 3m). Of note, the co-expression of GmzB/perforin was negligible in $2B4^-$ $CD8^+$ T cells (Supplementary Fig. 2n). However, the expression of TIGIT did not affect the expression of GzmB/perforin in $CD8^+$ T cells in CLL patients (Fig. 3k, and Supplementary Fig. 2o). Also, we found a significantly higher frequency of cytolytic molecules and CD107a expressing cells among $2B4^+$ $CD8^+$ T cells compared to their negative counterparts (Fig. 3i and Supplementary Fig. 2p). Finally, we found a higher frequency of CD107a expressing cells among $2B4^+CD8^+$ versus $TIGIT^+CD8^+$ T cells after stimulation with anti-CD3/CD28 antibodies for 5hr (Supplementary Fig. 2p).

Overall, the expression of 2B4 was associated with enhanced T cell effector functions in CLL patients but this was not the case for TIGIT.

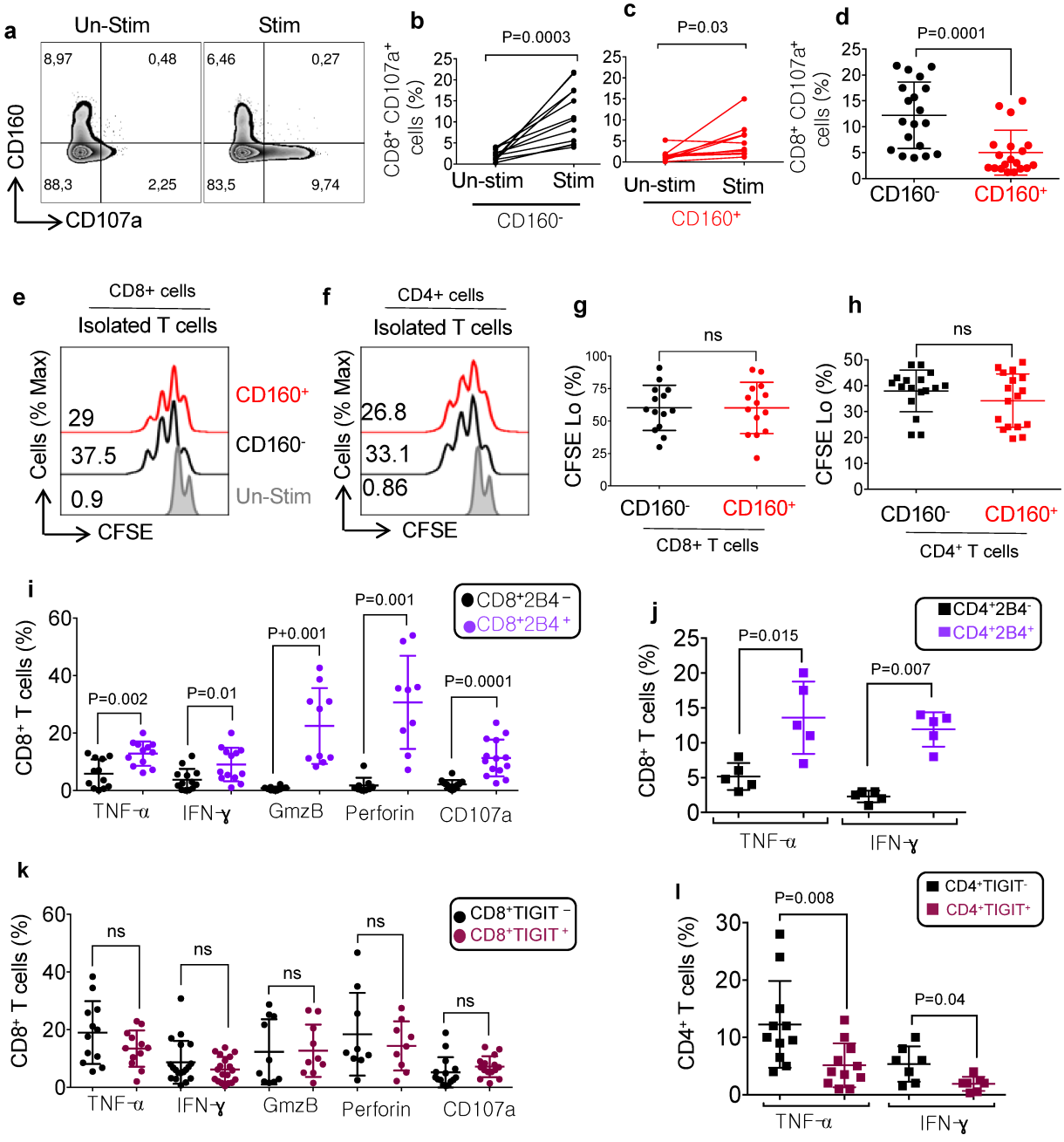


Fig. 3. The impact of CD160, 2B4 and TIGIT expression on T cell effector functions in CLL patients. (a) Representative flow cytometry plots, and **(b-d)** cumulative data of percentages of CD107a expressing cells among $CD8^+CD160^-$ and $CD160^+CD8^+$ T cells in unstimulated versus stimulated PBMCs with anti-CD3/CD28 antibodies for 5hr. **(e)** Representative flow cytometry plot of percentages of CFSELo (proliferated) $CD8^+CD160^-$ and $CD8^+CD160^+$, and **(f)** $CD4^+CD160^-$ and $CD4^+CD160^+$

T cells. **(g)** Cumulative data of percentages of CFSELo $CD8^+CD160^-$ versus $CD8^+CD160^+$ and **(h)** $CD4^+CD160^-$ versus $CD4^+CD160^-$ T cells after stimulation of PBMCs from CLL patients with anti-CD3/CD28 antibodies for 3 days. **(i)** Cumulative data of percentages of TNF-α, IFN-γ, GzmB, perforin and CD107a expressing cells among $2B4^-/2B4^+CD8^+$ T cells. **(j)** Cumulative data showing percentages of TNF-α and IFN-γ expressing cells among $2B4^-/2B4^+$ $CD4^+$ T cells of CLL patients. **(k)** Cumulative data showing percentages of TNF-α, IFN-γ, GzmB, perforin and CD107a expressing cells among $TIGIT^-/TIGIT^+$ $CD8^+$ T cells. **(l)** Cumulative data showing percentages of TNF-α and IFN-γ expressing cells among $TIGIT^-/TIGIT^+$ $CD4^+$ T cells of CLL patients. For TNF-α and IFN-γ and CD107a analysis, PBMC's were stimulated with the anti-CD3 (3 μg/ml) and the anti-CD28 (1 μg/ml) antibody in the presence of protein transporter inhibitor (1:1000) for 5hrs. Each dot represents data from a single CLL patient.

2.3.4 Co-expression of CD160 with other co-inhibitory receptors results in a more impaired $CD8^+$ T cell phenotype in CLL patients.

Since the co-expression of multiple co-inhibitory receptors dictates a more impaired T cell phenotype [10], we analyzed the co-expression of CD160 with other co-inhibitory receptors. We found that CD160 was highly co-expressed with 2B4 and TIGIT but to a lesser extent with PD-1, and BTLA in $CD8^+$ T cells (Fig. 4a, 4b). Conversely, CD160 was moderately co-expressed with TIGIT, PD-1 and BTLA but even lower with 2B4 on $CD4^+$ T cells (Fig. 4c, 4d). Besides, we analyzed the simultaneous co-expression of 2B4, CD160, and TIGIT using the SPICE software. We found that most of $CD8^+$ T cells expressed one or two of these co-inhibitory receptors but $20\pm5\%$ of $CD8^+$ T cells co-expressed CD160, 2B4, and TIGIT (Red-colored sector on the pie chart and the bar graph) (Fig. 4e). In contrast, the majority of $CD4^+$ T cells expressed only TIGIT (Gray sector colored on the pie chart and bar graph, or none of these co-inhibitory receptors (Purple sector colored on the pie chart and bar graph) (Fig. 4f). We found about 50% of $CD8^+$ and 10% of $CD4^+$ T cells co-expressed 2B4 and TIGIT (Supplementary Fig. 3a, 3b).

We also analysed the effect of CD160 co-expression with 2B4, TIGIT, and PD-1 on $CD8^+$ T cell effector functions. Co-expression of CD160 with 2B4 and TIGIT corresponded with impaired cytokine production (TNF-α and IFN-γ), lower levels of cytolytic molecules (GzmB and perforin) expression, and reduced degranulation (CD107a) capacity (Fig 4g, 4h). The reduced effector functions in $CD160^-/2B4^-$ and $CD160^-/TIGIT^-$ $CD8^+$ T cells could be explained by their exclusion from the effector T cell pool. Although the frequency of $CD160^+PD\text{-}1^+CD8^+$ T cell subset was low, these cells exhibited impaired cytokine production (Fig. 4i), which is consistent with a report [44]. These observations indicate that co-expression of CD160 with 2B4, TIGIT, and PD-1 dictates an impaired $CD8^+$ T cell phenotype in CLL patients (Fig. 4g-i).

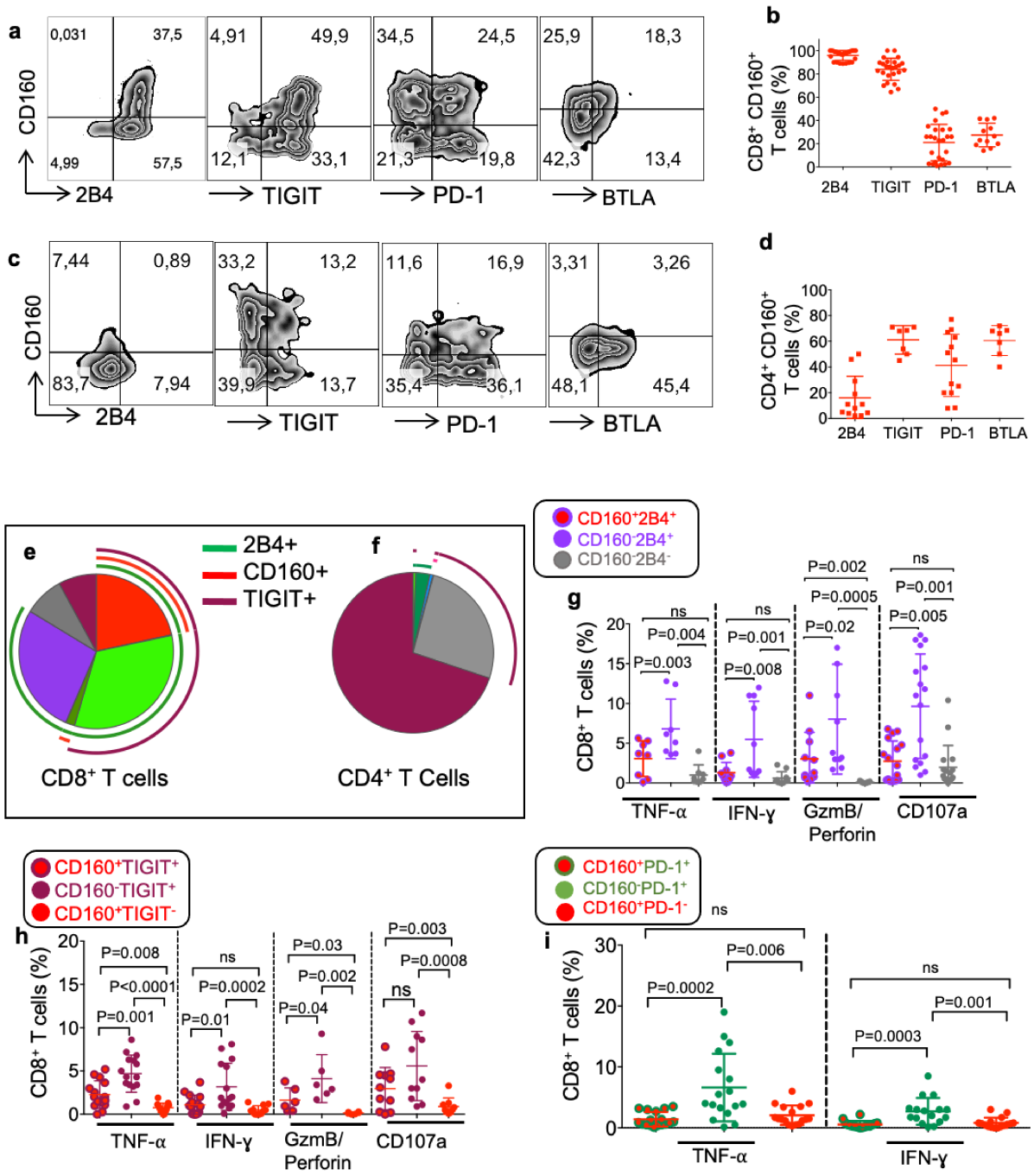


Fig. 4. Co-expression of CD160 with other co-inhibitory receptors on T cells in CLL patients.

(a) Representative flow cytometry plots, and **(b)** cumulative data showing percentages of CD160 co-expression with 2B4, TIGIT, PD-1, and BTLA in $CD8^+$ T cells. **(c)** Representative flow cytometry plots, and **(d)** cumulative data showing percentages of CD160 co-expression with 2B4, TIGIT, PD-1, and BTLA in $CD4^+$ T

cells. **(e)** The Pie chart showing percentages of $CD8^+$ T cells co-expressing CD160, 2B4, and TIGIT, simultaneously. **(f)** The Pie chart showing percentages of $CD4^+$ T cells co-expressing CD160, 2B4, and TIGIT, simultaneously. **(g)** Cumulative data showing percentages of TNF-α, IFN-γ, GzmB, perforin and CD107a expressing cells among $CD160^+2B4^+/CD160^-2B4^+/CD160^-2B4^-$ $CD8^+$ T cells. **(h)** Cumulative data showing percentages of TNF-α, IFN-γ, GzmB, perforin and CD107a expressing cells among $CD160^+TIGIT^+/CD160^-TIGIT^+/CD160^+TIGIT^-$ $CD8^+$ T cells. **(i)** Cumulative data showing percentages of TNF-α, IFN-γ expressing cells among $CD160^+PD\text{-}1^+/CD160^-$ $PD\text{-}1^+/CD160^+PD\text{-}1^-$ $CD8^+$ T cells. Each dot represents data from a single CLL patient.

2.3.5 CD160 expression is associated with different stages of T cell differentiation.

To determine the differentiation status of $CD160^+$ T cells, we stained them with CD45RA and CCR7. We observed that not only the majority of $CD8^+$ T cells but also $CD160^+CD8^+$ T cells were effector T cells (T_{EFF}) in CLL patients (Supplementary Fig. 3c-f). This was not the case for $CD4^+$ T cells, while total $CD4^+$ T cells were enriched with T_{EFF} cells (Supplementary Fig. 3g, 3h), $CD160^+CD4^+$ T cells were heterogeneous (Supplementary Fig. 3i, 3j). When we compared $CD8^+$ T cell subsets in CLL patients versus HCs, we found significantly higher percentages of T_{EFF} but lower naïve, CM, and EM in CLL patients (Supplementary Fig. 3d). The same pattern was the case for $CD4^+$ T cells except for percentages of naïve T cells remained unchanged in HCs versus CLL patients (Supplementary Fig. 3h). Also, we observed significantly higher replicative senescence in $CD8^+CD160^+$ T cells compared to their negative counterparts as evidenced by a lower CD28 but higher CD57 and PD-1 expression [43] (Supplementary Fig. 3k-n).

Next, we analysed the expression of CD160 in different generations of antigen-experienced $CD8^+$ T cells as follows: Naive ($CD27^+CD28^+CD45RA^+CCR7^+CD57^-$), Ag experienced level-1 ($CD27^+CD28^+CD45RA^-CCR7^+CD57^-$), Ag experienced level-2

($CD27^+CD28^+CD45RA^-CCR7^-CD57^{+/-}$), Ag experienced level-3 ($CD27^+CD28^-CD45RA^{+/-}CCR7^-CD57^{+/-}$), and Ag experienced level-4 ($CD27^-CD28^-CD45RA^-CCR7^-CD57^+$) [45,46] We found an increase in the expression of CD160 on $CD8^+$ T cells as the level of antigen experience progressed (Supplementary Fig. 3o, 3p). This was further confirmed by a higher expression of CD137, as a marker of antigen-experienced T cells [47], on $CD160^+CD8^+$ T cells (Supplementary Fig. 3q, 3r). Thus, $CD160^+CD8^+$ T cells have a characteristic of an antigen-experienced effector phenotype with very few senescent cells.

2.3.6 Differential expression of exhaustion transcriptional factors and IL-2 signaling in $CD160^+$ T cells in CLL patients.

T-bet and EOMES are T-box family transcription factors, whose intricate equilibrium delineates and maintains different subsets of exhausted $CD8^+$ T cells [48]. Tcf1 is another transcription factor related to preserving the effector function of exhausted T cells [49]. We found no change in the expression of EOMES at the gene level, but T-bet (TBX21) was significantly upregulated in $CD8^+$ T cells from CLL patients compared to HCs. However, the expression of the Tcf1 gene was downregulated in $CD8^+$ T cells of CLL patients versus HCs (Supplementary Fig. 4a-c). At the protein level, we found no change in EOMES and T-bet expressing $CD8^+$ T cells but significantly higher percentages of $CD160^+CD4^+$ T cells expressed EOMES compared to $CD4^+CD160^-$ T cells (Supplementary Fig. 4d-f).

Also, we measured the expression of IL-2 receptors, CD25 (IL-2Rα), and CD122 (IL-2Rβ) on $CD160^+$ T cells. We found that CD122 was significantly higher in $CD4^+CD160^+$ but not in $CD8^+CD160^+$ T cells (Supplementary Fig. 4g, 4h). On the other hand, CD25

was highly expressed on both $CD4^+$ and $CD8^+$ T cells expressing CD160 (Supplementary Fig. 3i, 3j). Considering the higher IL-2 receptor expression in $CD160^+$ T cells, we measured the signal transduction in response to IL-2 stimulation *in vitro*. We treated PBMCs from CLL patients with or without IL-2 and phosphorylation of STAT-5 was measured by Phospho-flow. We found that $CD160^+$ T cells had higher baseline phosphorylation of STAT-5 compared to $CD160^-$ population and after stimulation with IL-2 (Supplemental Fig. 4k-n).

2.3.7 Prolonged T cell stimulation upregulates surface CD160 expression on T cells.

To understand the mechanism underlying CD160 upregulation on T cell surface in CLL patients, we analyzed the effect of T cell stimulation on CD160 surface and intracytoplasmic expression. Interestingly, we observed that culture of PBMCs (72 hr) from CLL patients in the absence of any stimulation resulted in the expansion of CD160 expressing $CD8^+$ and $CD4^+$ T cells, respectively (Fig. 5a, 5b, and Supplementary Fig. 5a, 5b). To rule out the external source of CD160 such as NK cells [20], we cultured isolated $CD8^+$ T cells and again the same pattern was observed (Fig. 5c). We found that this was unrelated to the Fetal bovine serum (FBS) in the culture media (Fig. 5a, 5b, and Supplementary Fig. 5a, 5b). These observations suggested that the intracellular CD160 may translocate to the surface of T cells when cultured *in vitro*. To confirm this, brefeldin A (Endoplasmic Reticulum to Golgi inhibitor) and Monensin (Golgi to plasma membrane pathway inhibitor) were added to the culture media for 16 hours, which resulted in a significant reduction of cells expressing CD160 compared to non-treated T cells (Fig. 5d, e, and Supplementary

Fig. 5c, d). These observations suggested that CD160 gets transported to the surface via the ER-Golgi-Plasma membrane pathway.

Moreover, we observed that upon stimulation of PBMCs with anti-CD3/CD28 antibody (for 72 hours) the surface CD160 expression was significantly decreased while intracytoplasmic expression was increased in both $CD8^+$ (Fig. 5f-i) and $CD4^+$ T cells (Supplementary Fig. 5e-h). However, the prolonged stimulation with anti-CD3/CD28 antibody (for 6 days) resulted in a significant upregulation of surface CD160 on $CD8^+$ (Fig. 5j, k) and $CD4^+$ T cells (Supplementary Fig. 5i, 5j). Similarly, the intracytoplasmic expression of CD160 was increased in stimulated $CD8^+$ cells with anti-CD3/CD28 antibodies (Fig. 5l, 5m) and $CD4^+$ T cells (Supplementary Fig. 5k, l). To test the possibility of CD160 shedding from T cells, isolated $CD8^+$ T cells were stimulated with anti-CD3/CD28 antibodies, IL-2, or IL-15 for 72 hours. Interestingly, CD160 protein was undetectable in cell culture supernatants as measured by Western blotting, using an anti-CD160 antibody (Clone: BY55) (Supplementary Fig. 5m). Notably, we detected significantly lower levels of CD160 in the plasma of CLL patients compared to HCs (Supplementary Fig. 5n). These observations suggest that constant antigenic stimulation may result in the upregulation of CD160 in T cells of CLL patients.

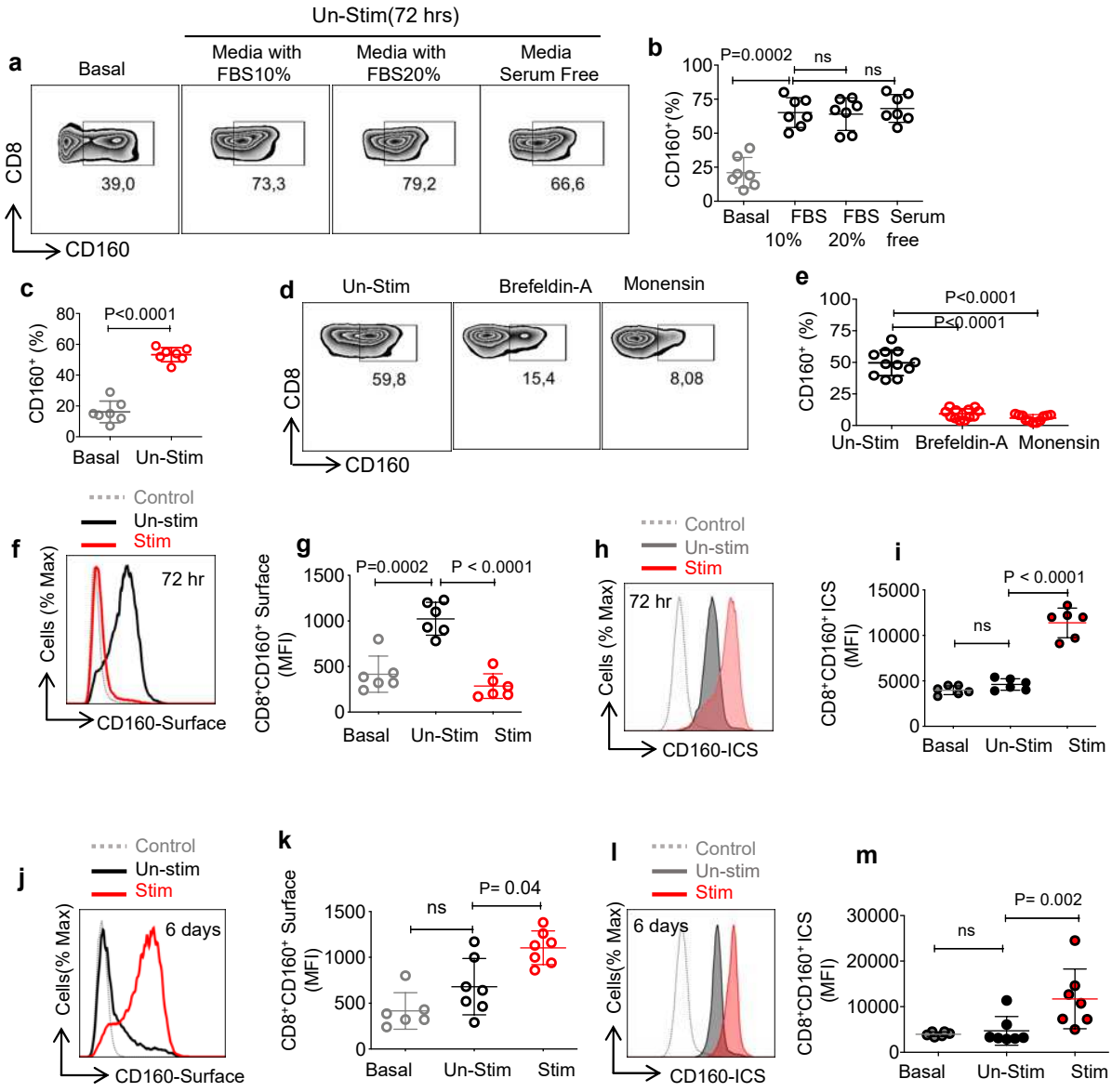


Fig. 5. Prolonged T cell stimulation upregulates CD160 expression. (a) Representative flow cytometry plots, and **(b)** cumulative data showing percentages of $CD8^+$ T cells expressing CD160 at the baseline, in the presence of 10%, 20% (Fetal Bovine Serum) or in the absence of FBS after 72 hr *in vitro* culture of total PBMCs from CLL patients. **(c)** Cumulative data showing percentages of isolated $CD8^+$ T cells expressing CD160 at the baseline and after 72 hr *in vitro* culture. **(d)** Representative flow cytometry plots, and **(e)** Cumulative data of percentages of $CD8^+$ T cells expressing CD160 in the absence or presence of Brefeldin A and Monensin after 6 hr culture of PBMCs. **(f)** Representative histogram, and **(g)** cumulative data of the mean fluorescence intensity (MFI) of surface CD160 expression on $CD8^+$ T cells stimulated with anti-CD3/CD28 antibodies (stim) versus unstimulated (Un-stim) for 72 hr. **(h)** Representative histogram, and **(i)** cumulative data of intracytoplasmic (ICS) CD160 expression in $CD8^+$ T cells unstimulated vs. stimulated with anti-CD3/CD28 for 72 hr. **(j)** Representative histogram, and **(k)** cumulative data of MFI for the surface

expression of CD160 in $CD8^+$ T cells, unstimulated versus stimulated with anti-CD3/CD28 after 6 days of *in vitro* culture. **(l)** Representative histogram, and **(m)** cumulative data of MFI for the intracytoplasmic expression of CD160 in $CD8^+$ T cells, unstimulated versus stimulated with anti-CD3/CD28 after 6 days of *in vitro* culture. Each dot represents data from a single study subject.

2.3.8 Plasma-derived EVs contain CD160 in CLL patients.

To determine the role of soluble mediators in CD160 expression on T cells, we added 5%,10%, and 20% of plasma from CLL or HCs to unstimulated PBMCs from CLL patients and HCs for 72 hours. Interestingly, we observed the upregulation of CD160 on both $CD8^+$ and $CD4^+$ T cells in a dose-dependent manner (Fig. 6a-c). To better understand the mechanism, we isolated EVs from the plasma of CLL patients and HCs. In contrast to a previous report [50], using the Exocet ELISA method we did not observe any difference in the quantity of EVs between CLL patients and HCs (Fig. 6d). We found that EVs as characterized by CD9, CD81, and CD63 markers, contained CD160 (Fig. 6e, 6f). This was further examined by western blotting (Fig. 6g-n). Due to the differential expression of actin in HC and CLL samples in western blot, we normalized the amount of CD9, CD81, CD63, and CD160 based on the actin expression and identified that CD160 expression was higher in CLL EVs compared to HCs (Fig. 6g, 6h). While CD9 and CD63 expression were remained unchanged (Fig. 6i-l), CD81 expression in EVs from CLL patients was higher than HCs (Fig. 6m, 6n). We further performed the EV uptake assay by labeling isolated EVs from CLL patients with the CFSE dye and then adding them into PBMCs from HCs overnight. We then investigated the uptake of EVs in T cells by using the ImageStream analysis. As shown in Supplementary Fig. 6a, we observed the uptake of EVs by T cells. These

data suggest that plasma derived EVs of CLL patients contain CD160 and could be taken up by T cells which may influence CD160 levels in T cells of CLL patients.

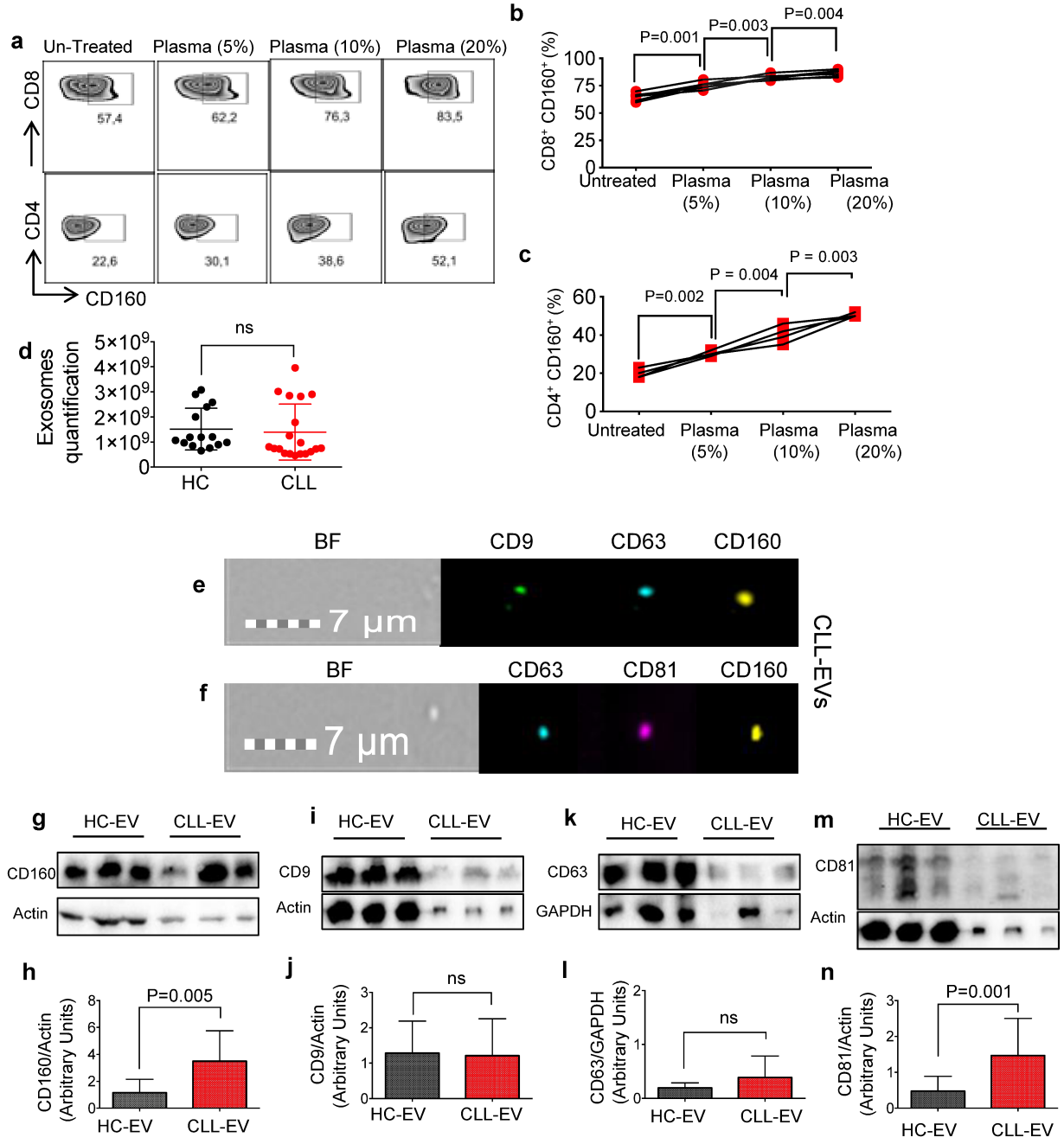


Fig. 6. Plasma derived EVs contain CD160. (a) Representative flow cytometry plots of CD160 expression in $CD8^+$ and $CD4^+$ T cells untreated versus treated with the plasma from CCL patients (using 5, 10 and 20% plasma) after 72 hr of *in vitro*

culture. **(b)** Cumulative data of percentages of CD160 expressing cells among $CD8^+$, and **(c)** $CD4^+$ T cells either untreated or treated with indicated plasma concentrations after 72 hr. **(d)** Quantification of EVs numbers isolated from the plasma of CLLs versus HCs by Exocet ELISA kit. **(e)** Image Stream plots of plasma derived EVs showing the expression of CD9, CD63 and CD160, bright field (BF). **(f)** Image stream plots of plasma derived EVs showing expression of CD63, CD81 and CD160. **(g)** Representative Western blot (WB) images of plasma derived EVs from HCs and CLL patients depicting CD160 presence. **(h)** Cumulative data showing normalised arbitrary units of CD160/Actin in plasma derived EVs in HCs versus CLL patients. **(i)** Representative WB images of plasma derived EVs depicting CD9 expression, and **(j)** cumulative data showing normalised arbitrary units of CD9/Actin in plasma derived EVs in HCs versus CLL patients. **(k)** Representative WB images of plasma derived EVs depicting CD63 expression, and **(l)** cumulative data showing normalised arbitrary units of CD63/GAPDH in plasma derived EVs in HCs versus CLL patients. **(m)** Representative WB images of plasma derived EVs depicting CD81 expression, and **(n)** cumulative data showing normalised arbitrary units of CD160/Actin in plasma derived EVs in HCs versus CLL patients. Actin was used as a loading control to normalise protein amounts of CD81, CD9, CD160 and GAPDH was used as a loading control to normalise protein amount of CD63. Each dot/band represents data from a subject.

2.3.9 A pro-inflammatory cytokine profile in the plasma of CLL patients.

Several studies have shown the impact of cytokines and chemokines on the modulation of co-inhibitory receptors and T cell effector functions [51]. For example, IL-10 has been reported to upregulate CD160 in $CD8^+$ T cells [28].

Plasma specimens from 37 CLL patients and 20 HCs were subjected to multiplex assay. Z-Scores were calculated for each cytokine and chemokine in each patient/HC and individual results are shown as a heatmap diagram for better comparison (Supplementary Fig. 6b, 6c). Plasma levels of TNF-β, IL-16, TNF-α, MIP-1α, MIP-1β (CCL4), IL-12/23p40, GM-CSF, IL-10, IL-8, IL-15, MCP-1, IL-1β, IL-17A and IL-2 were significantly elevated in CLL compared to HC samples (Fig. 7a). The Eotaxin-3 was significantly lower in CLL patients (Fig. 7a) but the other analyzed cytokines including IL-1α, IL-4, IL-5, IL-6, IL-7, IL12/23p70, IL-13, IFN-γ, VEGF, MCP-4 (CCL13), MDC-1, TARC (CCL17), IP-10, and Eotaxin were similar in both groups (Fig. 7a). Among

these cytokines, IL-16, TNF-α, TNF-β, MIP-1α and MIP-1β (CCL4) showed the highest deviation between CLL and HCs ($P < 0.0001$) followed by IL-8 ($P=0.0002$). We didn't find any significant difference in levels of IFN-γ, TARC, IL-5, and IL-6, as it has been reported [52]. MIP-1α and IL-16 concentration (pg/ml) showed a positive correlation with percentages of $CD160^{+}CD8^{+}$ T cells (Fig. 7b, 7c). In summary, our data indicate a more pro-inflammatory milieu in CLL patients.

2.3.10 B-CLLs are a major source of elevated IL-16 in the plasma of CLL patients and correlated to a high Rai Stage (III/IV).

Plasma cytokine levels were stratified based on the individual Rai stage, percentages of $CD160^{+}CD8^{+}$ T cells, and the lymphocyte count. Although we did not find any correlation between the measured cytokine levels and lymphocyte counts, we observed that IL-16 was higher in the high-risk Rai staging system (III/IV) compared to the low-risk group (Zero) without any difference between low/intermediate and intermediate/high-risk patients (Fig. 7d). Interestingly, CD160 expressing $CD8^{+}$ T cells were higher in the high risk (Rai stage: III/IV) compared to the low-risk group (Rai stage: Zero) (Fig. 7e). Because of the dramatically elevated levels of IL-16 in the plasma of CLL patients, and a positive correlation with the CD160 expressing $CD8^{+}$ T cells, we were curious about the possible role of IL-16 in CD160 up-regulation. Therefore, we treated PBMCs with different concentrations of IL-16 (50, 100, 250, and 500 pg/ml) and stimulated them with anti-CD3/CD28 antibodies for 3 days. Then we measured the surface expression of CD160. We noted that the addition of recombinant IL-16 (500 pg/ml) increased the gene expression of CD160 in $CD8^{+}$ T cells after 72 hours (Fig. 7f). Moreover, we measured the level of IL-16 in the culture

supernatant of malignant B cells when cultured without any stimulation for 12 hours. We found that malignant B cells secreted IL-16 when compared to non-B-CLL cells (Fig. 7g). Also, we assessed the viability of non-B-CLL versus B-CLL after 12 hours' culture, which indicated cell viability of >95% (Supplementary Fig. 6d). Finally, we observed a significantly lower intracytoplasmic IL-16 in B cells of CLL-patients (Fig. 7h, 7i), which suggests B-CLL may serve as a source of IL-16.

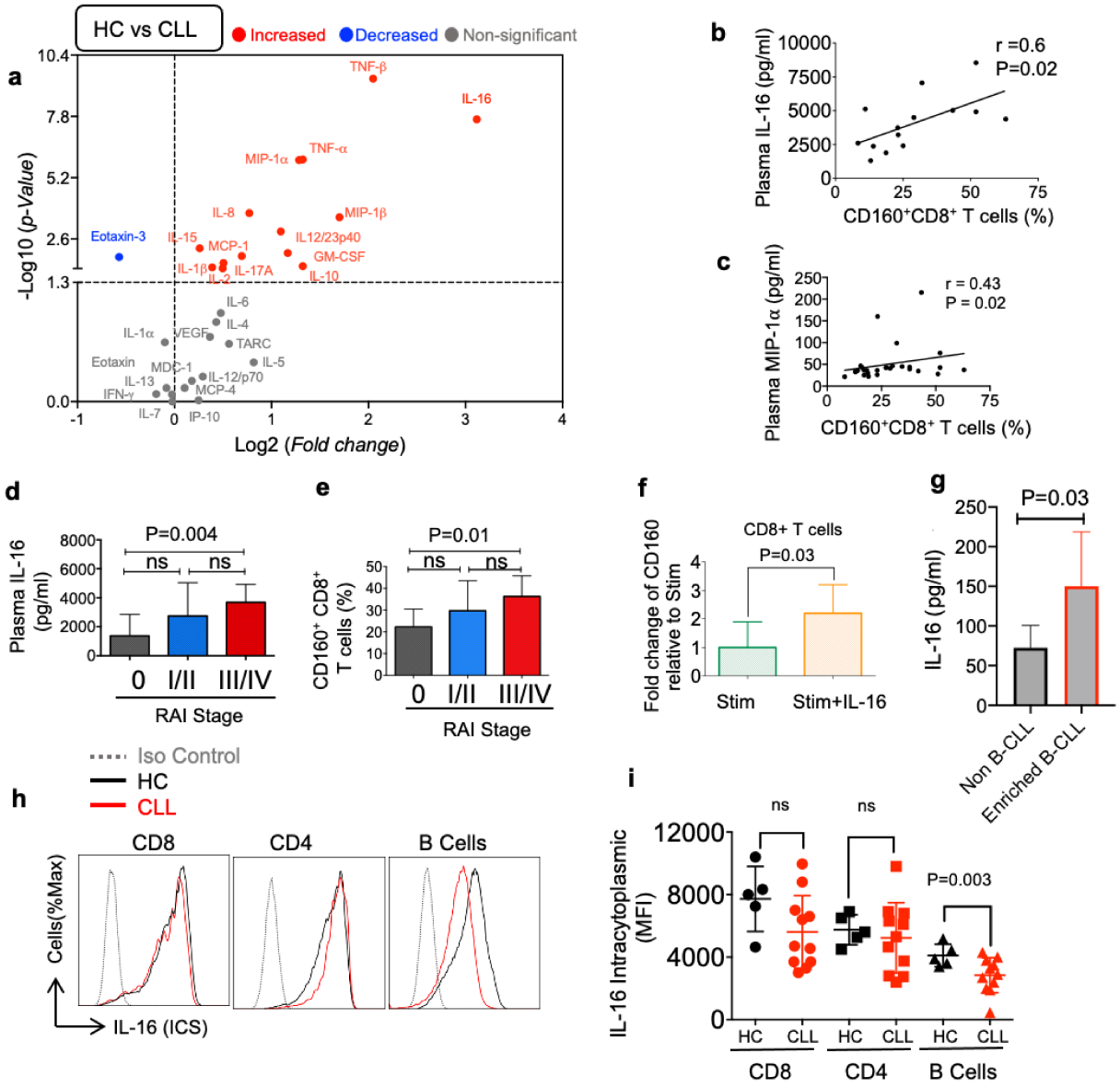


Fig. 7. The plasma cytokines/chemokines in the plasma of CLL patients versus HCs. (a) The Volcano plot illustrating the magnitude and significance of

differences in cytokine/chemokines plasma concentrations (measured by the Mesoplex assay) in CLL patients versus HCs. **(b)** Scattered plot of the correlation between percentages of $CD160^+CD8^+$ T cells in PBMCs with the IL-16, and **(c)** MIP-1α concentrations in the plasma of CLL patients. **(d)** Cumulative data showing IL-16 concentrations in the plasma of CLL patients in low (0), intermediate (I/II), and high (III/IV) RAI stages, 16, 24 and 7 patients/group respectively. **(e)** Cumulative data of percentages of $CD160^+$ expressing cells among $CD8^+$ T cells in PBMCs of CLL patients in low (0), intermediate (I/II), and high (III/IV) RAI stages, 9, 20 and 6 patients/group respectively. **(f)** Fold regulation of CD160 gene in $CD8^+$ T cells stimulated with the anti-CD3/CD28 antibodies in the absence or presence of rh-IL-16 (500 ng/ml) for 72 hours relative to stimulated as quantified by qPCR from 7 human subjects/group. **(g)** Cumulative data of IL-16 production in cell culture supernatants of isolated B-CLL vs. Non-B-CLLs after 12 hr culture as detected by ELISA from 4 patients. **(h)** Representative flow cytometry plots, and **(i**) cumulative data of intracytoplasmic IL-16 expression in $CD8^+$, $CD4^+$, and B Cells of CLL patients versus HCs.

2.4 Discussion

In this study, we found significantly higher percentages of CD160, 2B4, TIGIT, and PD-1 expressing T cells in CLL patients compared to HCs. However, a lower proportion of BTLA expressing T cells was observed, which can be explained by a decrease in naïve T cell population in CLL patients [40].

Although the frequency of $CD160^+CD8^+$ T cells was lower than $2B4^+CD8^+$ and $TIGIT^+CD8^+$ T cells in CLL patients, CD160 was associated with a prominent T cell impairment. Notably, we found that the intensity of CD160 expression was significantly higher in both T cell subsets in CLL patients compared to HCs. The role of CD160 as a co-inhibitory molecule in the induction of T cell dysfunction has been reported in chronic viral infections such as HIV [27], HCV [53], EBV, CMV [44], and in pancreatic cancer [54]. Likewise, the inhibitory nature of CD160 in T cells has been reported in the context of autoimmunity [55]. In contrast, some studies have shown a stimulatory role for CD160 in $CD8^+$ T cells in mucosal immunity and skin allograft in a mouse model [28, 29].

In our cohort, we did not find any association between the IgHV mutation and the upregulation of CD160 on T cells, however, the Trisomy-12 subjects were enriched with $CD160^{+}CD8^{+}$ T cells. These findings provide a novel and unreported, to our knowledge, insight into the relationship between the Trisomy-12 and CD160 expression. Our further characterization of $CD160^{+}$ T cells in CLL revealed that while $CD160^{+}CD8^{+}$ T cells were mainly T_{EFF}, $CD160^{+}CD4^{+}$ T cells were phenotypically heterogeneous and scattered through different T cell subsets. We also observed a higher expression of T-bet in $CD8^{+}$ T cells of CLL patients, which supports terminal differentiation of T_{EFF} cells [56]. However, a lower expression of Tcf1 mRNA in $CD8^{+}$ T cells in CLL patients may stem from a systemic inflammatory signal that suppresses Tcf1 expression in primed $CD8^{+}$ T cells or a lower number of naïve $CD8^{+}$ T cells in CLL patients. Although high EOMES expression is reported to be correlated with severe $CD8^{+}$ T cell exhaustion, we did not find a high mRNA level for EOMES in $CD8^{+}$ T cells of CLL patients. This can be explained by the complex reciprocity between IRs, T-bet, and EOMES in exhausted T cells [57]. In addition, we found that $CD160^{+}$ T cells in CLL patients had an impaired degranulation capacity as reported for mast cells [17]. This might be related to an impaired immunologic synapse formation with defective vesicle trafficking [7], which can decrease the cytotoxic ability of $CD160^{+}CD8^{+}$ T cells in CLL patients.

Similar to an exhausted phenotype, $CD160^{+}$ T cells exhibited an impaired cytokine production ability but surprisingly maintained their proliferative capability. This is in contrast with a report showing that CD160 blockade was associated with the restoration of virus-specific $CD8^{+}$ T cell proliferative capacity [27]. This discrepancy might be explained by the different status of $CD160^{+}$ T cells in a viral infection versus

CLL. Interestingly, CD160 was always co-expressed with 2B4 and highly co-expressed with TIGIT on $CD8^+$ T cells of CLL patients. We found that $2B4^+CD8^+$ T cells had a greater cytokine production ability, perforin/GzmB expression, and degranulation capacity compared to $2B4^-$ $CD8^+$ T cells in CLL patients. Interestingly, $2B4^-CD8^+$ T cells were almost devoid of perforin and GzmB expression. This demonstrates an important role for 2B4 in the cytolytic property of T cells in CLL. However, the co-expression of CD160 with 2B4 diminished their T cell effector functions. These findings indicate the dictated inhibitory role of CD160 on 2B4 expressing $CD8^+$ T cells in CLLs. Moreover, CD160 was highly co-expressed with TIGIT on $CD8^+$ T cells in CLLs. Despite previous reports on the inhibitory role of TIGIT on tumor-infiltrating $CD8^+$ T cells in follicular lymphoma [58] and multiple myeloma[59], this was not the case for CLL. Although TIGIT expression had no effects on $CD8^+$ T cells, it was associated with impaired cytokine production ability of $CD4^+$ T cells in CLL patients. Despite previous reports on the important role of TIM-3 and Gal-9 interactions in T cell exhaustion in haematological and non-haematological cancers [60,61], we observed a very low expression level of these co-inhibitory receptor/ligand in T cells of CLL patients.

Despite of the recognition of some tumor-associated antigens in CLL by $CD8^+$ T cells, identification of these cells is complex and might be inefficient due to the heterogeneity of tumor-associated antigens [62]. To overcome this limitation, we used an alternative immunophenotyping approach that enabled us to confirm that $CD160^+CD8^+$ T cells were highly antigen experienced. Adoptive T cell transfer studies have shown an enhanced antitumor efficacy in less antigen-experienced T cells [63].

This observation may explain impaired effector functions of $CD160^{+}CD8^{+}$ T cells in CLL patients as they appeared to be highly antigen experienced.

Another potential mechanism for dysfunctional $CD160^{+}CD8^{+}$ T cells in CLLs may be related to their high IL-2 and IL-15 dependent signaling which can restrict their survival and effector functions as reported for TIM-3^{+} T cells [51]. We noted spontaneous upregulation of CD160 on rested T cells *ex vivo*, as reported elsewhere [19]. Our further studies revealed that CD160 trafficking occurs via the ER/Golgi to the plasma membrane. This is in agreement with the GPI anchored protein trafficking that has been reported for CD59 [64]. GPI anchored proteins are synthesized in the ER and then selectively packaged into the coat protein complex II (COPII) vesicles and delivered via Golgi to the membrane in a clustered form partitioning in lipid rafts [65]. However, upon short-term TCR-dependent stimulation (anti-CD3/CD28) of T cells, we noted decreased surface but increased intracellular CD160, which is in contrast to one study [26] but consistent with another study [19]. Our further observations showed increased surface CD160 expression following prolonged T cell activation (6-days) which is in agreement with the concept of chronic antigenic stimulation and the upregulation of co-inhibitory receptors [66].

Intriguingly, for the very first time, we detected a high CD160 content in plasma derived EVs of CLL patients. Moreover, we found that $CD160^{+}$ EVs can be taken up by T cells and therefore, there is a possibility to propose that $CD160^{+}$ EVs serve as a potential source of CD160. However, it's unclear whether uptake of $CD160^{+}$EVs influences T cell effector functions. The inhibitory signal of PDL-1^{+}EVs with T cells expressing PD-1 has been reported [67]. Therefore, how these $CD160^{+}$EVs impact

the interaction of T cells with CD160 ligands is unknown and merits further investigation.

Cytokines and chemokines are other factors that can negatively influence T cell effector functions [48,67]. We observed a significant increase in pro-inflammatory cytokines (IL-1β, TNF-α) and Th_1 type cytokines (IL-2, TNF-α, TNF-β, IL-12/23p40, GM-CSF) in CLL patients. However, Th_2 type cytokines, IL-12/p70, IL-13, and IFN-γ were remained unchanged, inconsistent with other studies [52]. The dominancy of a pro-inflammatory cytokine profile in our study could be explained by the characteristics of our cohort that include mainly patients that were treatment naïve rather than treated (64.9% vs. 35%) and in the early stages (low/intermediate) of the disease (Supplementary Fig. 6e). In our study, CLL patients had elevated plasma IL-10 which is consistent with a previous study [69]. IL-10 prolongs B-CLL survival and reduces the generation of effector $CD4^+$ and $CD8^+$ T cells [70].

We also observed the elevation of chemokines [71] such as MIP-1α, MIP-1β, MCP-1, and IL-8 that can contribute to the survival of neoplastic B cells by maintaining various anti-apoptotic mechanisms [71,72]. For instance, MIP-1α is secreted by B-CLL cells and induces the recruitment of macrophage-lineage cells promoting the initiation of the leukemia niche [74]. Interestingly, we observed a positive relationship between percentages of $CD160^+CD8^+$ T cells and the plasma MIP-1α plasma concentrations. Although we were unable to investigate the source/role of these elevated chemokines, there is evidence that B-CLL cells constitutively express IL-8 [75] and it exhibits immunosuppressive on $CD8^+$ T cells in cancer [76]. Finally, we found a dramatic rise in IL-16 levels in the plasma of CLL patients compared to HCs that is consistent with another study [77]. IL-16 has been identified as a

chemotactic factor for $CD4^+$ T cells that binds to CD4 and CD9 receptors on T cells [78]. Similarly, in multiple myeloma, a strong expression of IL-16 in the BM that prolongs the survival of malignant cells has been reported [79]. Although we were unable to identify the main source of IL-16, our observations suggest that B-CLL cells could be considered as one of the potential sources of this cytokine in CLL patients. Importantly, we found a significant correlation between IL-16 levels with the cancer stage. This suggests that the plasma IL-16 could be used as a potential prognostic marker in CLL. However, further investigation on larger cohorts is required for the validity of our finding. Moreover, we found a positive correlation between the plasma IL-16 concentrations and percentages of $CD160^+CD8^+$ T cells, which suggests an intricate mechanism that calls for further investigations. Although our study provided a novel insight into the role of co-inhibitory receptors, in particular, CD160 in CLL, further studies in larger cohorts are warranted.

We are aware of multiple study limitations such as the lack of IgHV mutation results and the FISH analysis data for all our patients. Also, the differential proportion of T cell subpopulations (e.g., native, memory) in CLL patients versus HCs may influence our data regarding the expression of T-bet, EOMES, and Tcf1 expression in T cells. Obtaining the bone marrow aspirate from HCs was not possible in our studies, therefore, comparing immunological changes in the BM of HCs versus CLLs may assist in understanding the role of CD160 in the BM. Unfortunately, we were unable to obtain the whole blood cell count from HCs, therefore, there is a possibility that a large population of malignant B cells in CLL patients influences the T cell count reported in PBMCs of CLL patients.

2.5 Conclusions

Our data highlights the important role of CD160 in T cell exhaustion in CLL patients. In particular, the co-expression of CD160 with other co-inhibitory receptors dictates more impaired T cell effector function. Also, the abundance of IL-16 in the plasma of CLL patients and its impact on the upregulation of CD160 provides a novel insight into the mechanism underlying CD160 overexpression in CLL patients. The correlation of the plasma IL-16 levels with the Rai stage suggests that IL-16 could be used as a prognostic biomarker in CLL patients. Therefore, further studies on larger cohorts are warranted to determine whether targeting IL-16 and/or CD160 would have clinical implications in hematological cancers. Taken together, our findings provide a novel insight into the inhibitory role of CD160 alone and a synergistic inhibitory effect when co-expressed with other co-inhibitory receptors on T cells in CLL patients.

2.6 Ethics approval and consent to participate

This study was reviewed and approved by the Health Research Ethics Board of Alberta, Cancer Committee (HREBA#CC-17-0307). Also, recruitment of healthy controls was approved by the HREBA with protocol #Pro00063463. All participants provided informed written consent to participate in this study.

2.7 Acknowledgments

We thank all the volunteers who supported this study by donating their samples and dedicating their time, and the staff at the CLL clinic and medical laboratory at the Cross-Cancer Institute (CCI) for sample collection. We also would like to thank the flow core facility of the Faculty of Medicine and Dentistry, at the University of Alberta for supporting the study.

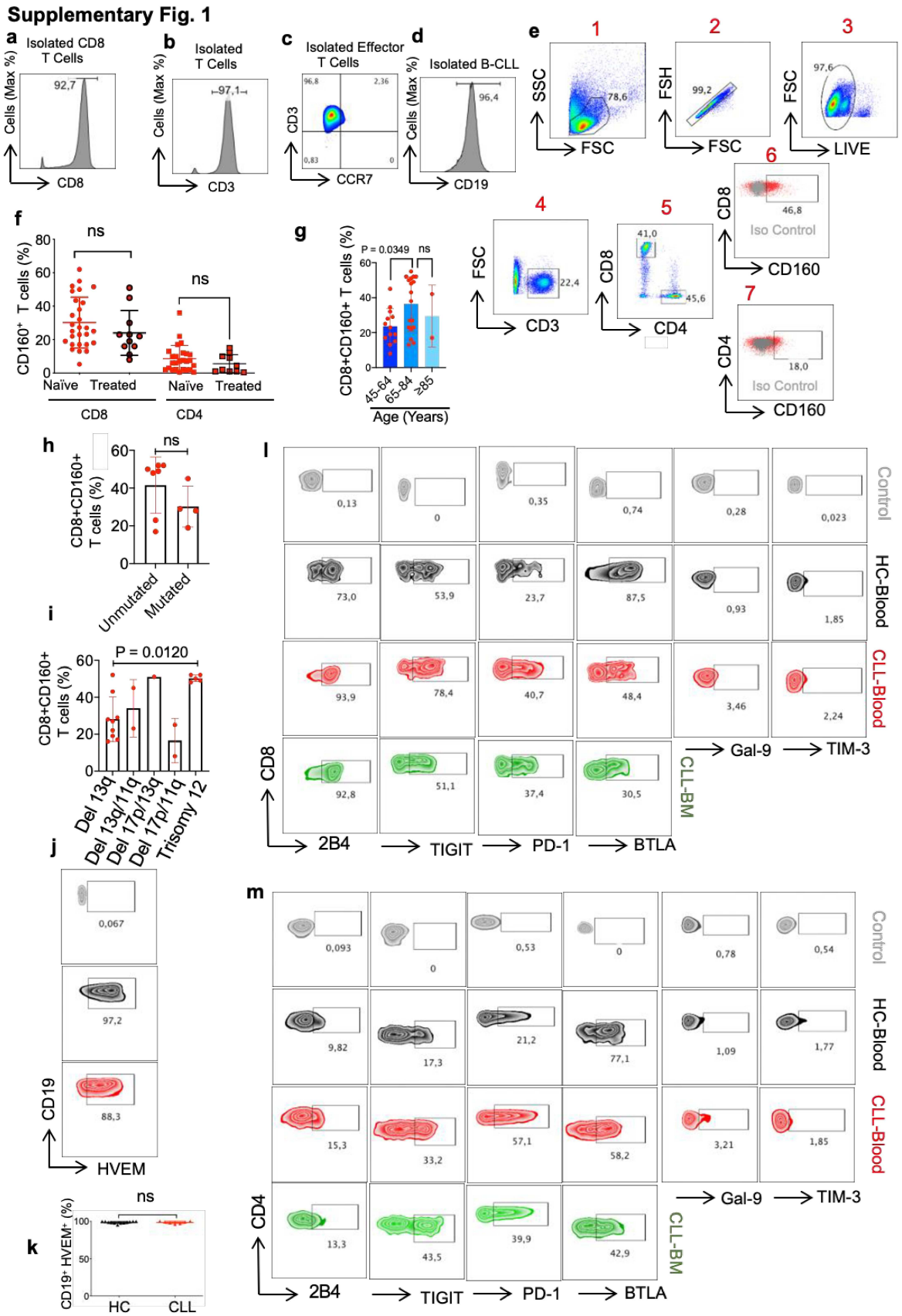
2.8 Supplementary Tables

No	Age (Yrs)	Sex	IgHV Mutation	FISH Analysis	Stage (Rai)	Treatment
1	70	M	Not done, ZAP70(+)	Del13q/11q	I	T
2	66	M	Not done, ZAP70(-)	Not done	II	N
3	77	M	Not done	Del17p/13q	III	T
4	76	M	Not done, ZAP70(-)	Del17p/13q	IV	T
5	72	M	Unmutated	Trisomy 12	II	N
6	51	F	Not done	Not done	I	N
7	54	M	Unmutated	Del13q	0	N
8	68	M	Not done	Del13q	II	N
9	75	M	Not done, ZAP70(-)	Del13q/17p (6.5%)	0	T
10	60	F	Not done, ZAP70(-)	Not done	0	N
11	86	F	Not done	Not done	0	N
12	85	M	Not done	N/A	0	N
13	78	F	Not done	Del13q	0	N
14	61	M	Not done	Not done	I	N
15	81	M	Not done	Not done	II	N
16	62	M	Not done	Del11q/17p	IV	T
17	77	M	Not done	Not done	0	N
18	80	F	Not done	Not done	I	N
19	63	M	Mutated ZAP70(+)	Del13q	III	N
20	65	M	Unmutated	Del 13q	II	N
21	73	M	Unmutated	Normal	I	N
22	82	M	Not done	Not done	III	T
23	74	M	Not done	Del13q	IV	T
24	71	F	Not done	Not done	IV	T
25	78	F	Not done	Not done	I	N
26	71	F	Not done	Del13q	0	T
27	62	M	Not done	Del17p Del11q	I	T
28	63	M	Not done	Normal	I	T

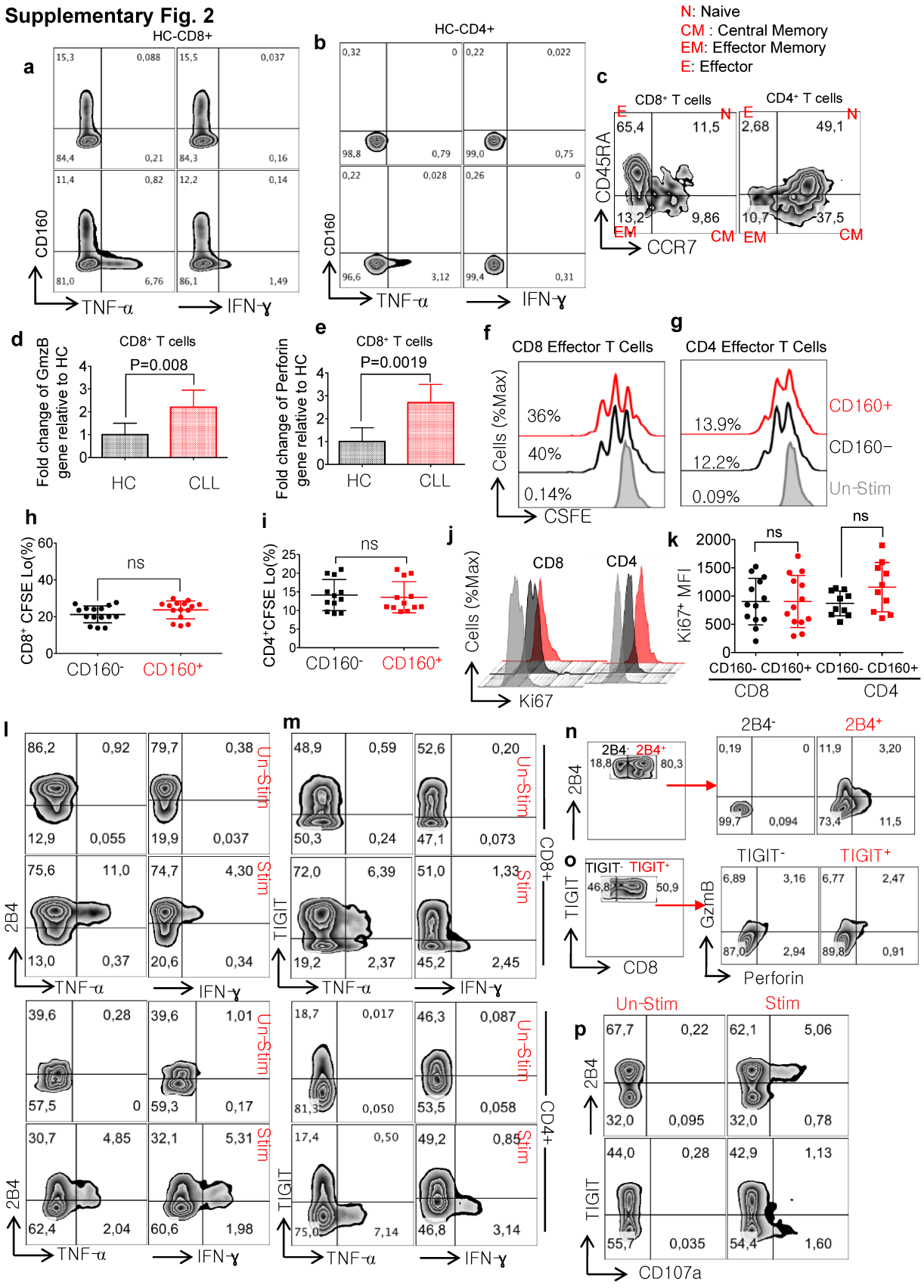
29	74	M	Unmutated	Del11q Del17p	0	N
30	70	M	Unmutated	Trisomy 12	I	N
31	63	F	Not done	Not done	0	N
32	68	M	Unmutated	Del11q Del13q	IV	T
33	53	M	Mutated	Del13q	II	N
34	54	M	Not done	Normal	II	N
35	71	M	Not done	Normal	II	T
36	91	F	Not done	Not done	I	T
37	68	M	Not done	Not done	II	N
38	72	M	Not done	Del13q Trisomy12	IV	T
39	63	M	Mutated	Del13q	II	T
40	65	F	Not done	Not done	0	N
41	55	F	Not done	Not done	0	N
42	59	M	Indeterminate	Normal	II	N
43	74	F	Not done	Del13q	I	N
44	79	F	Not done	Del13q	I	T
45	70	M	Unmutated	Trisomy12	III	N
46	56	F	Mutated	Del13q Del11q	III	N
47	72	M	Not done	Normal (2019) Del11q GAIN	IV	N
48	45	F	Mutated	Del13q	II	N
49	60	F	Indeterminate	Trisomy12	II	N
50	76	M	Unmutated	Trisomy12	I	N
51	74	M	Unmutated	Del13q Trisomy12	I	N
52	64	M	Mutated	Not done	0	T
53	64	M	Not done	Del13q	0	N
54	67	F	Not done	Not done	0	N
55	60	M	Not done	Not done	I	N
56	77	M	N/A	Del11q	I	T

Supplementary Table 1. Patients' characteristics. Sex: (M: Male, F: Female), Staging: based on Rai staging system, Treatment: Refers to the treatment condition when sample received for research (N: Naïve, T: Treated) N/A: Not Available.

2.9 Supplementary Figures

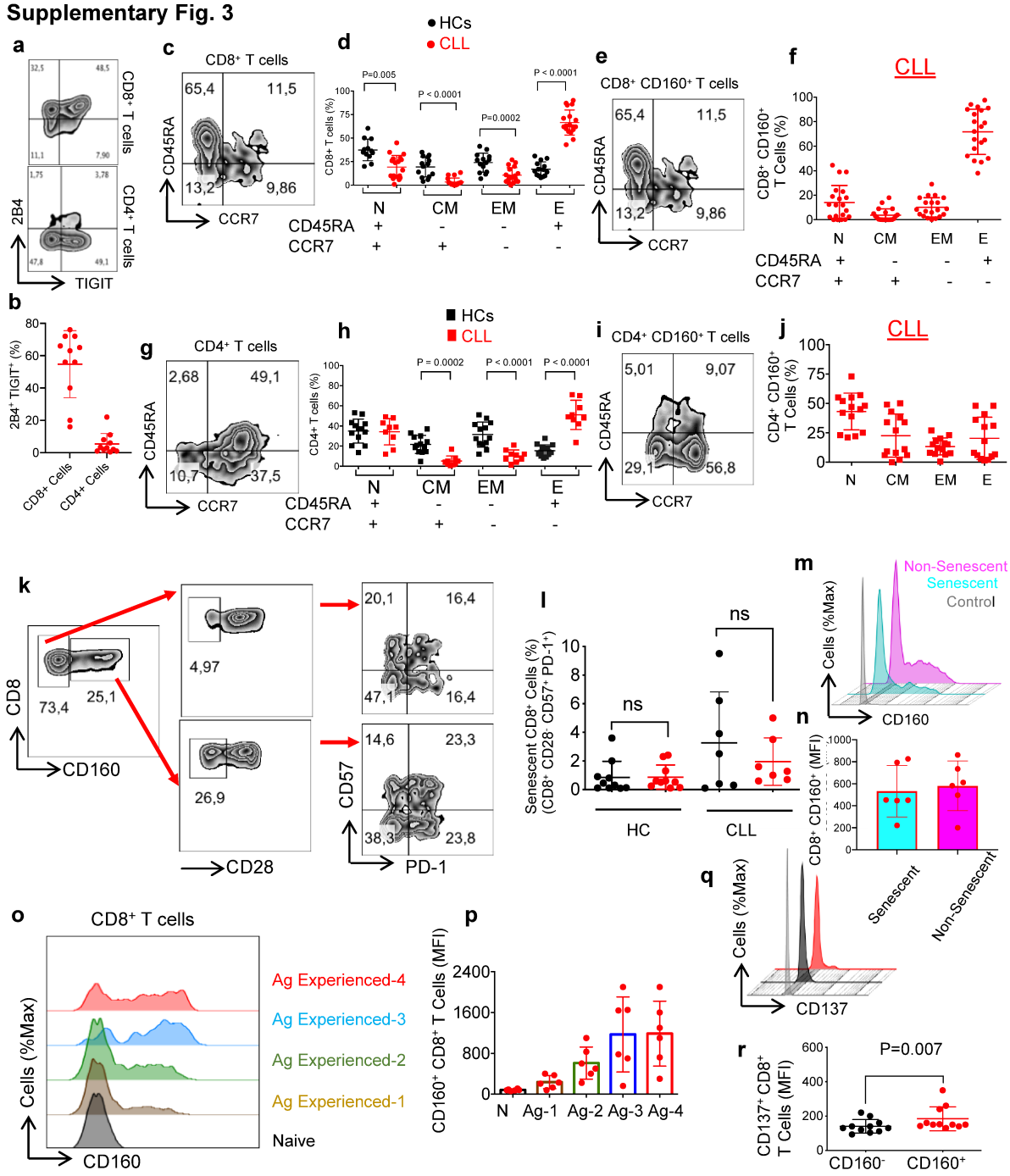

Supplementary Fig. 1
a
Isolated CD8 T Cells
Cells (Max %)
92,7
CD8
b
Isolated T Cells
Cells (Max %)
97,1
CD3
c
Isolated Effector T Cells
96,8
2,36
0,83
0
CD3
CCR7
d
Isolated B-CLL
Cells (Max %)
96,4
CD19
e
1
SSC
78,6
FSC
2
FSH
99,2
FSC
3
FSC
97,6
LIVE
6
CD8
46,8
Iso Control
CD160
4
FSC
22,4
CD3
5
CD8
41,0
45,6
CD4
7
CD4
18,0
Iso Control
CD160
f
CD160+ T cells (%)
ns
ns
Naïve
Treated
Naïve
Treated
CD8
CD4
g
CD8+CD160+ T cells (%)
P = 0.0349
ns
45-64
65-84
≥85
Age (Years)
h
CD8+CD160+ T cells (%)
ns
Unmutated
Mutated
i
CD8+CD160+ T cells (%)
P = 0.0120
Del 13q
Del 13q/11q
Del 17p/13q
Del 17p/11q
Trisomy 12
j
0,067
97,2
88,3
CD19
HVEM
k
CD19+ HVEM+ (%)
ns
HC
CLL
l
0,13
0
0,35
0,74
0,28
0,023
Control
73,0
53,9
23,7
87,5
0,93
1,85
HC-Blood
93,9
78,4
40,7
48,4
3,46
2,24
CLL-Blood
92,8
51,1
37,4
30,5
CLL-BM
CD8
2B4
TIGIT
PD-1
BTLA
Gal-9
TIM-3
m
0,093
0
0,53
0
0,78
0,54
Control
9,82
17,3
21,2
77,1
1,09
1,77
HC-Blood
15,3
33,2
57,1
58,2
3,21
1,85
CLL-Blood
13,3
43,5
39,9
42,9
CLL-BM
CD4
2B4
TIGIT
PD-1
BTLA
Gal-9
TIM-3

Supplementary Fig. 1 (a) Representative flow cytometry purity plots of isolated $CD8^+$ T cells, **(b)** $CD3^+$ T cells, **(c)** $CD8^+$ effector T cells ($CD3^+CCR7^-CD8^+$), and **(d)** B-CLLs. **(e)** Flow cytometry gating strategy for CD160+$CD8^+$ and $CD4^+$ T cells in CLL patients. **(f)** Cumulative data showing CD160 expression on $CD8^+$ and $CD4^+$ T cells in treatment naïve versus treated CLL patients. **(g)** Cumulative data of percentages of $CD160^+CD8^+$ in different age groups of HCs. **(h)** Cumulative data of percentages of $CD8^+CD160^+$ in CCL patients with or without IgHV mutation. **(i)** Cumulative data of the FISH analysis in CLL patients. **(j)** Representative flow cytometry plots, and **(k)** cumulative data of HVEM expression in B-CLL versus HCs B cells. **(l)** Representative flow cytometry plots of 2B4, TIGIT, PD-1, BTLA, Gal-9 and TIM-3 expression in $CD8^+$, and **(m)** $CD4^+$ T cells in blood of HCs versus blood and bone marrow of CLL patients. Each dot represents data from a human subject ± SD.

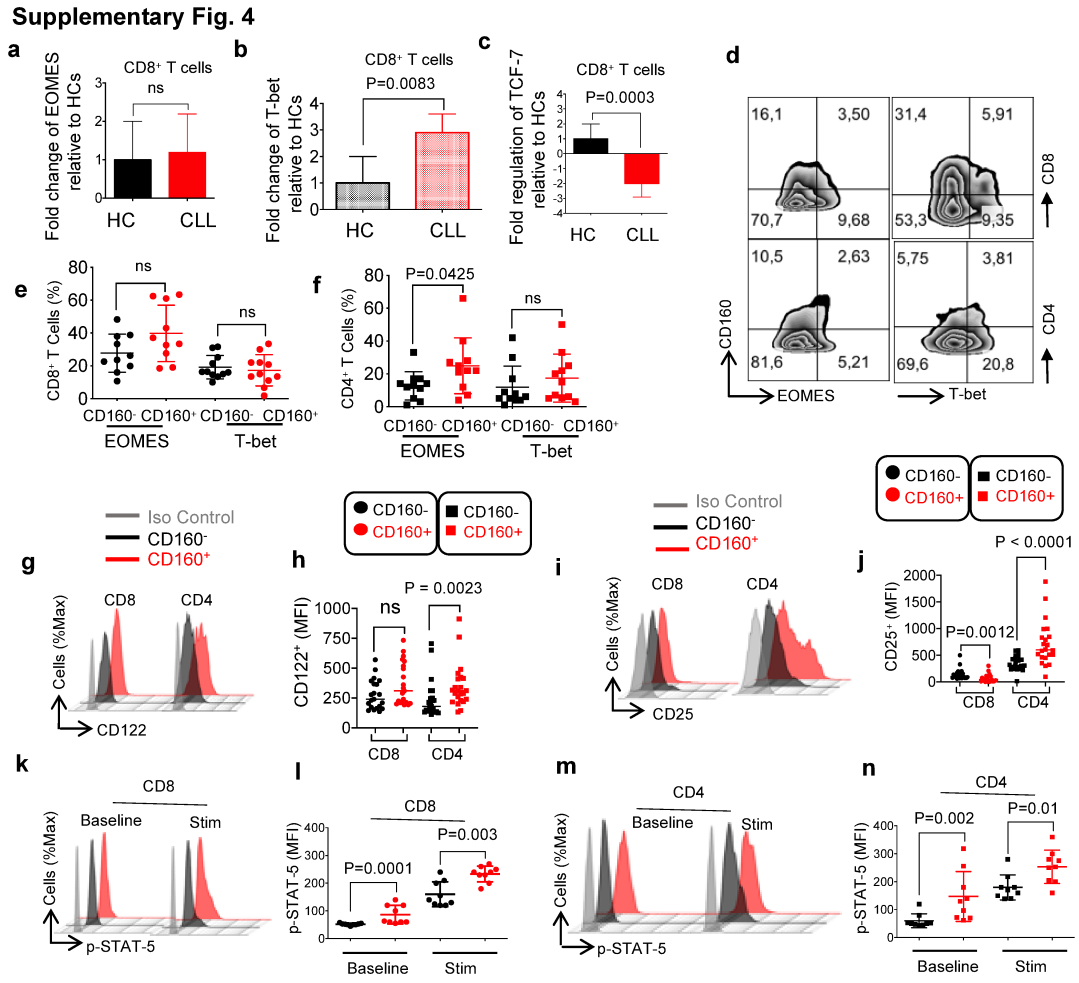

Supplementary Fig. 2
N: Naive
CM : Central Memory
EM: Effector Memory
E: Effector
a
HC-CD8+
CD160
TNF-α
IFN-γ
b
HC-CD4+
c
CD8+ T cells
CD4+ T cells
CD45RA
CCR7
d
CD8+ T cells
P=0.008
Fold change of GmzB gene relative to HC
HC
CLL
e
CD8+ T cells
P=0.0019
Fold change of Perforin gene relative to HC
f
CD8 Effector T Cells
CD4 Effector T Cells
Cells (%Max)
CSFE
CD160+
CD160−
Un-Stim
g
h
CD8+ CFSE Lo(%)
ns
CD160-
CD160+
i
CD4+CFSE Lo(%)
j
CD8
CD4
Ki67
k
Ki67+ MFI
CD160- CD160+ CD160- CD160+
CD8
CD4
l
2B4
TIGIT
Un-Stim
Stim
m
CD8+
CD4+
n
2B4-
2B4+
o
TIGIT-
TIGIT+
GzmB
Perforin
p
CD107a

Supplementary Fig. 2 (a) Representative plots of TNF-α and IFN-γ expression in $CD8^+CD160^+$ versus $CD8^+CD160^-$ T cells in HCs. **(b)** Representative plots of TNF-α and IFN-γ expression in $CD4^+CD160^+$ versus $CD4^+CD160^-$ T cells in a HC. **(c)** Representative flow plots of the gating strategy for T cell differential subsets in $CD8^+$ and $CD4^+$ cells. **(d)** Fold regulation of GzmB, and **(e)** perforin mRNA in $CD8^+$ T cells of CLLs relative to HCs blood, data are rom 6 individuals/group**. (f)** Representative plots of proliferation (CFSE lo) in $CD160^-$ and $CD160^+$ either $CD8^+$, or **(g)** $CD4^+$ T cells. **(h)** Cumulative data of proliferation (CFSE lo) in $CD160^-$ and $CD160^+$ either $CD8^+$, or **(i)** $CD4^+$ T cells. **(j)** Representative histograms, and **(k)** Cumulative data of Ki67 expression in $CD8^+$ and $CD4^+$ T cells in CLL patients. **(l)** Representative flow cytometry plots of TNF-α and IFN-γ expression in $2B4^-/2B4^+$ either $CD8^+$ or $CD4^+$ T cells. **(m)** Representative flow cytometry plots of TNF-α and IFN-γ expression in $TIGIT^-/TIGIT^+$ either $CD8^+$ or $CD4^+$ T cells. **(n)** Representative flow cytometry plots of GzmB and Perforin expression in $2B4^-/2B4^{+,}$ and **(o)** $TIGIT^-/TIGIT^+$ $CD8^+$ T cells. **(p)** Representative flow cytometry plots of CD107a expression in $2B4^-/2B4^+$ and $TIGIT^-/TIGIT^+$ $CD8^+$ T cells. Each dot represents data from a human subject ± SD.

Supplementary Fig. 3



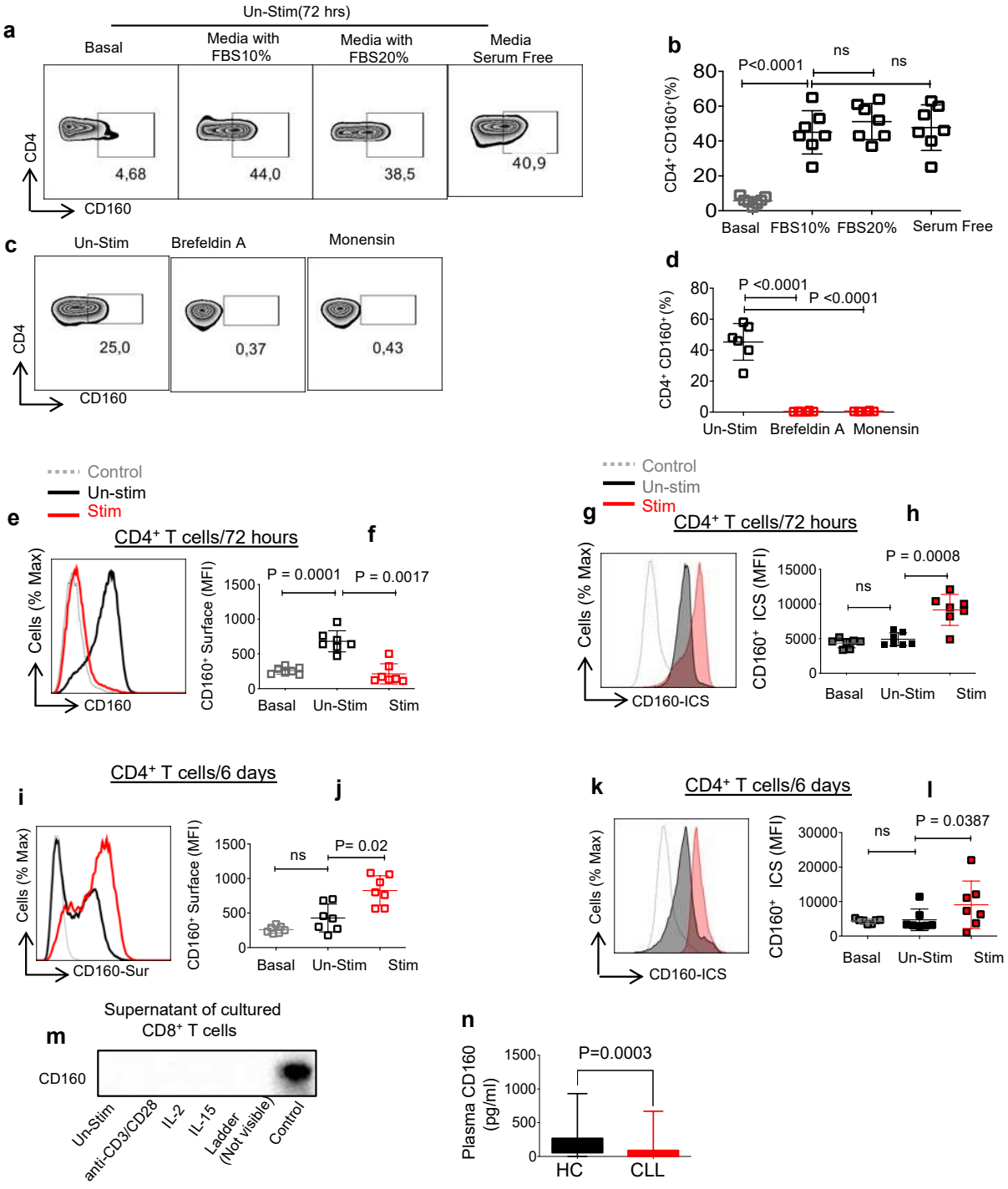
Supplementary Fig. 3 (a) Representative plots, and **(b)** cumulative data of 2B4 and TIGIT co-expression in $CD8^+$ and $CD4^+$ T cells of CLL patients. **(c)** Representative flow cytometry plot of CD45RA and CCR7 expression in $CD8^+$ T cells in the blood of CLLs**. (d)** Cumulative data showing expression of different subsets of $CD8^+$ T cells in CLLs (N: Naive, CM: Central memory, EM: Effector memory, E: Effector) vs HCs. **(e)** Representative flow cytometry plot of the expression of CD45RA and CCR7 in $CD160^+CD8^+$ T cells. **(f)** Cumulative data showing differential subset distribution of $CD160^+$ $CD8^+$ T cells in CLL patients. **(g)** Representative flow cytometry plot of CD45RA and CCR7 expression in $CD4^+$ T cells in CLLs**. (h)** Cumulative data showing the expression of different subsets of $CD4^+$ T cells in CLLs (N: Naive, CM: Central memory, EM: Effector memory, E: Effector) vs. HCs. **(i)** Representative flow cytometry plot of expression of CD45RA and CCR7 in $CD160^+$ $CD4^+$ T cells. **(j)** Cumulative data showing differential subset distribution of $CD160^+$ $CD4^+$ T cells. **(k)** Representative flow plots, and **(l)** cumulative data of CD28, CD57, PD-1 expression in $CD160^+$ $CD8^+$ T cells ($CD28^-$ $CD57^+$ $PD\text{-}1^+$ considered as senescent cells). **(m)** Histogram plots, and **(n)** cumulative data showing the mean fluorescence intensity (MFI) of senescent cells ($CD28^-CD57^+PD\text{-}1^+$) in $CD160^+CD8^+$ T cells. **(o)** Representative flow cytometry plot of percent expression of $CD160^+$ in Naive ($CD27^+CD28^+CD45RA^+CCR7^+CD57^-$), Ag experienced level-1 ($CD27^+$ $CD28^+CD45RA^-$ $CCR7^+$ $CD57^-$), Ag experienced level-2 ($CD27^+CD28^+CD45RA^-CCR7^-CD57^{+/-}$), Ag experienced level-3 ($CD27^+CD28^-CD45RA^{+/-}CCR7^-CD57^{+/-}$) and Ag experienced level-4 ($CD27^-CD28^-CD45RA^-CCR7^-CD57^+$) subsets of $CD8^+$ T cells. **(p)** Quantification of MFI of $CD160^+$ in Naive ($CD27^+CD28^+CD45RA^+CCR7^+CD57^-$), Ag experienced level-1 ($CD27^+CD28^+CD45RA^-CCR7^+CD57^-$), Ag experienced level-2 ($CD27^+CD28^+CD45RA^-CCR7^-CD57^{+/-}$), Ag experienced level-3 ($CD27^+CD28^-$ $CD45RA^{+/-}CCR7^-CD57^{+/-}$) and Ag experienced level-4 ($CD27^-CD28^-CD45RA^-CCR7^-$ $CD57^+$) subsets of $CD8^+$ T cells. **(q)** Representative flow cytometry plot, and **(r)** quantification of MFI of $CD137+CD160^-/CD160^+$ $CD8^+$ T cells. Each dot represents data from a human subject ± SD.



Supplementary Fig. 4 (a) Expression of EOMES mRNA in CD8$^+$ T cells of CLLs relative to HCs. **(b)** Expression of T-bet mRNA in CD8$^+$ T cells of CLLs relative to HCs. **(c)** Expression of TCF-7 mRNA in CD8$^+$ T cells of CLLs relative to HCs. **(d)** Representative flow cytometry plots of EOMEs and T-bet expression in CD160$^-$/CD160$^+$ CD8$^+$ and CD4$^+$ T cells. **(e)** Cumulative data showing percent expression of EOMEs and T-bet in CD160$^-$/CD160$^+$ CD8$^+$ T cells. **(f)** Cumulative data showing percent expression of EOMEs and T-bet in CD160$^-$/CD160$^+$ CD4$^+$ T cells. **(g)** Representative flow cytometry plots of CD122 (IL-2Rβ) expression in CD160$^-$/CD160$^+$ CD8$^+$ and CD4$^+$ T cells**. (h)** Quantification of MFI for CD122 in CD160$^-$/CD160$^+$ CD8$^+$ and CD4$^+$ T cells. **(i)** Representative flow cytometry plots of CD25 (IL-2Rα) expression in CD160$^-$/CD160$^+$ CD8$^+$ and CD4$^+$ T cells. **(j)** Quantification of MFI for CD25 in CD160$^-$/CD160$^+$ CD8$^+$ and CD4$^+$ T cells. **(k)** Representative flow cytometry plots of pSTAT-5 expression in CD160$^-$/CD160$^+$ CD8$^+$ T cells at the baseline and after *in vitro* stimulation with recombinant human IL-2 (100 IU/ml). **(l)** Quantification of MFI of pSTAT-5 in CD160$^-$/CD160$^+$ CD8$^+$ T cells at the baseline and after *in vitro* stimulation with recombinant human IL-2 (100 IU/ml). **(m)** Representative flow cytometry plots of pSTAT-5 expression in CD160$^-$/CD160$^+$ CD4$^+$ T cells at the baseline and after *in vitro* stimulation with recombinant human IL-2 (100 IU/ml). **(n)** Quantification of MFI for pSTAT-5 in CD160$^-$/CD160$^+$ CD4$^+$ T cells

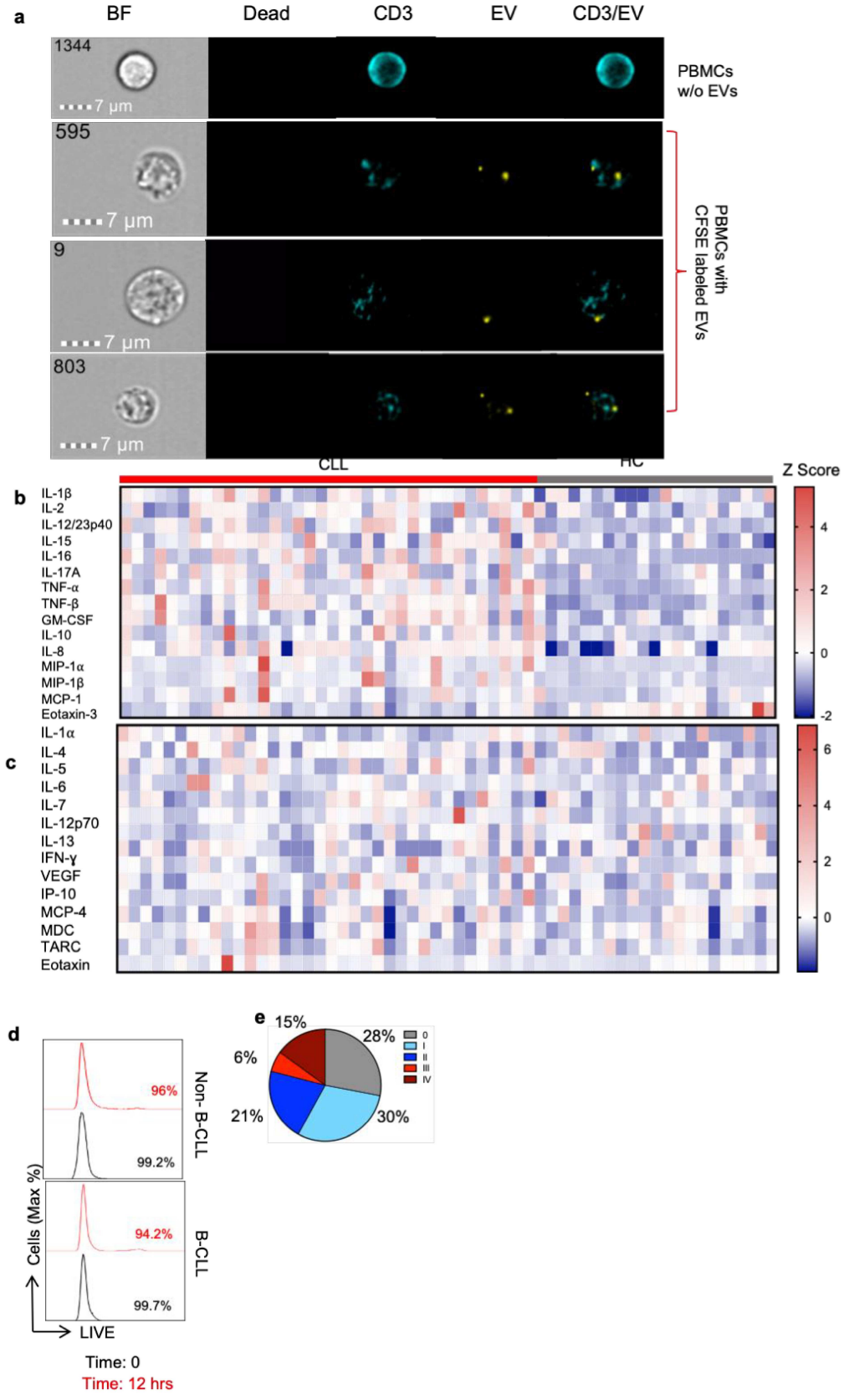
at the baseline and after *in vitro* stimulation with recombinant human IL-2 (100 IU/ml). Each dot represents data from a human subject ± SD, cumulative data in a-c are from 6 human subjects/group.

Supplementary Fig. 5



Supplementary Fig. 5 (a) Representative flow plots, and **(b)** cumulative data of CD160 expression at the baseline and following culture in RPMI media supplemented with FBS (10% and 20%) or serum free for 72 hours. **(c)** Representative flow plots, and **(d)** cumulative data of CD160 expression on CD4 T cells after 16 hours culture with supplementation of brefeldin A or Monensin. **(e)** Representative histogram, and **(f)** cumulative data of surface CD160 expression on $CD4^+$ T cells after 72 hours stimulation (Stim) with the anti-CD3/CD28 compared to unstimulated cells (un-Stim). **(g)** Representative histogram, and **(h)** and cumulative data of intra-cytoplasmic CD160 expression in $CD4^+$ T cells after 72 hours stimulation with the anti-CD3/CD28 compared to Un-Stim. **(i)** Representative histogram, and **(j)** cumulative data of surface CD160 expression on $CD4^+$ T cells after 6 days of stimulation with the anti-CD3/CD28. **(k)** Representative histogram, and **(l)** cumulative data of intracytoplasmic CD160 expression in $CD4^+$ T cells after 6 days of stimulation stimulated with the anti-CD3/CD28 compared to Un-Stim. Each dot represents data from a human subject ± SD. **(m)** The blot is showing CD160 protein expression in the culture supernatant from PBMCs of a CLL patient either un-stim or stimulated with the anti-CD3/CD28, IL-2, and IL-5 compared to the positive control (cell pellet) measured by the Western blotting. **(n)** Data of quantification of the plasma CD160 in HCs versus CLL patients measured by ELISA. Each dot represents data from a human subject/single experiment ± SD, cumulative data in **(n)** are from 23 HCs and 41 CLL human subjects.

Supplementary Fig. 6



Supplementary Fig. 6. (a) Image Stream plots of Evs uptake by live T cells following overnight culture of isolated and CFSE-labeled of EVs from a CLL patient with PBMCs of a HC compared with PBMCs cultured without (w/o) EVs but co-cultured with CFSE-labeled autologous B cells as control. Bright field (BF). **(b, c)** The heatmap showing differentially detected cytokines and chemokines in the plasma of CLLs and HCs. Each row represents one of the cytokines and each column represents a sample. The color scale calculated according to Z-score and means cytokine concentration standard deviation from the mean with red for the high expression and blue for the low expression levels. **(d)** Representative plots showing viability of non-B-CLL and B-CLL cells before and after 12 hr culture. **(e)** Pie chart showing percentages of patients in different RAI stages of CLL in our cohort.

2.10 References

1. Seifert M, Sellmann L, Bloehdorn J, Wein F, Stilgenbauer S, Dürig J, Küppers R. Cellular origin, and pathophysiology of chronic lymphocytic leukemia. J Exp Med. 2012 Nov 19;209(12):2183–98.

2. Scarfò L, Ferreri AJM, Ghia P. Chronic lymphocytic leukaemia. Crit Rev Oncol Hematol. 2016 Aug; 104:169–82.

3. Arruga F, Gyau BB, Iannello A, Vitale N, Vaisitti T, Deaglio S. Immune Response Dysfunction in Chronic Lymphocytic Leukemia: Dissecting Molecular Mechanisms and Microenvironmental Conditions. Int J Mol Sci [Internet]. 2020 Mar 6 [cited 2020 Oct 13];21(5). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7084946/

4. Christopoulos P, Pfeifer D, Bartholomé K, Follo M, Timmer J, Fisch P, Veelken H. Definition, and characterization of the systemic T-cell dysregulation in untreated indolent B-cell lymphoma and very early CLL. Blood. 2011 Apr 7;117(14):3836–46.

5. Porakishvili N, Roschupkina T, Kalber T, Jewell AP, Patterson K, Yong K, Lydyard PM. Expansion of CD4+ T cells with a cytotoxic phenotype in patients with B-chronic lymphocytic leukaemia (B-CLL). Clin Exp Immunol. 2001 Oct;126(1):29–36.

6. Ramsay AG, Clear AJ, Fatah R, Gribben JG. Multiple inhibitory ligands induce impaired T-cell immunologic synapse function in chronic lymphocytic leukemia that can be blocked with lenalidomide: establishing a reversible immune evasion mechanism in human cancer. Blood. 2012 Aug 16;120(7):1412–21.

7. Ramsay AG, Johnson AJ, Lee AM, Gorgün G, Le Dieu R, Blum W, Byrd JC, Gribben JG. Chronic lymphocytic leukemia T cells show impaired immunological synapse formation that can be reversed with an immunomodulating drug. J Clin Invest. 2008 Jul;118(7):2427–37.

8. Okoye IS, Houghton M, Tyrrell L, Barakat K, Elahi S. Coinhibitory Receptor Expression, and Immune Checkpoint Blockade: Maintaining a Balance in CD8+ T Cell Responses to Chronic Viral Infections and Cancer. Front Immunol. 2017; 8:1215.

9. Wherry EJ. T cell exhaustion. Nat Immunol [Internet]. 2011 Jun [cited 2020 Oct 13];12(6):492–9. Available from: https://www.nature.com/articles/ni.2035

10. McLane LM, Abdel-Hakeem MS, Wherry EJ. CD8 T Cell Exhaustion During Chronic Viral Infection and Cancer. Annu Rev Immunol. 2019;37(1):457–95.

11. Palma M, Gentilcore G, Heimersson K, Mozaffari F, Näsman-Glaser B, Young E, Rosenquist R, Hansson L, Österborg A, Mellstedt H. T cells in chronic lymphocytic leukemia display dysregulated expression of immune checkpoints and activation markers. Haematologica. 2017;102(3):562–72.

12. Riches JC, Davies JK, McClanahan F, Fatah R, Iqbal S, Agrawal S, Ramsay AG, Gribben JG. T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. Blood [Internet]. 2013 Feb 28 [cited 2020 Oct 13];121(9):1612–21. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3587324/

13. Hanna BS, Roessner PM, Yazdanparast H, Colomer D, Campo E, Kugler S, Yosifov D, Stilgenbauer S, Schmidt M, Gabriel R, Lichter P, Seiffert M. Control of chronic lymphocytic leukemia development by clonally-expanded CD8+ T-cells that undergo functional exhaustion in secondary lymphoid tissues. Leukemia. 2019;33(3):625–37.

14. Gassner FJ, Zaborsky N, Catakovic K, Rebhandl S, Huemer M, Egle A, Hartmann TN, Greil R, Geisberger R. Chronic lymphocytic leukaemia induces an exhausted T cell phenotype in the TCL1 transgenic mouse model. Br J Haematol. 2015 Aug;170(4):515–22.

15. Maïza H, Leca G, Leca G, Schiavon V, Boumsell L, Bensussan, A. A novel 80-kD cell surface structure identifies human circulating lymphocytes with natural killer activity. J Exp Med [Internet]. 1993 Sep 1 [cited 2020 Oct 13];178(3):1121–6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2191151/

16. Tsujimura K, Obata Y, Matsudaira Y, Nishida K, Akatsuka Y, Ito Y, Demachi-Okamura A, Kuzushima K, Takahashi T. Characterization of murine CD160+ CD8+ T lymphocytes. Immunol Lett. 2006 Jul 15;106(1):48–56.

17. Ortonne N, Ram-Wolff C, Giustiniani J, Marie-Cardine A, Bagot M, Mecheri S, Bensussan A. Human and mouse mast cells express and secrete the GPI-anchored isoform of CD160. J Invest Dermatol. 2011 Apr;131(4):916–24.

18. Farren TW, Giustiniani J, Liu F-T, Tsitsikas DA, Macey MG, Cavenagh JD, Oakervee HE, Taussig D, Newland AC, Calaminici M, Bensussan A, Jenner M, Gribben JG, Agrawal SG. Differential and tumor-specific expression of CD160 in B-cell malignancies. Blood. 2011 Aug 25;118(8):2174–83.

19. El-Far M, Pellerin C, Pilote L, Fortin J-F, Lessard IAD, Peretz Y, Wardrop E, Salois P, Bethell RC, Cordingley MG, Kukolj G. CD160 isoforms and regulation of CD4 and CD8 T-cell responses. J Transl Med. 2014 Sep 2; 12:217.

20. Giustiniani J, Marie-Cardine A, Bensussan A. A Soluble Form of the MHC Class I-Specific CD160 Receptor Is Released from Human Activated NK Lymphocytes and Inhibits Cell-Mediated Cytotoxicity. J Immunol [Internet]. 2007 Feb 1 [cited 2020 Oct 13];178(3):1293–300. Available from: https://www.jimmunol.org/content/178/3/1293

21. Maeda M, Carpenito C, Russell RC, Dasanjh J, Veinotte LL, Ohta H, Yamamura T, Tan R, Takei F. Murine CD160, Ig-like receptor on NK cells and NKT cells, recognizes classical and nonclassical MHC class I and regulates NK cell activation. J Immunol Baltim Md 1950. 2005 Oct 1;175(7):4426–32.

22. Tu TC, Brown NK, Kim T-J, Wroblewska J, Yang X, Guo X, Lee SH, Kumar V, Lee K-M, Fu Y-X. CD160 is essential for NK-mediated IFN-γ production. J Exp Med. 2015 Mar 9;212(3):415–29.

23. Rodriguez-Barbosa JI, Schneider P, Weigert A, Lee K-M, Kim T-J, Perez-Simon J-A, del Rio M-L. HVEM, a cosignaling molecular switch, and its interactions with BTLA, CD160 and LIGHT. Cell Mol Immunol [Internet]. 2019 Jul [cited 2020 Oct 13];16(7):679–82. Available from: https://www.nature.com/articles/s41423-019-0241-1

24. Kojima R, Kajikawa M, Shiroishi M, Kuroki K, Maenaka K. Molecular basis for herpesvirus entry mediator recognition by the human immune inhibitory receptor CD160 and its relationship to the cosignaling molecules BTLA and LIGHT. J Mol Biol. 2011 Nov 4;413(4):762–72.

25. Šedý JR, Bjordahl RL, Bekiaris V, Macauley MG, Ware BC, Norris PS, Lurain NS, Benedict CA, Ware CF. CD160 activation by herpesvirus entry mediator augments inflammatory cytokine production and cytolytic function by NK cells. J Immunol Baltim Md 1950. 2013 Jul 15;191(2):828–36.

26. Cai G, Anumanthan A, Brown JA, Greenfield EA, Zhu B, Freeman GJ. CD160 inhibits activation of human CD4 + T cells through interaction with herpesvirus entry mediator. Nat Immunol [Internet]. 2008 Feb [cited 2020 Oct 13];9(2):176–85. Available from: https://www.nature.com/articles/ni1554

27. Peretz Y, He Z, Shi Y, Yassine-Diab B, Goulet J-P, Bordi R, Filali-Mouhim A, Loubert J-B, El-Far M, Dupuy FP, Boulassel MR, Tremblay C, Routy J-P, Bernard N, Balderas R, Haddad EK, Sékaly R-P. CD160 and PD-1 co-expression on HIV-specific CD8 T cells defines a subset with advanced dysfunction. PLoS Pathog. 2012;8(8):e1002840.

28. Tan CL, Peluso MJ, Drijvers JM, Mera CM, Grande SM, Brown KE, Godec J, Freeman GJ, Sharpe AH. CD160 Stimulates CD8+ T Cell Responses and Is Required for Optimal Protective Immunity to Listeria monocytogenes. ImmunoHorizons

[Internet]. 2018 Aug 1 [cited 2020 Oct 13];2(7):238–50. Available from: https://www.immunohorizons.org/content/2/7/238

29. del Rio M-L, Bravo Moral AM, Fernandez-Renedo C, Buhler L, Perez-Simon J-A, Chaloin O, Alvarez Nogal R, Fernandez-Caso M, Rodriguez-Barbosa J-I. Modulation of cytotoxic responses by targeting CD160 prolongs skin graft survival across major histocompatibility class I barrier. Transl Res [Internet]. 2017 Mar 1 [cited 2020 Oct 13];181:83-95.e3. Available from: http://www.sciencedirect.com/science/article/pii/S1931524416302018

30. Zhang Y, Liu Y, Liu H, Tang WH. Exosomes: biogenesis, biologic function and clinical potential. Cell Biosci [Internet]. 2019 Feb 15 [cited 2020 Oct 13];9(1):19. Available from: https://doi.org/10.1186/s13578-019-0282-2

31. Whiteside TL. Exosomes carrying immunoinhibitory proteins and their role in cancer. Clin Exp Immunol [Internet]. 2017 [cited 2020 Oct 13];189(3):259–67. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/cei.12974

32. Stages of chronic lymphocytic leukemia (CLL) - Canadian Cancer Society [Internet]. www.cancer.ca. [cited 2021 Feb 18]. Available from: https://www.cancer.ca:443/en/cancer-information/cancer-type/leukemia-chronic-lymphocytic-cll/staging/?region=on

33. Elahi S, Ertelt JM, Kinder JM, Jiang TT, Zhang X, Xin L, Chaturvedi V, Strong BS, Qualls JE, Steinbrecher KA, Kalfa TA, Shaaban AF, Way SS. Immunosuppressive CD71 + erythroid cells compromise neonatal host defence against infection. Nature [Internet]. 2013 Dec [cited 2020 Oct 23];504(7478):158–62. Available from: https://www.nature.com/articles/nature12675

34. Shahbaz S, Dunsmore G, Koleva P, Xu L, Houston S, Elahi S. Galectin-9 and VISTA Expression Define Terminally Exhausted T Cells in HIV-1 Infection. J Immunol Baltim Md 1950. 2020 May 1;204(9):2474–91.

35. Dunsmore G, Bozorgmehr N, Delyea C, Koleva P, Namdar A, Elahi S. Erythroid Suppressor Cells Compromise Neonatal Immune Response against Bordetella pertussis. J Immunol [Internet]. 2017 Aug 4 [cited 2020 Oct 23]; Available from: https://www.jimmunol.org/content/early/2017/08/04/jimmunol.1700742

36. Namdar A, Dunsmore G, Shahbaz S, Koleva P, Xu L, Jovel J, Houston S, Elahi S. CD71+ Erythroid Cells Exacerbate HIV-1 Susceptibility, Mediate trans-Infection, and Harbor Infective Viral Particles. mBio [Internet]. 2019 Dec 24 [cited 2020 Oct 23];10(6). Available from: https://mbio.asm.org/content/10/6/e02767-19

37. Morales-Kastresana A, Telford B, Musich TA, McKinnon K, Clayborne C, Braig Z, Rosner A, Demberg T, Watson DC, Karpova TS, Freeman GJ, DeKruyff RH, Pavlakis GN, Terabe M, Robert-Guroff M, Berzofsky JA, Jones JC. Labeling Extracellular Vesicles for Nanoscale Flow Cytometry. Sci Rep [Internet]. 2017 May 12 [cited 2021 Mar 3];7(1):1878. Available from: https://www.nature.com/articles/s41598-017-01731-2

38. Roederer M, Nozzi JL, Nason MC. SPICE: exploration and analysis of post-cytometric complex multivariate datasets. Cytom Part J Int Soc Anal Cytol. 2011 Feb;79(2):167–74.

39. Yun X, Zhang Y, Wang X. Recent progress of prognostic biomarkers and risk scoring systems in chronic lymphocytic leukemia. Biomark Res [Internet]. 2020 Sep 7 [cited 2021 Mar 3];8(1):40. Available from: https://doi.org/10.1186/s40364-020-00222-3

40. Fuertes Marraco SA, Neubert NJ, Verdeil G, Speiser DE. Inhibitory Receptors Beyond T Cell Exhaustion. Front Immunol. 2015; 6:310.

41. Podack ER, Young JD, Cohn ZA. Isolation and biochemical and functional characterization of perforin 1 from cytolytic T-cell granules. Proc Natl Acad Sci U S A. 1985 Dec;82(24):8629–33.

42. Lieberman J. The ABCs of granule-mediated cytotoxicity: new weapons in the arsenal. Nat Rev Immunol. 2003 May;3(5):361–70.

43. Zhao Y, Shao Q, Peng G. Exhaustion and senescence: two crucial dysfunctional states of T cells in the tumor microenvironment. Cell Mol Immunol. 2020 Jan;17(1):27–35.

44. Viganò S, Banga R, Bellanger F, Pellaton C, Farina A, Comte D, Harari A, Perreau M. CD160-Associated CD8 T-Cell Functional Impairment Is Independent of PD-1 Expression. PLOS Pathog. 2014 Sep 25;10(9):e1004380.

45. Romero P, Zippelius A, Kurth I, Pittet MJ, Touvrey C, Iancu EM, Corthesy P, Devevre E, Speiser DE, Rufer N. Four functionally distinct populations of human effector-memory CD8+ T lymphocytes. J Immunol Baltim Md 1950. 2007 Apr 1;178(7):4112–9.

46. Walker EB, Haley D, Petrausch U, Floyd K, Miller W, Sanjuan N, Alvord G, Fox BA, Urba WJ. Phenotype and functional characterization of long-term gp100-specific memory CD8+ T cells in disease-free melanoma patients before and after boosting immunization. Clin Cancer Res Off J Am Assoc Cancer Res. 2008 Aug 15;14(16):5270–83.

47. Elahi S, Dinges WL, Lejarcegui N, Laing KJ, Collier AC, Koelle DM, McElrath MJ, Horton H. Protective HIV-specific CD8+ T cells evade Treg cell suppression. Nat Med. 2011 Jul 17;17(8):989–95.

48. Lazarevic V, Glimcher LH, Lord GM. T-bet: a bridge between innate and adaptive immunity. Nat Rev Immunol [Internet]. 2013 Nov [cited 2020 Oct 13];13(11):777–89. Available from: https://www.nature.com/articles/nri3536

49. Chen Z, Ji Z, Ngiow SF, Manne S, Cai Z, Huang AC, Johnson J, Staupe RP, Bengsch B, Xu C, Yu S, Kurachi M, Herati RS, Vella LA, Baxter AE, Wu JE, Khan O, Beltra J-C, Giles JR, Stelekati E, McLane LM, Lau CW, Yang X, Berger SL, Vahedi G, Ji H, Wherry

EJ. TCF-1-Centered Transcriptional Network Drives an Effector versus Exhausted CD8 T Cell-Fate Decision. Immunity [Internet]. 2019 Nov 19 [cited 2020 Oct 13];51(5):840-855.e5. Available from: http://www.sciencedirect.com/science/article/pii/S1074761319304091

50. Yeh Y-Y, Ozer HG, Lehman AM, Maddocks K, Yu L, Johnson AJ, Byrd JC. Characterization of CLL exosomes reveals a distinct microRNA signature and enhanced secretion by activation of BCR signaling. Blood. 2015 May 21;125(21):3297–305.

51. Yang Z-Z, Grote DM, Ziesmer SC, Niki T, Hirashima M, Novak AJ, Witzig TE, Ansell SM. IL-12 upregulates TIM-3 expression and induces T cell exhaustion in patients with follicular B cell non-Hodgkin lymphoma. J Clin Invest. 2012 Apr;122(4):1271–82.

52. Yan X-J, Dozmorov I, Li W, Yancopoulos S, Sison C, Centola M, Jain P, Allen SL, Kolitz JE, Rai KR, Chiorazzi N, Sherry B. Identification of outcome-correlated cytokine clusters in chronic lymphocytic leukemia. Blood. 2011 Nov 10;118(19):5201–10.

53. Bengsch B, Seigel B, Ruhl M, Timm J, Kuntz M, Blum HE, Pircher H, Thimme R. Coexpression of PD-1, 2B4, CD160 and KLRG1 on exhausted HCV-specific CD8+ T cells is linked to antigen recognition and T cell differentiation. PLoS Pathog. 2010 Jun 10;6(6):e1000947.

54. Liu S, Zhang W, Liu K, Wang Y. CD160 expression on CD8+ T cells is associated with active effector responses but limited activation potential in pancreatic cancer. Cancer Immunol Immunother [Internet]. 2020 May 1 [cited 2020 Oct 13];69(5):789–97. Available from: https://doi.org/10.1007/s00262-020-02500-3

55. He W, Wang B, Li Q, Yao Q, Jia X, Song R, Li S, Zhang J. Aberrant Expressions of Co-stimulatory and Co-inhibitory Molecules in Autoimmune Diseases. Front Immunol [Internet]. 2019 Feb 20 [cited 2020 Oct 13];10. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6391512/

56. Reiser J, Banerjee A. Effector, Memory, and Dysfunctional CD8+ T Cell Fates in the Antitumor Immune Response [Internet]. Vol. 2016, Journal of Immunology Research. Hindawi; 2016 [cited 2020 Oct 24]. p. e8941260. Available from: https://www.hindawi.com/journals/jir/2016/8941260/

57. Jia B, Zhao C, Rakszawski KL, Claxton DF, Ehmann WC, Rybka WB, Mineishi S, Wang M, Shike H, Bayerl MG, Sivik JM, Schell TD, Drabick JJ, Hohl RJ, Zheng H. Eomes $^{+}$ T-bet ^{low} CD8 $^{+}$ T Cells Are Functionally Impaired and Are Associated with Poor Clinical Outcome in Patients with Acute Myeloid Leukemia. Cancer Res [Internet]. 2019 Apr 1 [cited 2020 Oct 24];79(7):1635–45. Available from: http://cancerres.aacrjournals.org/lookup/doi/10.1158/0008-5472.CAN-18-3107

58. Josefsson SE, Huse K, Kolstad A, Beiske K, Pende D, Steen CB, Inderberg EM, Lingjærde OC, Østenstad B, Smeland EB, Levy R, Irish JM, Myklebust JH. T Cells Expressing Checkpoint Receptor TIGIT Are Enriched in Follicular Lymphoma Tumors

and Characterized by Reversible Suppression of T-cell Receptor Signaling. Clin Cancer Res Off J Am Assoc Cancer Res. 2018 15;24(4):870–81.

59. Guillerey C, Harjunpää H, Carrié N, Kassem S, Teo T, Miles K, Krumeich S, Weulersse M, Cuisinier M, Stannard K, Yu Y, Minnie Sa, Hill Gr, Dougall Wc, Avet-Loiseau H, Teng Mwl, Nakamura K, Martinet L, Smyth Mj. TIGIT immune checkpoint blockade restores CD8 + T-cell immunity against multiple myeloma. Blood [Internet]. 2018 Oct 18 [cited 2020 Oct 14];132(16):1689–94. Available from: https://pubmed.ncbi.nlm.nih.gov/29986909/

60. Tan J, Yu Z, Huang J, Chen Y, Huang S, Yao D, Xu L, Lu Y, Chen S, Li Y. Increased PD-1+Tim-3+ exhausted T cells in bone marrow may influence the clinical outcome of patients with AML. Biomark Res [Internet]. 2020 Feb 13 [cited 2021 Mar 5];8(1):6. Available from: https://doi.org/10.1186/s40364-020-0185-8

61. Okoye I, Xu L, Motamedi M, Parashar P, Walker JW, Elahi S. Galectin-9 expression defines exhausted T cells and impaired cytotoxic NK cells in patients with virus-associated solid tumors. J Immunother Cancer [Internet]. 2020 Dec [cited 2021 Mar 5];8(2):e001849. Available from: https://jitc.bmj.com/lookup/doi/10.1136/jitc-2020-001849

62. Krackhardt AM, Witzens M, Harig S, Hodi FS, Zauls AJ, Chessia M, Barrett P, Gribben JG. Identification of tumor-associated antigens in chronic lymphocytic leukemia by SEREX. Blood [Internet]. 2002 Sep 15 [cited 2020 Oct 14];100(6):2123–31. Available from: https://ashpublications.org/blood/article/100/6/2123/16598/Identification-of-tumor-associated-antigens-in

63. Ca K, L G, Np R. Sorting through subsets: which T-cell populations mediate highly effective adoptive immunotherapy? J Immunother Hagerstown Md 1997 [Internet]. 2012 Dec [cited 2020 Oct 14];35(9):651–60. Available from: https://pubmed.ncbi.nlm.nih.gov/23090074/

64. Bonnon C, Wendeler MW, Paccaud J-P, Hauri H-P. Selective export of human GPI-anchored proteins from the endoplasmic reticulum. J Cell Sci [Internet]. 2010 May 15 [cited 2020 Oct 14];123(10):1705–15. Available from: https://jcs.biologists.org/content/123/10/1705

65. Satoa, Ken, Nakano, Akohiko. Mechanisms of COPII vesicle formation and protein sorting - PubMed. FEBS Lett [Internet]. [cited 2020 Oct 14];581(11):2076–82. Available from: https://pubmed.ncbi.nlm.nih.gov/17316621/

66. A L, De S, H P, D Z, Sa FM. Inhibitory Receptor Expression Depends More Dominantly on Differentiation and Activation than "Exhaustion" of Human CD8 T Cells [Internet]. Vol. 4, Frontiers in immunology. Front Immunol; 2013 [cited 2020 Oct 14]. Available from: https://pubmed.ncbi.nlm.nih.gov/24391639/

67. Xie F, Xu M, Lu J, Mao L, Wang S. The role of exosomal PD-L1 in tumor progression and immunotherapy. Mol Cancer [Internet]. 2019 Oct 23 [cited 2020 Oct 13];18(1):146. Available from: https://doi.org/10.1186/s12943-019-1074-3

68. Sabzevary-Ghahfarokhi M, Shirzad H, Rafieian-Kopaei M, Ghatreh-Samani M, Shohan M. The Role of Inflammatory Cytokines in Creating T Cell Exhaustion in Cancer. Cancer Biother Radiopharm. 2018 Sep;33(7):267–73.

69. Alhakeem SS, McKenna MK, Oben KZ, Noothi SK, Rivas JR, Hildebrandt GC, Fleischman RA, Rangnekar VM, Muthusamy N, Bondada S. Chronic Lymphocytic Leukemia–Derived IL-10 Suppresses Antitumor Immunity. J Immunol [Internet]. 2018 Jun 15 [cited 2020 Oct 14];200(12):4180–9. Available from: https://www.jimmunol.org/content/200/12/4180

70. Kitabayashi A, Hirokawa M, Miura AB. The role of interleukin-10 (IL-10) in chronic B-lymphocytic leukemia: IL-10 prevents leukemic cells from apoptotic cell death. Int J Hematol. 1995 Aug;62(2):99–106.

71. Schröttner P, Leick M, Burger M. The role of chemokines in B cell chronic lymphocytic leukaemia: pathophysiological aspects and clinical impact. Ann Hematol. 2010 May;89(5):437–46.

72. Ghamlouch H, Ouled-Haddou H, Damaj G, Royer B, Gubler B, Marolleau J-P. A Combination of Cytokines Rescues Highly Purified Leukemic CLL B-Cells from Spontaneous Apoptosis In Vitro. PLoS ONE [Internet]. 2013 Mar 26 [cited 2020 Oct 14];8(3). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3608602/

73. Schulz A, Toedt G, Zenz T, Stilgenbauer S, Lichter P, Seiffert M. Inflammatory cytokines and signaling pathways are associated with survival of primary chronic lymphocytic leukemia cells in vitro: a dominant role of CCL2. Haematologica. 2011 Mar;96(3):408–16.

74. Baba T, Mukaida N. Role of macrophage inflammatory protein (MIP)-1α/CCL3 in leukemogenesis. Mol Cell Oncol [Internet]. 2014 Jul 15 [cited 2020 Oct 14];1(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4905173/

75. Wierda WG, Johnson MM, Do K-A, Manshouri T, Dey A, O'Brien S, Giles FJ, Kantarjian H, Thomas D, Faderl S, Lerner S, Keating M, Albitar M. Plasma interleukin 8 level predicts for survival in chronic lymphocytic leukaemia. Br J Haematol. 2003 Feb;120(3):452–6.

76. Somasundaram A, Cillo A, Oliveri L, Herman J, Kirkwood J, Ferris RL, Bruno TC, Vignali D. IL-6, IL-8 drive LAG3/PD1 immune suppression on effector and naïve, peripheral blood CD8+ T cells in cancer patients. J Immunol. 2019 May 1;202(1 Supplement):195.9-195.9.

77. Bellomo G, Allegra A, Alonci A, Quartarone E, Pizzoleo MA, Cincotta M, Del Fabro V, Guglielmo S, Musolino C. Serum levels of interleukin-16 in lymphoid malignancies. Leuk Lymphoma. 2007 Jun;48(6):1225–7.

78. Cruikshank WW, Kornfeld H, Center DM. Interleukin-16. J Leukoc Biol [Internet]. 2000 [cited 2020 Oct 24];67(6):757–66. Available from: https://jlb.onlinelibrary.wiley.com/doi/abs/10.1002/jlb.67.6.757

79. Atanackovic D, Hildebrandt Y, Templin J, Cao Y, Keller C, Panse J, Meyer S, Reinhard H, Bartels K, Lajmi N, Sezer O, Zander AR, Marx AH, Uhlig R, Zustin J, Bokemeyer C, Kröger N. Role of interleukin 16 in multiple myeloma. J Natl Cancer Inst. 2012 Jul 3;104(13):1005–20.

3 Chapter 3: Depletion of polyfunctional $CD26^{high}CD8^{+}$ T cell repertoire in chronic lymphocytic leukemia.

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3.1 Background

CD26 also known as DPP4 (dipeptidyl peptidase 4) is a 110kDa homodimer transmembrane glycoprotein with enzymatic activity [1]. It has extracellular, transmembrane, and intracytoplasmic domain [1]. The extracellular domain contains catalytic, cysteine-rich, and glycosylated regions. The catalytic region has serine protease activity that cleaves off amino-terminal dipeptides from many peptide hormones and chemokines that have proline or alanine at their N-terminus [2]. Also, CD26 stabilizes glucose levels by inactivating glucagon-peptide-1 (GLP) and gastric-inhibitory protein (GIP) [3]. The glycosylated and cysteine-rich regions of CD26 interact with different binding partners [4].

CD26 is widely expressed by various cells in different tissues including fibroblasts, endothelial, epithelial, mesothelial, and immune cells [5]. Among immune cells, T cells are the major CD26 expression cells [6]. Nevertheless, B cells [7], natural killer cells (NK) [8], dendritic cells (DCs), [9] and macrophages [10] express lower levels of CD26. It is worth mentioning that erythroid precursors/progenitors ($CD71^+$ erythroid cells) also express substantial levels of CD26 [11].

The role of CD26 in the immune system particularly T cell development and differentiation has been widely studied. For example, CD26 is considered a thymus maturation marker for T cells since most single-positive $CD4^+$ and $CD8^+$ T cells express this glycoprotein [12]. Notably, deletion of CD26 in mice is associated with decreased frequency and functionality of $CD4^+$ T cells, which subsequently impairs cytokine and immunoglobulin production in CD26 knock-out (KO) mice [13]. In line with these observations, CD26 (DPP4) inhibitors, as glycemic controllers have been

associated with Th1, Th2, and Th17 cell suppression but regulatory T cells (Tregs) expansion in diabetic patients [14].

CD26 modulates T cell activation and proliferation via interaction with its binding partners such as Adenosine Deaminase (ADA), which is an essential enzyme in the adenosine pathway [15]. Upon binding to CD26, ADA converts adenosine to inosine with a wide range of anti-inflammatory effects [16,17]. Moreover, the interaction of ADA with CD26 transduces a stimulatory signal to T cells [18]. For instance, Caveolin-1 on antigen -presenting cells upon interaction with CD26 via CARMA-1 enhances T cell activation [19].

CD26 is also involved in T cell trafficking by modulating chemokines and the tendency for binding to extracellular matrix molecules and endothelial cells [20]. The enzymatic activity of CD26 regulates diverse chemokines including RANTES (CCL5), Eotaxin, Stromal-derived Factor-α (SDF-1α/CXCL12), and macrophage-derived chemokine (MDC/CCL22) [21]. For example, CD26 increases CCR5-dependent but reduces CCR1-dependent migration of monocytes [22]. Additionally, the CD26 molecule has binding sites for extracellular matrix components such as fibronectin and collagen [23]. These capabilities support $CD26^+$ T cell activity, homing and trans-endothelial migration [24].

It appears that $CD26^{high}CD4^+$ T cells are dominantly Th17 cells and exhibit effective anti-tumor immunity in different cancer models [25-27]. $CD26^{high}CD4^+$ T cells, due to their increased migration and persistence capacities, are desirable for T cell-based immunotherapies [26]. It is worth mentioning that CD26 expressing $CD4^+$ T cells play a crucial role in the differentiation of B cells into plasma cells [6].

However, compared to $CD4^+$ T cells, the role of CD26 in $CD8^+$ T cells is not fully understood. Some studies have reported that CD26 provides a costimulatory signal and increases cytokine production in $CD8^+$ T cells [28]. Given its costimulatory role, CD26 blockade attenuates organ transplantation [29,30] and skin allograft rejections in animal models [31,32]. Moreover, CD26 plays an essential role in the formation of memory $CD8^+$ T cells in viral infections [33] but their frequency is reduced in HIV-infected individuals [34]. Nevertheless, the loss of CD26 expression in malignant T cells in cutaneous T cell lymphoma is reported [35].

Recently it has been shown that CD26 expression is a distinctive surrogate marker for Mucosal Associated Invariant T (MAIT) cells [36]. Human MAIT cells are unconventional innate-like T cells that are present in blood circulation and tissues [37]. These cells are defined by the expression of a semi-invariable T-cell receptor-α chain (TCR-α) composed of TVα7.2 [38]. MAIT cells are restricted to MHC class I related Protein 1 (MR-1) which enables them a unique opportunity to recognize riboflavin (Vitamin B12) metabolites in microbial components [39]. In addition, MAIT cells exhibit a distinct phenotype evidenced by high levels of surface markers such as CD161, IL-18α, and CD26 [36,40,41]. However, to our knowledge, the frequency and functionality of $CD26^+CD8^+$ T cells in Chronic Lymphocytic Leukemia (CLL) have never been investigated.

CLL is a hematologic malignancy with clonal expansion of malignant B cells in the bone marrow, lymph nodes, and peripheral blood [42]. CLL patients usually suffer from secondary immunodeficiency due to hypogammaglobulinemia and abnormal cellular immunity [43]. In CLL, circulating malignant B cells deleteriously affect the T cell anti-tumor immunity [44,45]. CLL-associated mortalities are mainly due to

disease progression, secondary solid malignancy, and/or infections [46] that are governed by the compromised immune system in predisposed patients [47,48]. T cell impairment/exhaustion is one aspect of the compromised anti-tumor immunity in CLL patients. Unfortunately, current immunotherapies targeting PD-1 and CTLA-4 pathways have not been encouraging in CLL patients [49]. This might be related to the differential nature of exhausted T cells in hematological cancers versus solid tumors. For example, we have shown that CD160, not PD-1 is the dominant co-inhibitory receptor associated with $CD8^+$ T cell exhaustion in CLL patients [45]. These examples provide an urgent need for a better understanding of T cell repertoire in CLL patients. Although CAR T cell therapy has been associated with promising results in CLL patients [50], further T cell-related studies will assist us in identifying potential novel immunotherapies.

In this study, we investigated the frequency of $CD26^+CD8^+$ T cells in a cohort of CLL patients in comparison with age-sex-matched healthy controls (HCs). We further performed extensive immunophenotyping on $CD26^{neg}$, $CD26^{low}$, and $CD26^{high}$ $CD8^+$ T cell subsets in both cohorts. Moreover, we conducted comprehensive studies on the effector functions of different subpopulations of $CD26^+CD8^+$ T cells in CLL versus HCs. Notably, we investigated the mechanism underlying the depletion of $CD26^+$ T cells in CLL patients. Therefore, our studies provide a novel insight into the role of $CD26^+CD8^+$ T cells in CLL patients and suggest that $CD26^{high}CD8^+$ T cells may have promising potential for adoptive T cell transfer and CAR T cell therapies.

3.2 Materials and methods

3.2.1 Study population

We recruited 55 patients with confirmed CLL for this study (Supplementary Table 3.8.1, 3.8.2), along with 44 age-and-sex-matched healthy controls for comparison. We collected peripheral blood specimens and bone-marrow aspirates in EDTA-containing tubes. The clinical data including IGHV mutation status, FISH analysis, clinical staging (Rai staging system) [51], and treatment state/course were collected for further analysis.

3.2.2 Cell isolation and purification

The peripheral blood mononuclear cells (PBMCs) and the bone-marrow cells were isolated using Ficoll-Paque gradients (GE Healthcare). $CD3^+$ T cells were enriched by a negative selection kit (EasySep isolation kit, Stem Cell Technologies) with a purity of > 97% (Supplementary Fig.1a). For effector T cell ($CD3^+CCR7^-$) isolation, $CD3^+$ T cells were stained with the PE-conjugated anti-CCR7 antibody followed by the anti-PE-conjugated microbeads (Miltenyi) with a purity of > 96% (Supplementary Fig.1b). For B cell enrichment, B cells were stained with FITC-conjugated anti-CD19 antibody and then isolated by the anti-FITC microbeads (Miltenyi) with a purity of > 91% (Supplemental Fig.1c).

3.2.3 Flow cytometry

The fluorochrome-conjugated antibodies were purchased from BD Biosciences, Thermo Fisher Scientific or Biolegend including human anti-CD3 (SK7), anti-CD4 (RPA-T4), anti-CD8 (RPA-T8), anti-CD26 (M-A261), anti-CD161(HP-3G10), anti-TVα7.2 (3C10), anti-IL-18Rα (H44), anti-CD5 (UCHT2), anti-CD19 (HIB19), anti-CD160 (BY55), anti-2B4 (eBioDM244), anti-TIGIT (MBSA43), anti-PD1 (EH12.1),

anti-TIM-3 (7D3), anti-CD39 (TU66), anti-CD73 (AD2), anti-CD95 (DX2), anti-CD127 (HIL-7R-M21), anti-ROR$\gamma\delta$ (Q21-559), anti-CD45RA (HL100), anti-CCR7 (3D12), anti-CD27 (G3H69), anti-CD28 (CD28.2), anti-ICOS (C398.4A), anti-CD57 (NK-1), anti-CD16 (B73.1), anti-CD56 (B159), anti-KLRG1 (2F1/KLRG1), anti-CD69 (N50), anti-CD107a (H4A3), anti-IL-2 (MQ1-17H12), anti-TNF- α (MAB11), anti-IFN-γ (45.B3), anti-Perforin (dG9), anti-Granzyme-B (GB11), anti-Granzyme-K (G3H69), anti-CLA (HECA452, Miltenyi), anti-CCR4 (1G1), anti-CCR5 (2D7/CCR5), anti-CCR6 (11A9), anti-Integrin-β7 (FIB504), anti-CXCR3 (1C6/CXCR3), anti-CXCR4 (12G5), anti-Galectin-9 (9M1-3), anti-Annexin-V (Annexin-V), anti-TOX (TXRX10), anti-FOXP3 (150D/E4), and anti-T-bet (4B10). We also used mouse anti-CD26 (H194-112), anti-CD8 (53-6.7), and anti-CD3 (17A2).

antibodies. Surface staining was performed, as we have reported elsewhere [52,53]. Data were acquired on an LSR Fortessa-SORP (BD Bioscience) and subsequently analyzed using Flow Jo software (V.10.8.1). Cell viability was analyzed using the LIVE/DEAD kit (Life technologies).

3.2.4 Cell culture and ex vivo cytokine measurement

Isolated PBMCs were cultured and stimulated with soluble Purified NA/LE anti-human CD3 (UCHT1, 3 μg/ml)/CD28 (CD28.2, 1 μg/ml) or PMA (20 ng/ml)/Ionomycin (1 μg/ml) (Cell stimulation cocktail, Biolegend) in the presence of the protein transport inhibitor Brefeldin A (BD Biosciences, 1/1000) for 5 hours. Intracellular cytokine staining was performed according to our protocols [54]. For cytokine-dependent cultures, PBMCs were treated with a cocktail of cytokines including recombinant human IL-12 (Cedarlane,100 ng/ml), IL-18 (Biolegend,100 ng/ml), and IL-15

(Biolegend, 100 ng/ml) for 18 hours. Brefeldin A (1/1000) was added 5 hours before the intracellular staining. In other experiments, the effects of different cytokines including TNF-α (50 ng/ml), IFN-γ (100 ng/ml), IL-10 (100 ng/ml), IL-16 (500 ng/ml), IFN-α (100 ng/ml), IL-2 (20 ng/ml), IL-6 (100 ng/ml), and TGF-β (20 ng/ml) on CD26 expression was analyzed.

3.2.5 qPCR analysis

The RNA was isolated from $CD8^+$ T cells from HCs and CLL patients using the Direct-zol RNA MicroPrep kit (Zymo Research). cDNA was synthesis using the Quantitect Reverse Transcription Kit (Qiagen). RT-PCR was carried out using the Quantitect primer Kit (Qiagen) to measure the expression of CD26 mRNA. Each sample was run in duplicate, using the CFX96 Touch Real-Time PCR Detection System (BioRad). Beta-2-microglobulin was used as a reference gene and the relative fold change of the targeted genes was calculated by the $\Delta\Delta$ CT method.

3.2.6 Proliferation assay

Isolated effector T cells ($CD3^+$ $CCR7^-$) were labeled with the CFSE dye (Life Technologies) before stimulation using the Dyna beads Human T-activator CD3/CD28 (Thermo Fisher Scientific) according to the manufacturer's instruction and our protocols [53,54]. After 72 hours cells were stained and analyzed.

3.2.7 Migration assay

The migration assay was performed using the CytoSelect migration assay kit (Cell Biolabs), as we have reported elsewhere [55,56]. PBMCs were starved overnight in

FBS-free culture media. The next day, FBS (10%), recombinant human RANTES (CCL5) (R & D, 10 nM), and recombinant human IL-18 (Biolegend, 100 ng/ml) were used as chemoattractants. Cell suspension of starved cells (0.5 X 10^6 cells/well) was added to the upper chamber and incubated in the incubator (37℃, 5% CO25) for 24 hours. Migrated cells in the lower chamber were harvested and quantified by flow cytometry according to the manufacturer's instructions. The migration ratio was calculated compared to the wells lacking the chemoattractant.

3.2.8 Multiple and ELISA assay

The plasma concentration of cytokines/chemokines was measured using the MesoScale Discovery (MSD) multiplex kit, as we have reported elsewhere [45]. Data were acquired on the V-plex® Sector Imager 2400 plate reader and analyzed using the MSD Workbench 3.0 software. In addition, soluble CD26, IL-18, TGF-β, and Galectin-9 (Gal-9) were detected using the DuoSet ELISA kit (R&D) according to the manufacturer's protocol. The microplate reader (Synergy H1 Biotek) was used for acquiring ELISA data and analyzed by Gen5 V.2.07 software.

3.2.9 Statistical analysis

GraphPad Prism software (version 9.3.1) was used for statistical analysis. Mann-Whitney U test or Wilcoxon signed rank test was used for non-paired or paired comparisons, respectively. For multiple comparisons, the Kruskal-Wallis one-way analysis of variance test was used. Data were presented as median with an interquartile range. P-values less than 0.05 was considered statistically significant. The visual summary was prepared using the Biorender software.

3.3 Results

3.3.1 A significant reduction in $CD26^+CD8^+$ T cells in CLL patients.

To determine the frequency of $CD26^+CD8^+$ T cells, PBMCs from CLL patients (n=55) and healthy controls (HCs) (n=44) were subjected to CD26 expression analysis. (Supplementary Fig.1d, the gating strategy). These studies revealed that the frequency of $CD26^+CD8^+$ T cells was significantly declined in CLL patients compared to HCs (Fig.1a, 1b). While on average half of $CD8^+$ T cells in PBMCs of HCs expressed CD26 (Mean $\pm$ SD: 45.88 $\pm$ 21.36) this was substantially lower (Mean $\pm$ SD: 26.84$\pm$16.64) in CLL (Fig.1a, 1b). Although the majority of CD26 expressing $CD8^+$ T cells were $CD26^{low}$, the proportions of both $CD26^{high}$ and $CD26^{low}$ were significantly reduced in CLL patients compared to HCs (Fig.1a, 1c, 1d). We also measured the cell number in both groups, which confirmed a significant reduction in the number of $CD26^{low}$ and $CD26^{high}$ T cells in CLL patients (Supplementary Fig. 1e). Moreover, the intensity of CD26 expression was significantly decreased in $CD8^+$ T cells of CLL patients compared to HCs (Fig.1e, 1f). As expected, the $CD26^{low}$ subpopulation had a significantly lower intensity of CD26 expression than their $CD26^{high}$ counterparts in CLL patients (Supplementary Fig.1f).

Given the reported impact of age and sex on T cell repertoire [57], we found these variables did not influence the frequency of $CD26^+CD8^+$ T cells (Supplementary Fig.1g, 1h). Also, we did not observe any difference in the frequency of $CD26^+CD8^+$ T cells among treated (n=18) versus untreated (n=37) CLL patients (Supplementary Fig.1i, 1j). To determine the potential correlation between the clinical staging of CLL patients with the frequency of $CD26^+CD8^+$ T cells, we stratified our patients according

to the Rai staging system for CLL into three groups [51]; however, we did not observe any significant difference between them (Supplementary Fig.1k). Moreover, our analysis revealed that the lymphocyte count in the whole blood did not correlate with the frequency of $CD26^+CD8^+$ T cells (Supplementary Fig.1l). To determine whether CD26 downregulation was $CD8^+$ T cells specific or a general phenomenon of CLL, we measured the expression of CD26 on other blood mononuclear cells. Although $CD4^+$ T cells were the most dominant CD26 expressing cells, their frequency in CLL patients was also significantly reduced compared to HCs (Supplementary Fig.1m-o). Of note, NK cells exhibited a very small proportion of CD26-expressing cells without any difference between HCs and CLL patients (Supplementary Fig.1p). Also, we compared the mean fluorescence intensity (MFI) of CD26 in malignant B cells in CLL with non-malignant B cells. These studies revealed a significant increase in the intensity of CD26 in malignant B cells, which is consistent with previous reports [58,59] (Supplementary Fig.1q). These observations suggested that CD26 may get shed from the cell surface resulting in the elevation of soluble CD26 in the plasma. However, the plasma levels of CD26 in CLL patients did not support this hypothesis (Supplementary Fig.1r). Moreover, we compared $CD26^+CD8^+$ frequencies in the bone marrow and blood of CLL patients. Despite a trend towards lower $CD26^+CD8^+$ T cells in the bone marrow, it was not significant (Supplementary Fig.1s, 1t). Finally, to understand the stage of CD26 reduction, we quantified CD26 mRNA levels in $CD8^+$ T cells from CLL and HCs. However, we did not find any significant difference between the groups at the gene level (Supplementary Fig.1u). Overall, these observations support the notion that CLL is associated with a substantial decline in the frequency

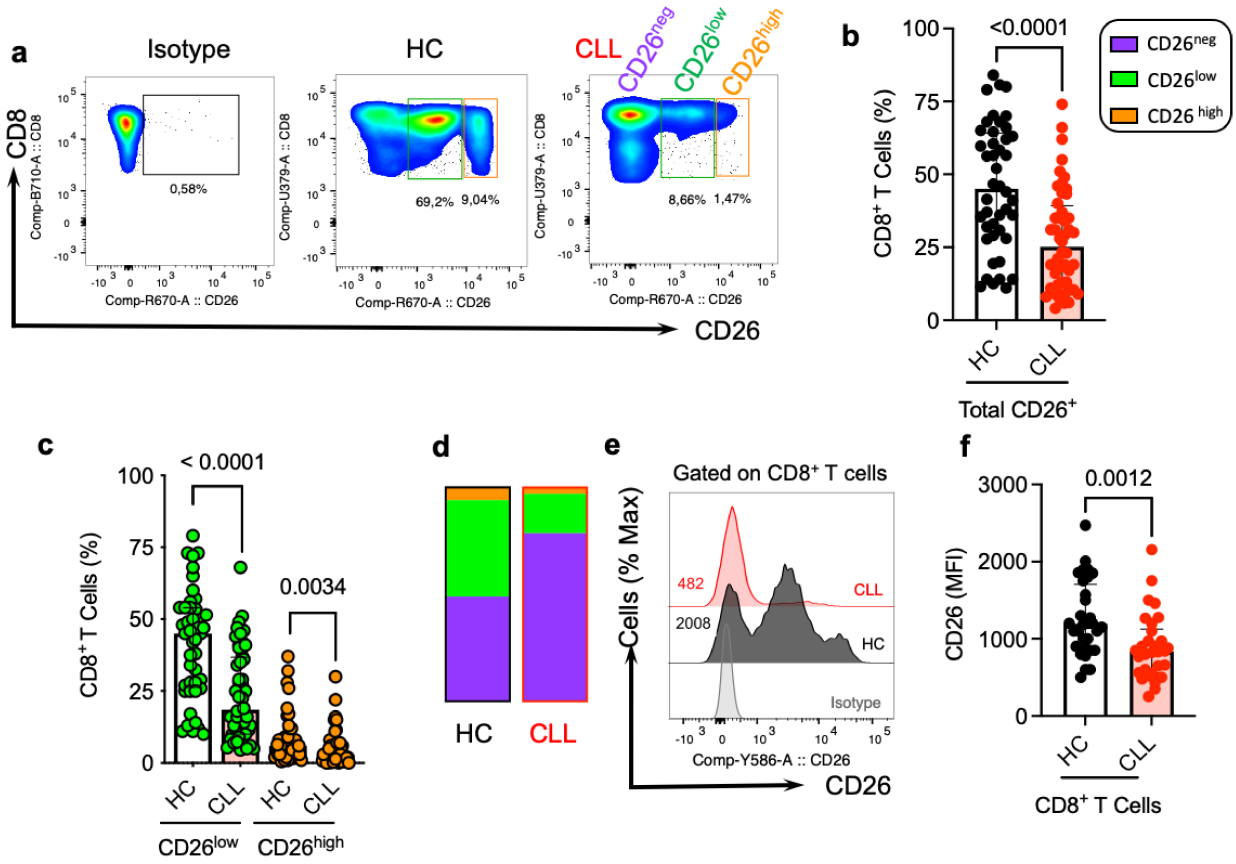
of $CD26^{+}$ T cells, particularly $CD26^{low}CD8^{+}$ T cells, without any changes in the plasma levels of soluble CD26.

3.3.2 The differential expression pattern of CD26 in $CD8^{+}$ T cell subsets in CLL.

To better phenotype $CD26^{+}CD8^{+}$ T cells in CLL, we conducted a detailed *ex vivo* analysis of these cells. Based on CD45RA, CCR7, CD95, and CD27 markers, we characterized T cell subsets such as naïve ($CD45RA^{+}CCR7^{+}CD95^{-}$), stem cell memory ($CD45RA^{+}CCR7^{+}CD95^{+}$), central memory ($CD45RA^{-}CCR7^{+}$), transitional memory ($CD45RA^{-}CCR7^{-}CD27^{+}$), effector memory ($CD45RA^{-}CCR7^{-}CD27^{-}$), and effectors ($CD45RA^{+}CCR7^{-}$) [60-62]. We found that $CD8^{+}CD26^{low}$ T cells were mainly naïve, stem cell memory, and central memory (Fig.1g). As illustrated in this figure, the frequency of $CD8^{+}CD26^{low}$ expressing T cells declines as T cells differentiate to other subsets (e.g., transitional memory, effector memory, and effectors) in both HCs and CLL patients. In contrast, $CD8^{+}CD26^{high}$ T cells were uniquely populated in transitional and effector memory subsets with very low frequency in other subsets and absent in the naïve population (Fig.1g). In particular, we observed that the frequency of $CD26^{low}$ was significantly lower in stem cell memory, effector memory, and effectors but unchanged in other T cell subsets in CLL patients compared to HCs (Fig.1h-n). However, the frequency of $CD26^{high}CD8^{+}$ T cells was significantly lower in all T cell subsets except the naïve subset in CLL patients compared to HCs (Fig.1h-n). Despite the expansion of total effector memory and effector $CD8^{+}$ T cell subsets in CLL patients (Supplementary Fig 2a, 2b), the frequency of those expressing $CD26^{high}$ was significantly lower in CLL patients (Fig.1m, 1n).

Moreover, to better characterize $CD26^{+}CD8^{+}$ T cells, we subjected them to CD27 expression analysis. CD27 is involved in $CD8^{+}$ T cell activation and memory formation that augments anti-tumor activity [63,64]. Our further studies confirmed the abundance of transitional and effector memory subsets in $CD26^{low}$ and $CD26^{high}CD8^{+}$ T cells (Supplementary Fig. 2c, 2d).

Overall, our results indicate that $CD26^{+}CD8^{+}$ T cells are in distinct stages of differentiation. Considering the substantial co-expression of CD27 and CD26, a considerable decline in the proportion of $CD26^{+}CD8^{+}$ T cells may deprive CLL patients of the potent anti-tumor activity of this T cell subset [64].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CD26neg
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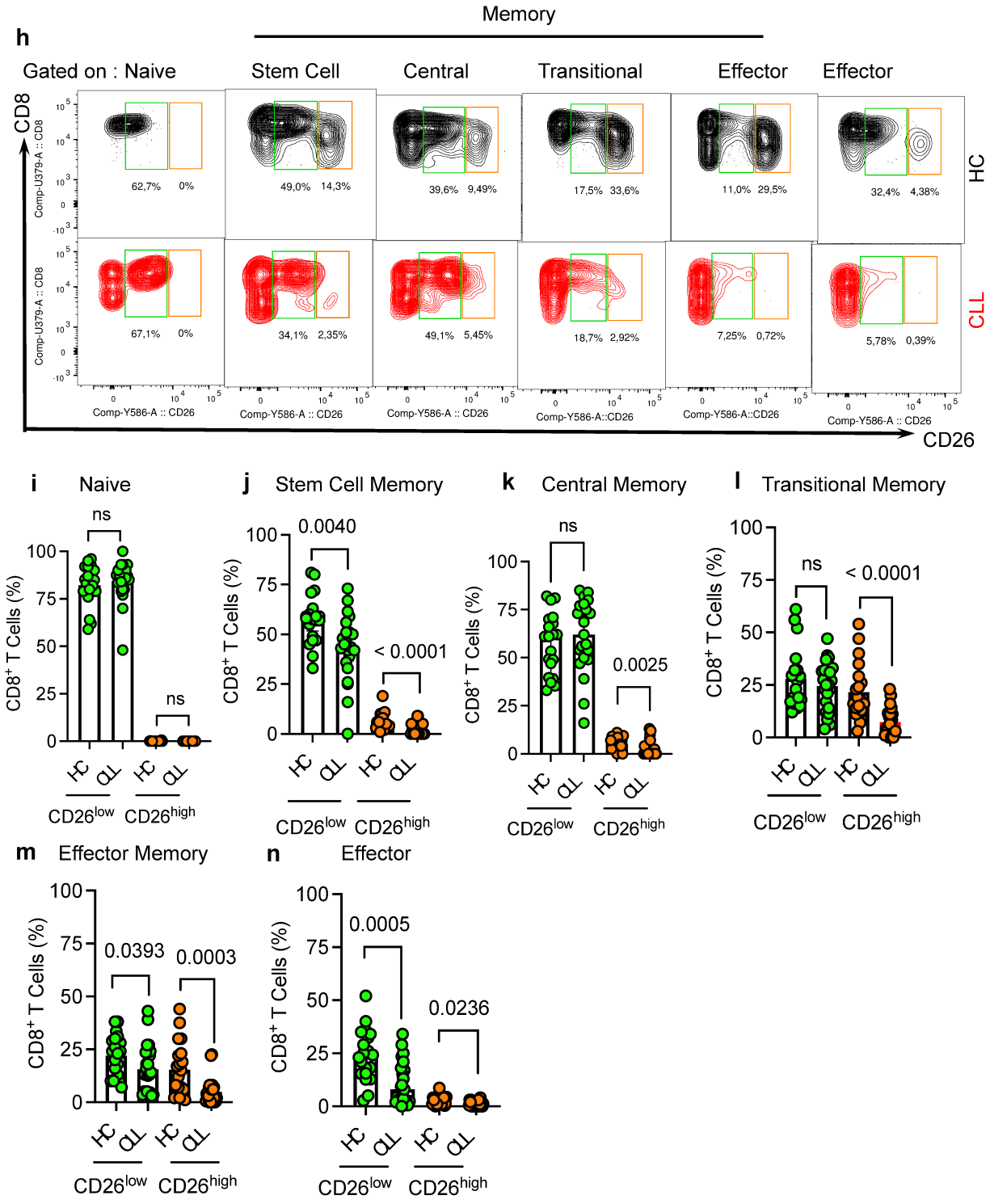


Fig. 1. CD26^{low} and CD26^{high} CD8^{+} T cells are reduced in CLL. (a) Representative flow cytometry plots, and **(b)** Cumulative data comparing the frequency of CD26^{+}CD8^{+} T cells in PBMCs from HCs (n=44) and CLL (n=55) patients. **(c)** Cumulative data comparing the frequency of CD26^{low} and CD26^{high} CD8^{+} T cells in HC

and CLL. **(d)** Bar plots illustrating the proportion of $CD26^{neg}$, $CD26^{low}$ and $CD26^{high}CD8^{+}$ T cells in HC and CLL **(e)** Representative histogram plots, and **(f)** Cumulative data comparing the Mean Fluorescence Intensity (MFI) of CD26 in $CD8^{+}$ T cells in HCs and CLLs. **(g)** The pie charts represent the median frequency of $CD26^{neg/low/high}$ in different subsets of $CD8^{+}$ T cells (e.g., Naïve, Stem cell memory, Central memory, Transitional memory, Effector memory, and Effector) in HCs versus CLLs. **(h)** Representative flow cytometry plots of $CD26^{low/high}$ in different $CD8^{+}$ T cell subsets of HCs (black) and CLLs (red). **(i)** Cumulative data of $CD26^{low/high}$ in naïve, **(j)** stem cell memory, **(k)** central memory, **(l)** transitional memory, **(m)** effector memory, and **(n)** effector $CD8^{+}$ T cells in HC and CLL. Statistics are assessed by Mann-Whitney or the Kruskal-Wallis multiple comparison tests. P-value < 0.05 was considered as significant. Error bars represent the median with an interquartile range. Each dot represents an individual human sample.

3.3.3 $CD26^{high}CD8^{+}$ T cells are enriched with MAIT cells in CLL.

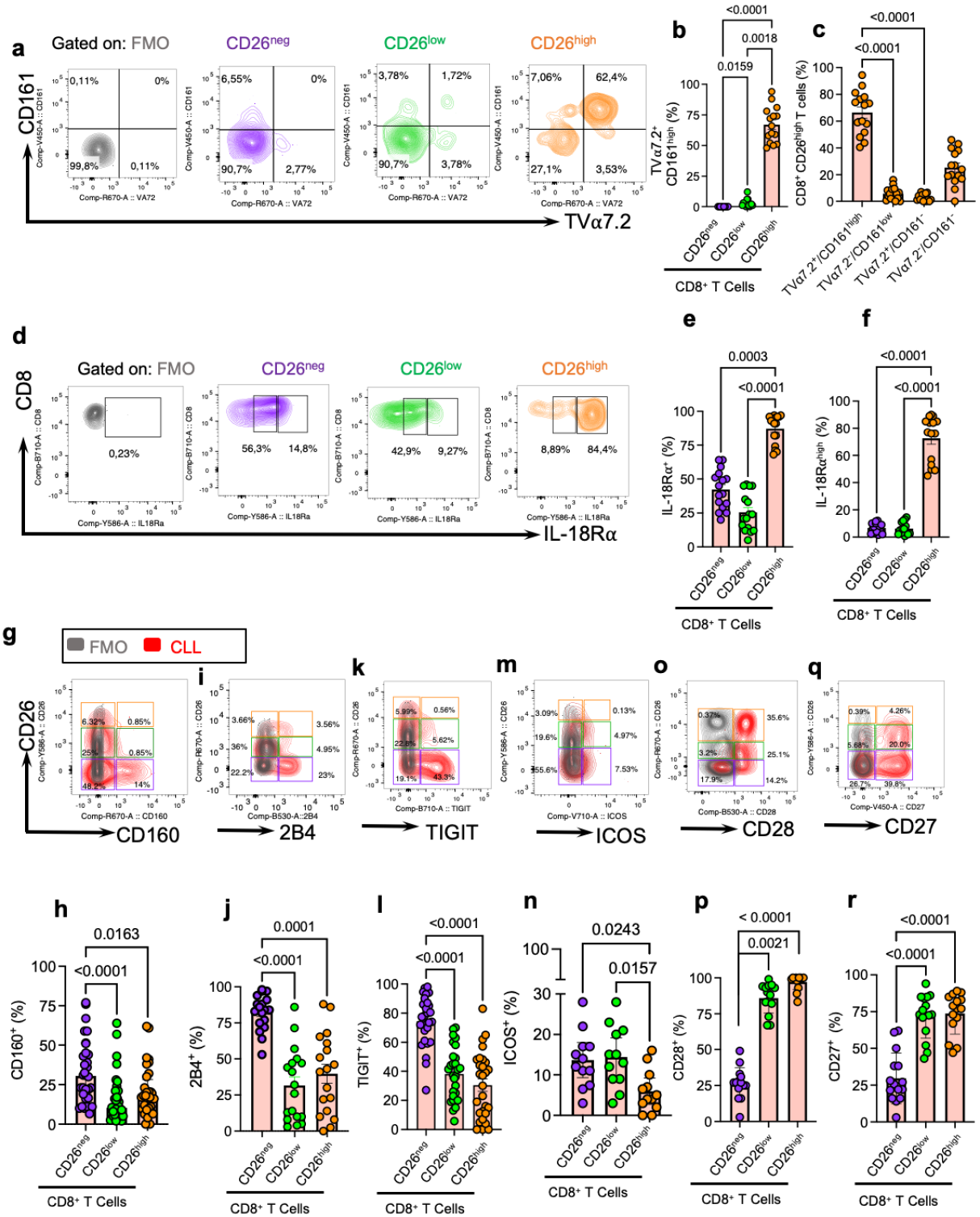
MAIT cells express high levels of CD161 [40], IL-18Rα, and CD26 [36,41]. In particular, the most specific surrogate marker for MAIT cells is the co-expression of $CD161^{high}$ and TVα7.2 [65]. As such, we decided to determine whether $CD26^{+}CD8^{+}$ T cells were MAIT cells. We observed that the majority (Mean $\pm$SD:67 ± 14.48) of $CD26^{high}CD8^{+}$ T cells co-expressed TVα7.2^{+} & $CD161^{high}$ in CLL patients (Fig. 2a-c). However, a portion of $CD26^{high}$ did not express TVα7.2 and $CD161^{high}$ (Fig.2a, 2c). Of note, the frequency of MAIT-like cells expressing TVα7.2 and $CD161^{high}$ was significantly lower among $CD8^{+}CD26^{+}$ T cells in CLL patients (Supplementary Fig. 2e-g). Moreover, we investigated the expression levels of IL-18Rα in three subsets of $CD26^{neg}$, $CD26^{low}$, and $CD26^{high}CD8^{+}$ T cells, which elucidated that $CD26^{high}$ cells were the dominant cells expressing IL-18Rα in both HCs and CLL patients. However, a portion of $CD26^{high}$ $CD8^{+}$ T cells lacked the expression of this cytokine receptor (Fig. 2d-f). These observations suggest that $CD26^{high}CD8^{+}$ T cells are enriched with MAIT-like cells, but they are a heterogeneous T cell subset. It is worth mentioning that we found a considerable frequency of MAIT-like cells that did not express $CD26^{high}$. As

such, we speculate that CLL may impact the expression of MAIT surrogate markers. Therefore, $CD26^{high}$ might not be a definite marker for MAIT cell identification in CLLs. However, the majority of $CD26^{high}CD8^{+}$ T cells displayed the MAIT-like phenotype ($CD161^{high}$ $TV\alpha7.2^{+}$) and significantly declined in CLL patients compared to HCs.

3.3.4 The heterogeneous expression of co-inhibitory and co-stimulatory receptors in $CD26^{+}CD8^{+}$ T cells.

To better characterize $CD26^{+}$ versus their negative counterparts, we subjected them to further analysis for the expression of co-stimulatory/inhibitory molecules. Previously, we have reported that the co-inhibitory receptor, CD160, was selectively overexpressed on $CD8^{+}$ T cells in CLL patients [45]. In agreement, we found that $CD26^{neg}CD8^{+}$ T cells were significantly enriched with CD160 (Fig. 2g, 2h), 2B4 (Fig. 2i, 2j), TIGIT (Fig. 2k, 2l), and ICOS (Fig. 2m, 2n) expressing T cells than their $CD26^{low}$ and $CD26^{high}$ siblings. On the contrary, $CD26^{low}$ and $CD26^{high}$ T cells were significantly populated with CD28 and CD27 expressing $CD8^{+}$ T cells (Fig. 2o-r). Interestingly, we did not observe any difference in the proportion of PD-1 expressing $CD8^{+}$ T cells between $CD26^{neg}$, CD26low, and $CD26^{high}$ subsets (Fig. 2s, 2t). Although HCs, in general, have a lower frequency of T cells expressing co-inhibitory/stimulatory receptors, we observed a significant reduction in the proportion of CD160, 2B4, and PD-1 expressing cells in $CD26^{low}CD8^{+}$ T cell subset compared to their $CD26^{neg}$ or $CD26^{high}$ siblings (Supplementary Fig. 2h-j). The frequency of TIGIT-expressing T cells was significantly lower in $CD26^{low}$/$CD26^{high}$ subsets in HCs (Supplementary Fig. 2k). Although ICOS and CD28 had similar expression patterns in $CD26^{-/+}$ subsets, $CD27^{+}CD8^{+}$ T cells were significantly abundant in the $CD26^{low}$

subset in HCs (Supplementary Fig. 2l-n). Owing to the tandem contribution of ectonucleotidases CD39, CD73, and CD26 in the adenosine pathway [66], we measured the expression of these two ectoenzymes in different subsets of CD26-expressing $CD8^+$ T cells. We found that CD39 was highly expressed in $CD26^{neg}CD8^+$ T cells (Fig. 2u, 2v), whereas CD73 was prominently expressed in $CD26^{low}$ followed by $CD26^{high}$ and $CD26^{neg}$ $CD8^+$ T cells (Fig. 2w, 2x). Similarly, we analyzed the expression of CD39 and CD73 in $CD26^+CD8^+$T cells of HCs, which showed no differential expression pattern for CD39, but we found a higher abundance of $CD73^+$ T cells among $CD26^{low}$ compared to their $CD26^{neg}$/$CD26^{high}$ counterparts (Supplementary Fig. 2o, 2p). Our further analysis revealed that $CD26^{low}CD73^+$ T cells predominantly displayed naïve T cell phenotype (Supplementary Fig. 2q, 2r). However, the subpopulation of $CD26^+CD73^+$ with effector and effector memory phenotype ($CD8^+CCR7^-$ T cells) was significantly reduced in CLL patients versus HCs (Supplementary Fig. 2s). Overall, we found that CD160, 2B4, TIGIT, and PD-1 expressing $CD26^{low}CD8^+$ T cells were significantly enriched in CLL versus HCs (Fig. 2y). In contrast, the proportion of $CD27^+CD73^+$ expressing cells was significantly reduced in the $CD26^{low}CD8^+$ T cell subset without any changes in the frequency of ICOS, CD28, CD39 expressing $CD8^+$ T cells in CLL versus HCs (Fig. 2y). However, this pattern was different for $CD26^{high}$ T cells, and they showed higher expression of $TIGIT^+$ and $CD27^+$ cells in CLL patients compared to HCs (Supplementary Fig. 2t). Interestingly, we found that the proportion of $2B4^+$ T cells was significantly increased but CD28, CD27 and CD73-expressing cells were decreased among $CD26^{neg}CD8^+$ T cells in CLL patients (Supplementary Fig. 3a).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Gated on: FMO
CD26neg
CD26low
CD26high
CD161
TVα7.2
0,11%
0%
99,8%
0,11%
6,55%
0%
90,7%
2,77%
3,78%
1,72%
90,7%
3,78%
7,06%
62,4%
27,1%
3,53%
b
<0.0001
0.0018
0.0159
TVα7.2+ CD161high (%)
CD26neg
CD26low
CD26high
CD8+ T Cells
c
<0.0001
<0.0001
CD8+ CD26high T cells (%)
TVα7.2+/CD161high
TVα7.2+/CD161low
TVα7.2+/CD161-
TVα7.2-/CD161-
d
Gated on: FMO
CD26neg
CD26low
CD26high
CD8
IL-18Rα
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56,3%
14,8%
42,9%
9,27%
8,89%
84,4%
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0.0003
<0.0001
IL-18Rα+ (%)
CD26neg
CD26low
CD26high
CD8+ T Cells
f
<0.0001
<0.0001
IL-18Rαhigh (%)
CD26neg
CD26low
CD26high
CD8+ T Cells
g
FMO
CLL
CD26
CD160
i
2B4
k
TIGIT
m
ICOS
o
CD28
q
CD27
h
0.0163
<0.0001
CD160+ (%)
CD26neg
CD26low
CD26high
CD8+ T Cells
j
0.0001
<0.0001
2B4+ (%)
CD26neg
CD26low
CD26high
CD8+ T Cells
l
<0.0001
<0.0001
TIGIT+ (%)
CD26neg
CD26low
CD26high
CD8+ T Cells
n
0.0243
0.0157
ICOS+ (%)
CD26neg
CD26low
CD26high
CD8+ T Cells
p
< 0.0001
0.0021
CD28+ (%)
CD26neg
CD26low
CD26high
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r
<0.0001
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CD27+ (%)
CD26neg
CD26low
CD26high
CD8+ T Cells

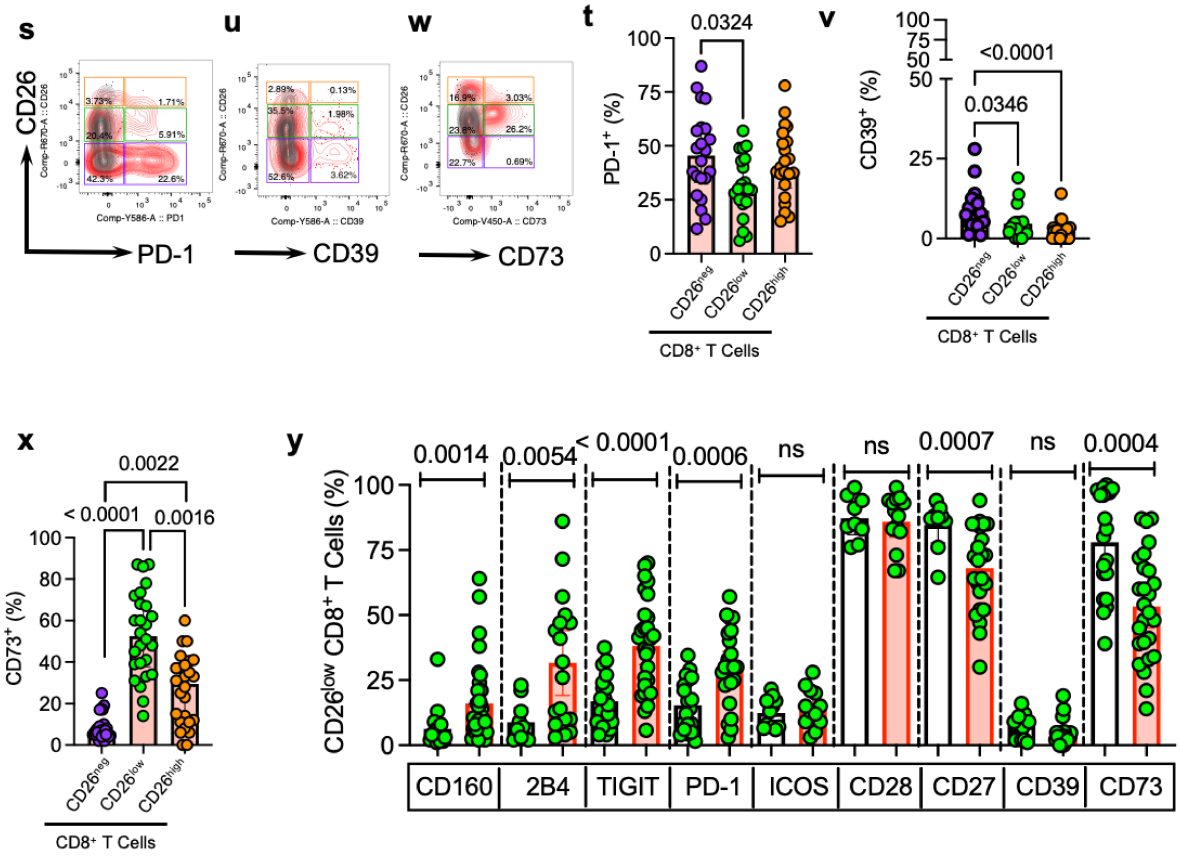


Fig. 2. Phenotypic profile of CD26$^{neg/low/high}$ CD8^{+} T cells in CLL. (a) Representative flow plots, and **(b)** cumulative data showing the frequency of CD161^{+} TVα7.2^{+} in CD26$^{neg/low/high}$ CD8^{+} T cell subsets in CLL. **(c)** Cumulative data of the frequency CD161/ TVα7.2 co-expressing cells in CD26^{high}CD8^{+} T cells in CLL. **(d)** Representative flow plots, and **(e)** cumulative data of the frequency of IL-18Rα expressing cells among CD26$^{neg/low/high}$ CD8^{+} T cells in CLL. **(f)** Cumulative data of the frequency of IL-18Rα^{high} expressing cells among CD26$^{neg/low/high}$ CD8^{+} T cells in CLL. **(g)** Representative plots, and **(h)** the frequency of CD160^{+} cells among CD26$^{neg/low/high}$ CD8^{+} T cells in CLL. **(i)** Representative plots, and **(j)** cumulative data of the frequency of 2B4^{+} cells among CD26$^{neg/low/high}$ CD8^{+} T cells in CLL. **(k)** Representative plots, and **(l)** cumulative data of the frequency of TIGIT^{+} cells among CD26$^{neg/low/high}$ CD8^{+} T cells in CLL. **(m)** Representative plots, and **(n)** cumulative data of the frequency of ICOS^{+} cells among CD26$^{neg/low/high}$ CD8^{+} T cells in CLL. **(o)** Representative plots, and **(p)** cumulative data of the frequency of CD28^{+} among CD26$^{neg/low/high}$ CD8^{+} T cells in CLL. **(q)** Representative plots, and **(r)** cumulative data of the frequency of CD27^{+} cells among CD26$^{neg/low/high}$ among CD8^{+} T cells in CLL. **(s)** Representative plots, and **(t)** cumulative data of the frequency of PD-1^{+} cells among CD26$^{neg/low/high}$ CD8^{+} T cells in CLL. **(u)** Representative plots, and **(v)** cumulative data of the frequency of CD39^{+} cells among CD26$^{neg/low/high}$ among CD8^{+} T cells in CLL. **(w)** Representative plots, and **(x)** cumulative data of the frequency of CD73^{+} cells among

$CD26^{neg/low/high}$ $CD8^+$ T cells in CLL. **(y)** Cumulative data showing the frequency of co-inhibitory/co-stimulatory expressing cells in $CD26^{low}CD8^+$ T cells in HC and CLL. Error bars represent the median with an interquartile range. Each dot represents an individual human sample. Florescence minus one (FMO).

3.3.5 $CD26^{neg}CD8^+$ T cells exhibit higher cytotoxic properties.

$CD8^+$ T cells as cytotoxic T lymphocytes play an essential role against virally infected and tumor cells [67,68] via granule-mediated cytotoxicity, FAS-FASL interaction, and the release of cytokines (e.g. TNF-α and IFN-γ) [69]. Granzymes as cytolytic molecules and their harmonized action with perforin (pore forming protein) are required for effective granule-mediated cytotoxicity [70]. To determine the granule-mediated cytotoxic ability of $CD26^{+/-}CD8^+$ T cells, we measured the intracytoplasmic expression of perforin and granzyme-B (GzmB) in $CD26^{neg}$, $CD26^{low}$, and $CD26^{high}$ $CD8^+$ T cells in CLL patients *ex vivo*. We found that in contrast to the $CD26^{neg}$ subset, $CD26^{low}$ and $CD26^{high}$ cells were devoid of perforin and expressed very low levels of GzmB (Fig. 3a-e). Considering the heterogeneous nature of $CD8^+$ T cells, we observed that even $CD26^{low/high}CD8^+$ T cells with effector and effector memory phenotype showed substantial downregulation of perforin/GzmB compared to their $CD26^{neg}$ counterparts in CLL patients (Supplemental Fig. 3b-d). We found the same phenotype in terms of GzmB and perforin expression in $CD26^{low/high}CD8^+$ T cells in HCs (Supplementary Fig. 3e-g). To better characterize the potential cytolytic role of $CD26^+CD8^+$T cells, we subjected them to GzmK expression analysis. Interestingly, we observed that $CD26^{high}CD8^+$ T cells expressed substantial levels of intracytoplasmic GzmK compared to $CD26^{low}$ and $CD26^{neg}$ T cells (Fig. 3f, 3g). Moreover, we assessed the degranulation capacity of $CD8^+$ T cells by measuring

CD107a expression (Lysosomal-associated membrane protein I (LAMP-I)) [71] in response to the global stimulation with anti-CD3/CD28 antibodies. These studies revealed that $CD26^{neg}CD8^{+}$ T cells had higher degranulation capacity following *in vitro* stimulation than $CD26^{low/high}$ $CD8^{+}$ T cells (Fig. 3h, 3i). To characterize the functional properties of $CD26^{+}CD8^{+}$ T cells in response to stimulation, we stimulated them either via T Cell receptor (TCR) (anti-CD3/CD28) or a cytokine cocktail (IL-18+IL-12+IL-15) for 18 hours. We found that TCR-mediated stimulation significantly increased GzmB expression in $CD26^{low}$ and $CD26^{high}$ $CD8^{+}$ T cell populations, while GzmK expression levels remained unchanged (Fig. 3j-m). Of note, TCR stimulation did not change the expression levels of GzmB and GzmK in $CD26^{neg}$ $CD8^{+}$ T cells (Fig. 3j-m). However, cytokine-mediated stimulation significantly enhanced GzmB/GzmK co-expression in all T cell subsets but was more pronounced in the $CD26^{high}CD8^{+}$ T cell subset (Fig. 3j-m). The same expression pattern was observed for the upregulation of perforin in different T cell subsets after stimulation (Supplementary Fig. 3h, 3i). Overall, these observations revealed differential expression of GzmB and GzmK in $CD26^{+/-}CD8^{+}$ T cell subsets in CLL patients. Collectively, while $CD26^{neg}CD8^{+}$ T cells exhibited higher GzmB and perforin expression at the baseline, $CD26^{high}$ and $CD26^{low}$ $CD8^{+}$ T cells acquired a greater cytolytic molecules expression upon stimulation.

3.3.6 $CD26^{high}CD8^{+}$ T cells display higher cytokine-induced responsiveness.

To better delineate the effector functions of $CD26^{+}CD8^{+}$ T cells, we analyzed their cytokine production capacity (e.g., IFN-γ and TNF-α). We found that $CD26^{high}$ exhibited significantly higher IFN-γ, TNF-α, and IFN-γ/TNF-α expression than their

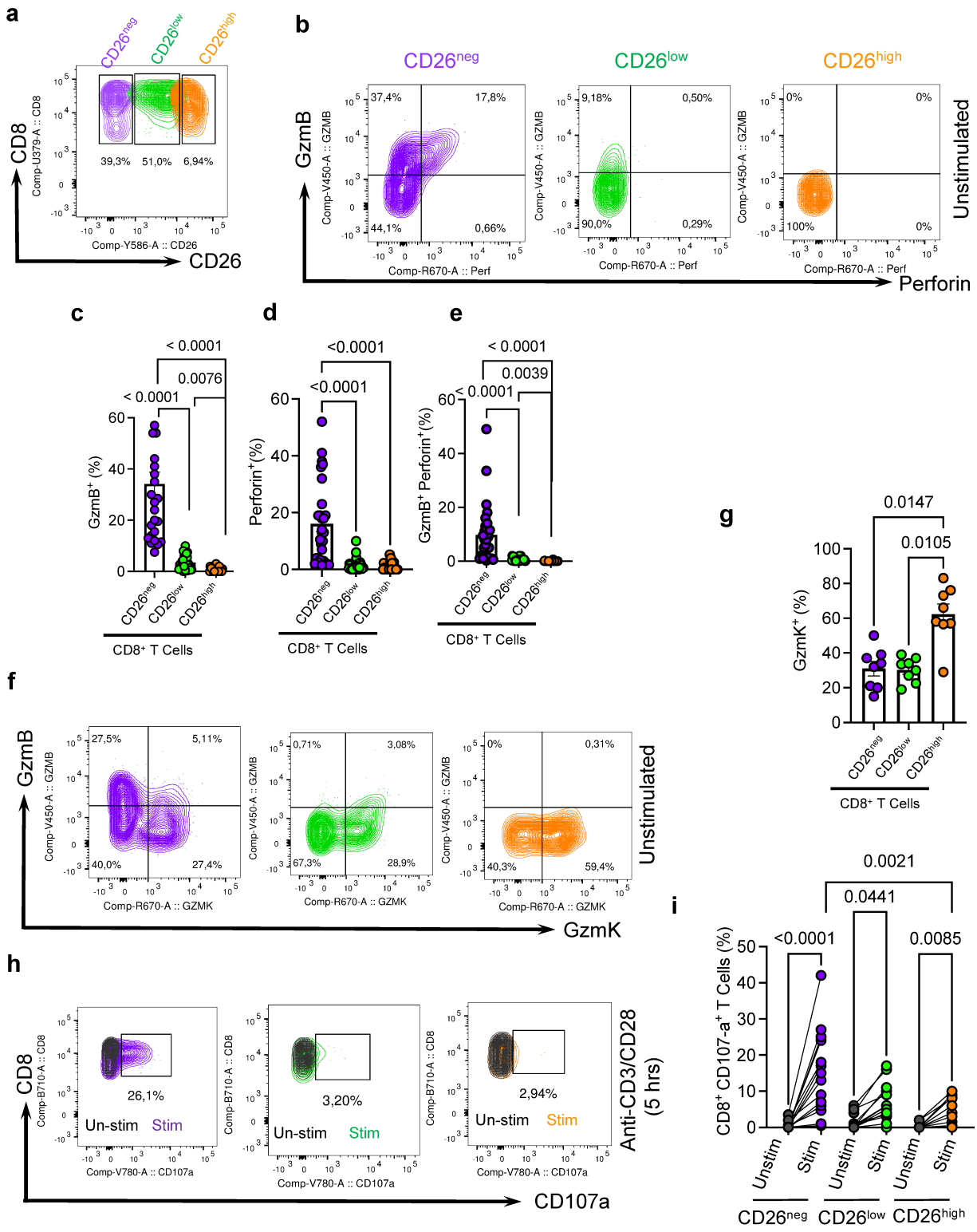
$CD26^{neg}$ counterparts following 5-hour stimulation with anti-CD3/CD28 antibodies *in vitro* (Fig. 4a-d). However, stimulation of PBMCs with PMA for the same period induced higher IFN-γ, TNF-α, and IFN-γ/TNF-α in $CD26^{neg}$ and $CD26^{high}$ compared to $CD26^{low}CD8^{+}$ T cells, while the magnitude of cytokine response was more pronounced in $CD26^{high}CD8^{+}$ T cells (Supplementary Fig. 3j-l). Next, we stimulated PBMCs via TCR-dependent (anti-CD3/CD28) or cytokine-dependent (IL-18+IL-12+IL-15) manners for 18 hours. Interestingly, we found that $CD26^{high}CD8^{+}$ T cells exhibited a greater cytokine production capacity than their other counterparts (e.g., $CD26^{neg}$ and $CD26^{low}$) within the same CLL patients (Fig. 4e-h).

We also measured IL-2 expression following 5-hour PMA stimulation and found that $CD26^{high}$ followed by $CD26^{low}$ T cells had a superior capacity for IL-2 production compared to $CD26^{neg}$ $CD8^{+}$ T cells in CLL patients (Fig. 4i, 4j). However, we found that the proliferative capability of different $CD8^{+}$ T cell subsets did not correspond with their cytokine production capacity. As such, $CD26^{low}CD8^{+}$ effector T cells displayed a significantly greater proliferation compared to their $CD26^{neg/high}$ siblings following 72-hour TCR stimulation *in vitro* (Fig. 4k, 4l). Moreover, we assessed the expression of cytokines in different $CD8^{+}$ T cell subsets in HCs and noted that $CD26^{neg}$ and $CD26^{high}$ cells had higher levels of IFN-γ and TNF-α compared to $CD26^{low}CD8^{+}$ T cells (Supplementary Fig. 3m-o).

Although CTLs are the best-characterized subpopulation of $CD8^{+}$ T cells to kill infected cells or tumor cells, $CD8^{+}$ T cells are highly heterogeneous. For example, it has been reported that environmental cues induce transcriptional factors to differentiate $CD8^{+}$ T cells into Tc1, Tc2, Tc17, and Tc1/Tc17 cells that can be stratified based on the surface expression of CXCR3, CCR6, and CCR4 [72]. Therefore, we further

characterized $CD26^{+}$ and their $CD26^{neg}$ counterparts in CLL patients, which showed $CD26^{high}$ cells were enriched with Tc17 ($CCR4^{+}CCR6^{+}$) and Tc1/Tc17 ($CCR6^{+}CXCR3^{+}$) $CD8^{+}$ T cell phenotype but Tc2 ($CCR4^{+}CCR6^{-}$) $CD8^{+}$ T cells were more abundant in $CD26^{neg}$ and $CD26^{low}$ T cell subsets (Supplementary Fig. 3p-u). In addition, we evaluated the expression of different transcriptional factors in CD26-expressing subsets. We found a higher ROR$\gamma\delta$ expression [72] in $CD26^{high}$ $CD8^{+}$ T cells, which supports the Tc17 skewed phenotype of this T cell subset in CLL patients (Fig. 4m, 4n). However, we did not find any significant difference in T-bet and FOXP3 expression among CD26 subsets in CLL patients (Supplementary Fig. 4a, 4b). Interestingly, we noted a higher expression of TOX transcription factor in $CD26^{high}$ than $CD26^{neg}$ T cells in CLL patients (Supplementary Fig. 4c, 4d). Although TOX may be involved in T cell exhaustion, recent studies suggested that it is expressed by most effector memory/polyfunctional $CD8^{+}$ T cells and not exclusively exhausted T cells in humans [73]. Therefore, our observations support this notion that TOX is not linked to exhaustion but polyfunctionality.

Overall, our observations support the heterogenous nature of $CD26^{high}CD8^{+}$ T cells with a greater cytokine production capacity compared to their $CD26^{neg/low}$ counterparts.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CD8
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CD26
b
CD26neg
CD26low
CD26high
GzmB
Comp-V450-A :: GZMB
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44,1%
0,66%
9,18%
0,50%
90,0%
0,29%
0%
0%
100%
0%
Comp-R670-A :: Perf
Unstimulated
Perforin
c
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GzmB+ (%)
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d
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Perforin+(%)
CD8+ T Cells
e
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0.0039
< 0.0001
GzmB+ Perforin+(%)
CD8+ T Cells
f
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Comp-V450-A :: GZMB
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5,11%
40,0%
27,4%
0,71%
3,08%
67,3%
28,9%
0%
0,31%
40,3%
59,4%
Comp-R670-A :: GZMK
Unstimulated
GzmK
g
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CD26neg
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Stim
3,20%
Un-stim
Stim
2,94%
Un-stim
Stim
Comp-V780-A :: CD107a
Anti-CD3/CD28
(5 hrs)
CD107a
i
0.0021
0.0441
<0.0001
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CD8+CD107-a+ T Cells (%)
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Stim
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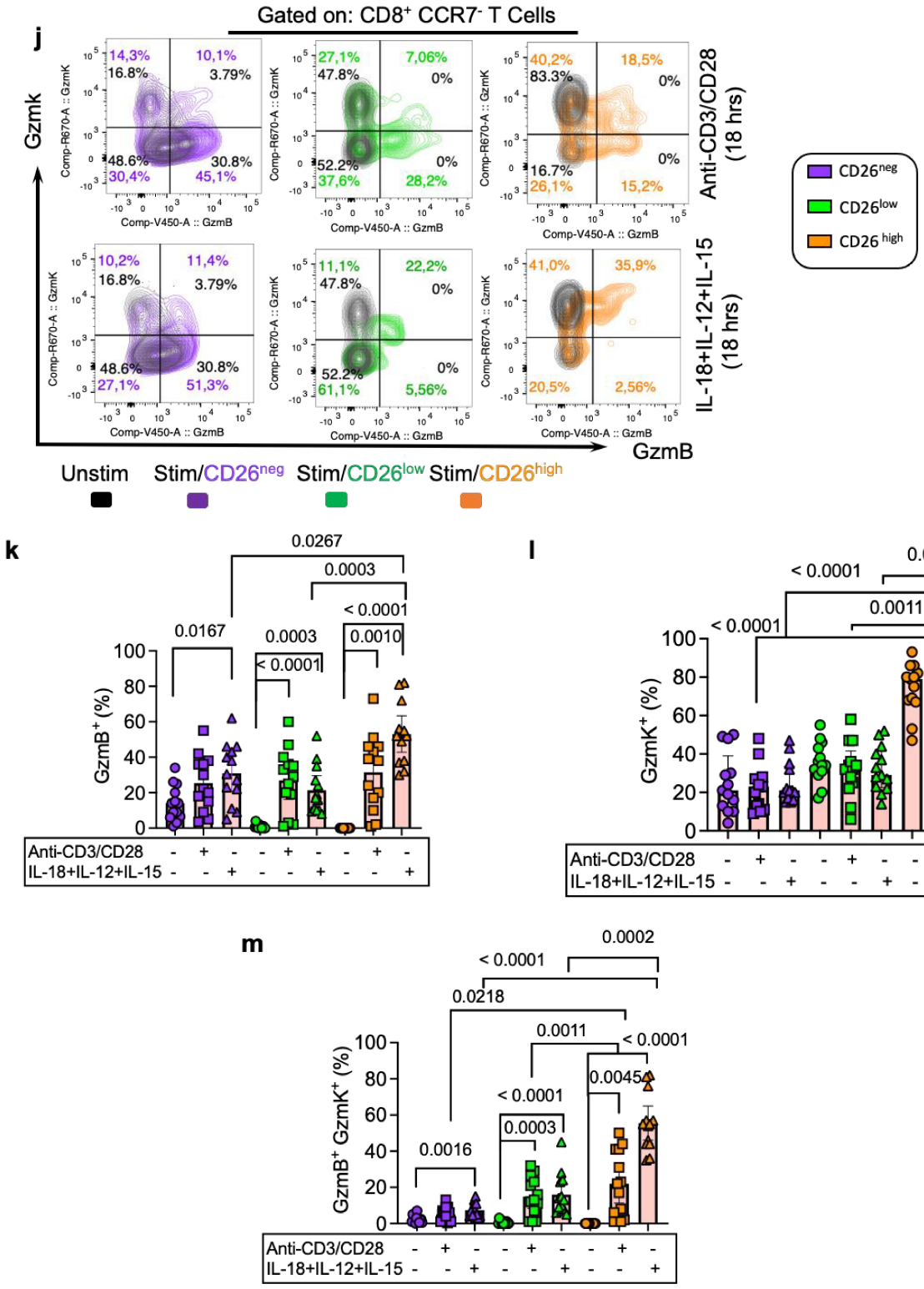
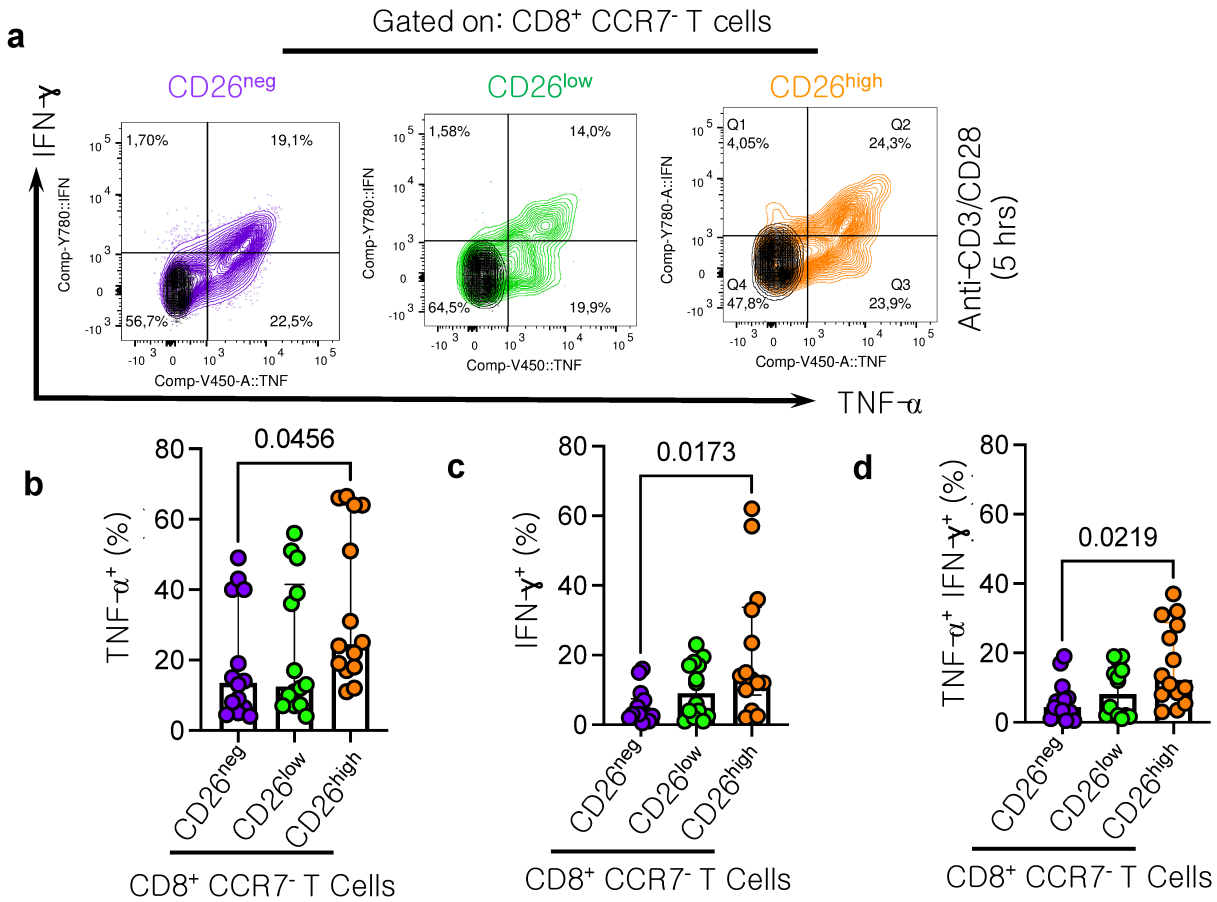


Fig. 3. Cytotoxic properties of CD26$^{neg/low/high}$ CD8^{+} T cells in CLL. (a) Representative flow plot of the gating strategy for CD26^{neg}, CD26^{low}, and CD26^{high} CD8^{+} T cells. **(b)** Representative flow plots of the co-expression of Granzyme-B (GzmB)/Perforin in CD26$^{neg/low/high}$ CD8^{+} T cells in CLL. **(c)** Cumulative data showing

the frequency of $GzmB^+$, **(d)** $Perforin^+$, and **(e)** $GzmB^+Perforin^+$ cells among $CD26^{neg/low/high}$ $CD8^+$ T cells in CLL. **(f)** Representative flow plots, and **(g)** cumulative data of the frequency of $GzmB^+GzmK^+$ cells among $CD26^{neg/low/high}$ $CD8^+$ T cells in CLL. **(h)** Representative flow plots, and **(i)** cumulative data of the frequency of $CD107a^+$ cells among $CD26^{neg/low/high}$ $CD8^+$ T cells in CLL either unstimulated (unstim) or stimulated (stim) with anti-CD3/CD28 (3 μg/ml, 1 μg/ml) in the presence of protein transport inhibitor (1/1000). **(j)** Representative flow plots, and cumulative data of the frequency of **(k)** $GzmB^+$ **(l)** $GzmK^+$, and **(m)** $GzmB^+GzmK^+$ cells among $CD26^{neg/low/high}$ $CCR7^-CD8^+$ T cells in CLL either unstimulated or stimulated with anti-CD3/CD28(3 μg/ml, 1 μg/ml), and a cocktail of IL-18+IL-12+IL-15 (100 ng/ml of each). Error bars represent median with interquartile range. Each dot represents an individual human sample.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CD26low
CD26high
IFN-γ
TNF-α
Anti-CD3/CD28
(5 hrs)
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Comp-V450-A::TNF
Comp-V450::TNF
Comp-Y780-A::IFN
1,70%
19,1%
56,7%
22,5%
1,58%
14,0%
64,5%
19,9%
Q1
4,05%
Q2
24,3%
Q4
47,8%
Q3
23,9%
b
0.0456
TNF-α+ (%)
CD26neg
CD26low
CD26high
CD8+ CCR7- T Cells
c
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IFN-γ+ (%)
CD8+ CCR7- T Cells
d
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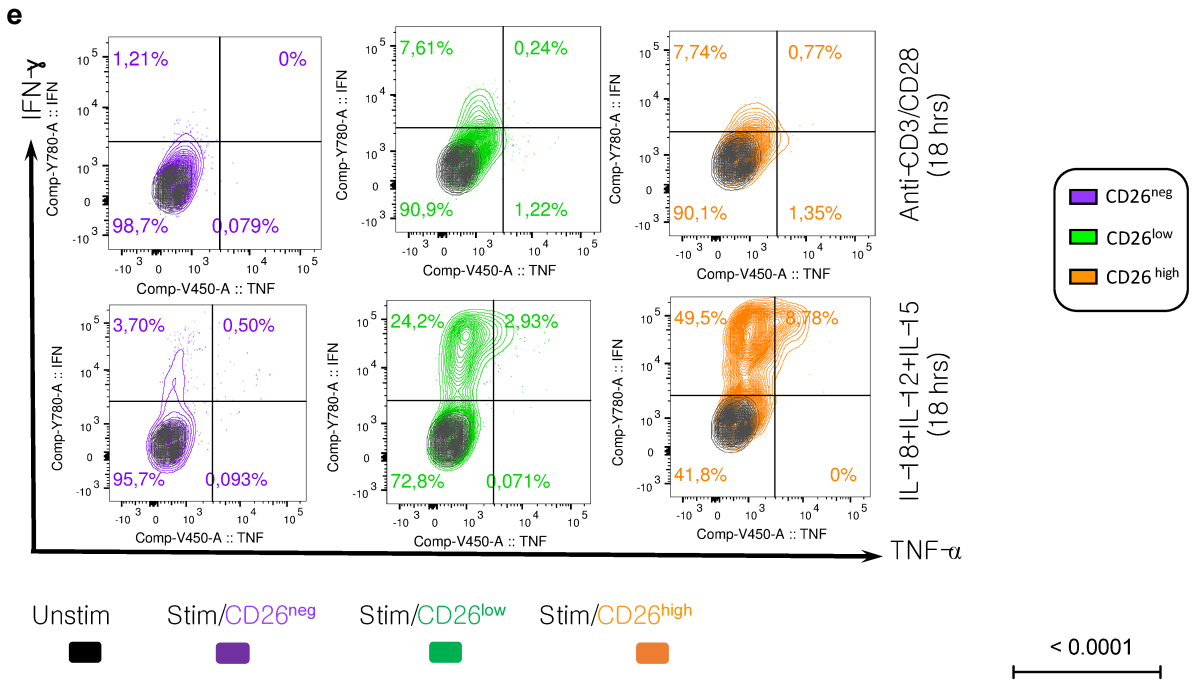
TNF-α+ IFN-γ+ (%)
CD8+ CCR7- T Cells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IFN-γ
TNF-α
Comp-Y780-A :: IFN
Comp-V450-A :: TNF
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0%
98,7%
0,079%
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0,24%
90,9%
1,22%
7,74%
0,77%
90,1%
1,35%
3,70%
0,50%
95,7%
0,093%
24,2%
2,93%
72,8%
0,071%
49,5%
8,78%
41,8%
0%
Anti-CD3/CD28 (18 hrs)
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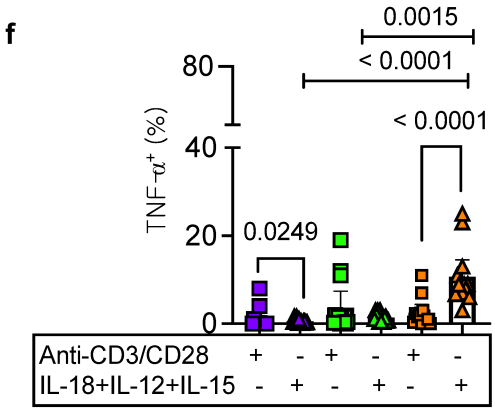
Stim/CD26low
Stim/CD26high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TNF-α+ (%)
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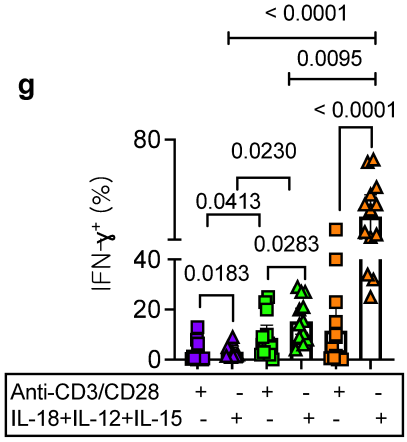
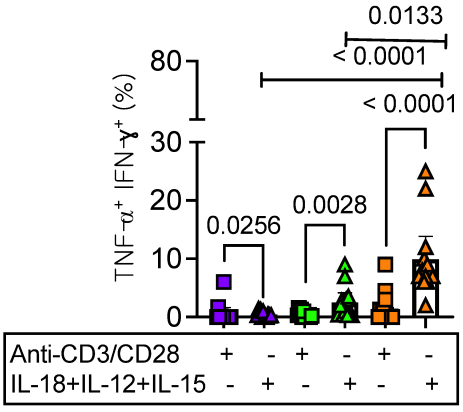
< 0.0001
0.0249
Anti-CD3/CD28
IL-18+IL-12+IL-15


g
IFN-γ+ (%)
< 0.0001
0.0095
< 0.0001
0.0230
0.0413
0.0283
0.0183
Anti-CD3/CD28
IL-18+IL-12+IL-15


h
TNF-α+ IFN-γ+ (%)
0.0133
< 0.0001
< 0.0001
0.0256
0.0028
Anti-CD3/CD28
IL-18+IL-12+IL-15

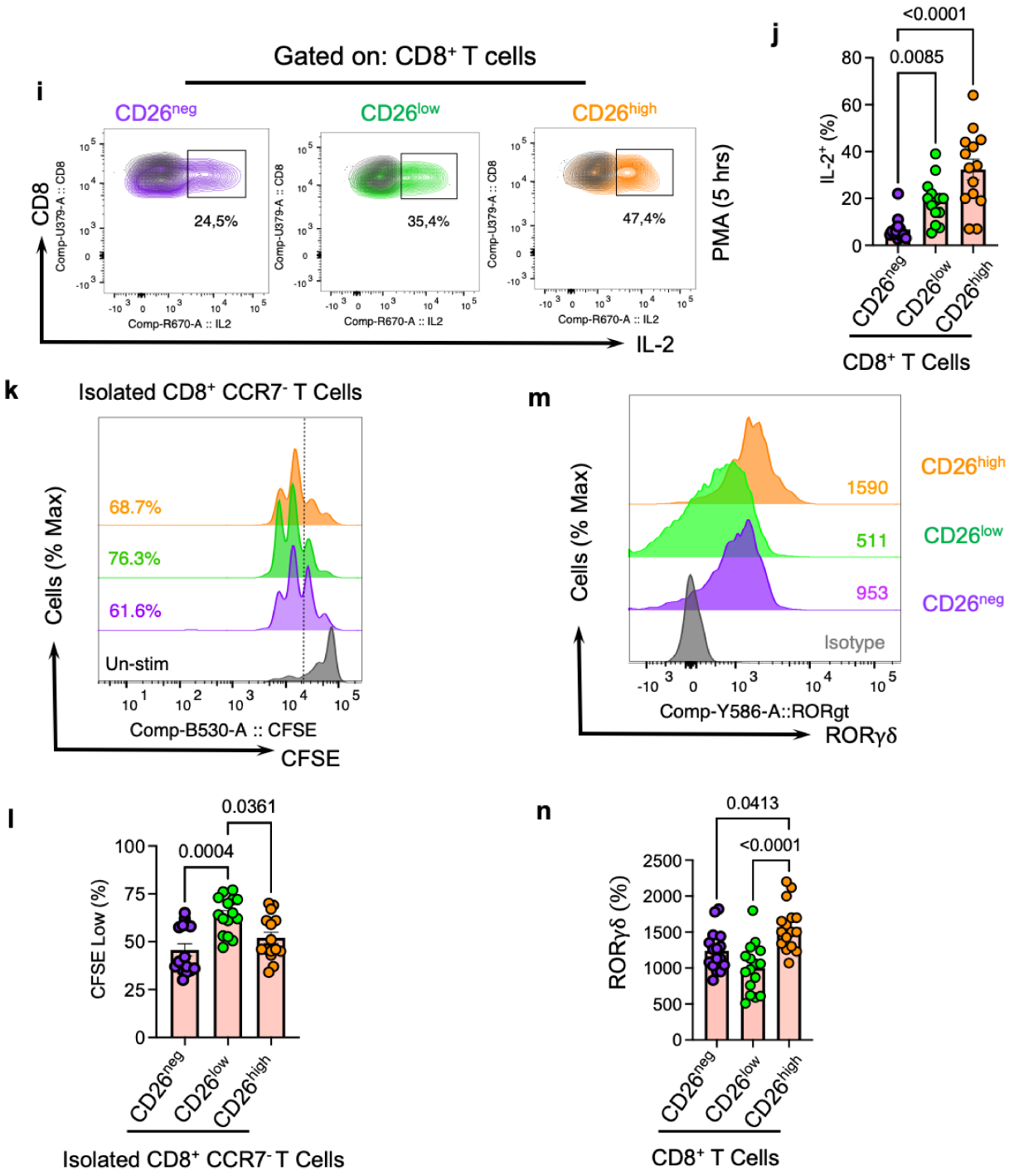


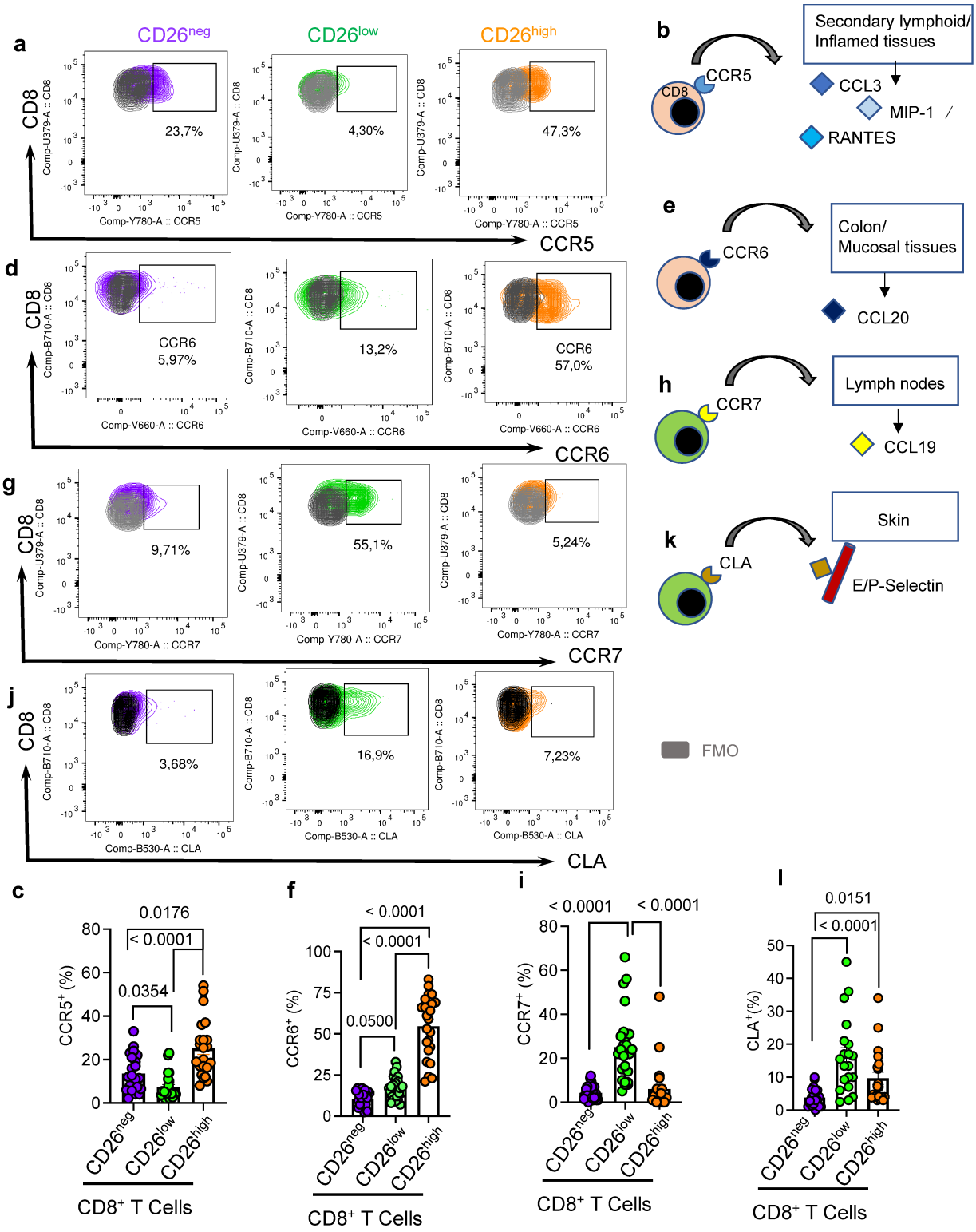
Fig. 4. Cytokine production and proliferation ability of CD26$^{neg/low/high}$ CD8^{+} T cells in CLL. (a) Representative flow plots, **(b)** the frequency of TNF-α, **(c)** IFN-γ, and **(d)** TNF-α^{+}IFN-γ^{+} cells among CD26$^{neg/low/high}$ CCR7^{-}CD8^{+} T cells. **(e)** Representative plots, **(f)** cumulative data showing the frequency of TNF-α^{+}, **(g)** IFN-γ^{+}, and **(h)** TNF-α^{+}IFN-γ^{+} among CD26$^{neg/low/high}$ CCR7^{-}CD8^{+} T cells in unstimulated or stimulated with anti-CD3/CD28 (3μg/ml, 1μg/ml) and a cocktail of IL-18+IL-12+IL-

15 (100 ng/ml of each). **(i)** Representative flow plots and, **(j)** cumulative data of the frequency of IL-2 expressing cells among $CD26^{neg/low/high}$ $CD8^+$ T cells in unstimulated (black color) versus 5 hours after *in-vitro* stimulation with PMA/ionomycin cocktail (Biolegend, 2 ng/ml) in the presence of Brefeldin A (1 μg/ml). **(k)** Representative flow plots and, **(l)** cumulative data of the frequency of $CFSE^{low}$ (proliferated) cells among $CD26^{neg/low/high}$ $CCR7^-CD8^+$ T cells, unstimulated (black color) versus 72 hours stimulation. **(m)** Representative flow plots, and **(n)** cumulative data showing the MFI for RORγδ in $CD26^{neg}$, $CD26^{low}$, and $CD26^{high}$ $CD8^+$ T cells in CLL. Error bars represent the median with an interquartile range. Each dot represents an individual human sample.

3.3.7 $CD26^{high}CD8^+$ T cells possess a greater migratory capacity.

$CD8^+$ T cell migratory ability is crucial for accessing tumor sites, and distinct homing receptors are involved in this process [74]. To better characterize the migratory capacity of $CD26^+CD8^+$ T cell subpopulations, we subjected them to further analysis for the expression of various homing receptors. We found that $CD26^{high}CD8^+$ T cells had significantly higher frequency and intensity of CCR5 expression than $CD26^{neg}$ and $CD26^{low}CD8^+$ T cells in CLL patients (Fig. 5a-c, and Supplementary Fig.4e, 4f). We made similar observations for the proportion/intensity of CCR6 (Fig. 5d-f, Supplementary Fig.4g, 4h), and β7 Integrin in $CD26^{high}CD8^+$ T cells (Supplementary Fig. 4i, 4j). While CCR5 directs migration along RANTES, CCL3, and MIP-1α/β to the secondary lymphoid organs and inflammation sites [75], CCR6 and integrin-β7 traffic T cells towards mucosal tissues such as the gut in response to CCL20 and MadCAM-1 [76,77]. In contrast to $CD26^{high}$, we found significantly a higher proportion of CCR7 expressing cells with greater intensity among $CD26^{low}CD8^+$ T cells (Fig. 5g-i, and Supplementary Fig. 4k, 4l), which implies these cells tend to home to lymph nodes in response to CCL19 [78]. Similar observations were made for the Cutaneous Lymphocyte Antigen (CLA), a skin homing-receptor via binding to E selectins [79], in $CD26^{low}CD8^+$ T cells (Fig. 5j-l, and Supplementary Fig.4m, 4n). It is worth mentioning

that we did not find any significant difference in the frequency/intensity of T cells expressing either CXCR3 or CXCR4 among $CD26^{neg}/CD26^{low}/CD26^{high}$ $CD8^+$ T cells in CLL patients (Supplementary Fig. 4o-r). Finally, we investigated the expression of CCR4 and found a higher advantage of $CD26^+$ versus their $CD26^{neg}$ $CD8^+$ T cells counterparts for the expression of this skin homing receptor [75] (Supplementary Fig.4s). To better delineate the migration capabilities of $CD26^{neg}/CD26^+CD8^+$ T cells, we performed a trans-well migration assay on PBMCs from CLL patients. We observed that $CD26^{neg}$ and $CD26^{high}$ exhibited equally but significantly higher migratory capacity than their $CD26^{low}$ counterparts toward a general chemoattractant (Fetal bovine serum 10%) when examined after 18 hr (Fig.5m, 5n). However, $CD26^{high}$ cells displayed an enhanced migration ability towards RANTES and IL-18 compared to $CD26^{neg}/CD26^{low}$ $CD8^+$ T cells, possibly due to higher expression of CCR5 and IL-18Rα (Fig. 5m, 5o, 5p). We also measured the proportion of CD69-expressing cells among different $CD26^{+/-}$ subsets. CD69, as an early activation marker, is reported to regulate the retention of T cells from the periphery into tissues to generate tissue-resident memory T cells [80]. Interestingly, we noted that $CD26^{high}$ subset was significantly enriched with CD69 expressing cells compared to $CD26^{neg/low}$ $CD8^+$ T cells in CLL patients (Supplementary Fig. 5a, 5b). Collectively, our results suggest that $CD26^{high}CD8^+$ T cells have a greater migratory trait to peripheral organs such as the gut, mucosal surfaces, and inflamed tissues. In contrast, the $CD26^{low}$ subset has a higher homing capacity to lymph nodes and skin.


a
CD26neg
CD26low
CD26high
CD8
23,7%
4,30%
47,3%
Comp-Y780-A :: CCR5
CCR5
b
CCR5
CD8
Secondary lymphoid/
Inflamed tissues
CCL3
MIP-1 /
RANTES
d
CCR6
5,97%
13,2%
57,0%
Comp-V660-A :: CCR6
CCR6
e
CCR6
Colon/
Mucosal tissues
CCL20
g
9,71%
55,1%
5,24%
Comp-Y780-A :: CCR7
CCR7
h
CCR7
Lymph nodes
CCL19
j
3,68%
16,9%
7,23%
Comp-B530-A :: CLA
CLA
k
CLA
Skin
E/P-Selectin
FMO
c
0.0176
< 0.0001
0.0354
CCR5+ (%)
CD26neg
CD26low
CD26high
CD8+ T Cells
f
< 0.0001
< 0.0001
0.0500
CCR6+ (%)
CD8+ T Cells
i
< 0.0001
< 0.0001
CCR7+ (%)
CD8+ T Cells
l
0.0151
< 0.0001
CLA+(%)
CD8+ T Cells

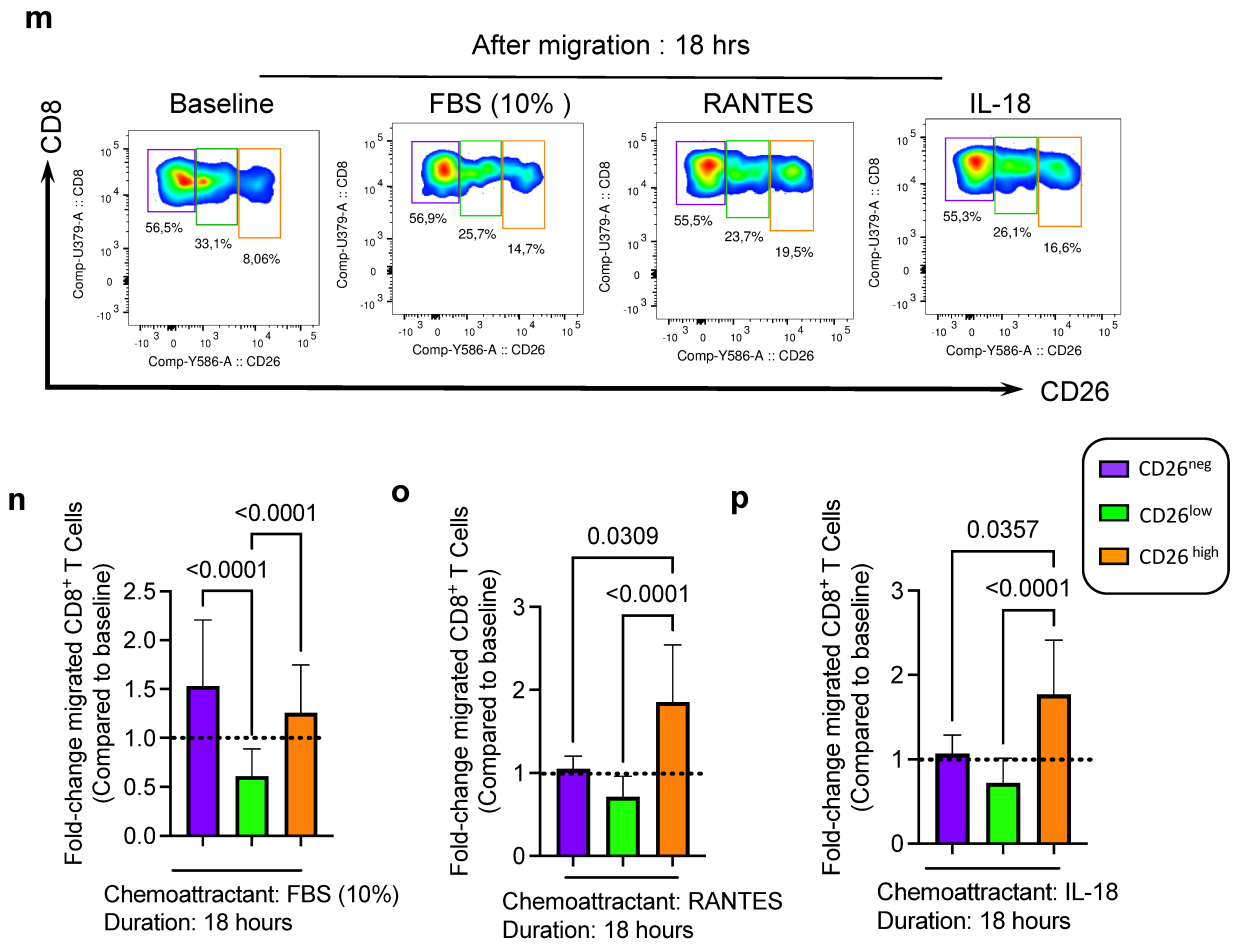


Fig. 5. CD26^{+}CD8^{+} T cells exhibit a greater migratory capacity in CLL. **(a)** Representative plots of the frequency of CCR5^{+}CD8^{+} T cells among CD26$^{neg/low/high}$ subsets. **(b)** The graphical illustrates the tendency of CCR5^{+}CD8^{+} T cells homing to secondary lymphoid organs or inflamed tissues in response to CCL3, MIP-1α/β, and RANTES. **(c)** Cumulative data of the frequency of CCR5^{+}CD8^{+} T cells among CD26$^{neg/low/high}$ CD8+ T cells in CLL. **(d)** Representative flow plots of the frequency CCR6^{+}CD8^{+} T cells among CD26$^{neg/low/high}$ subsets. **(e)** The graphical illustrates the tendency of CCR6^{+}CD8^{+} T cells homing to colon, and mucosal tissues in response to CCL20. **(f)** Cumulative data of the frequency of CCR6^{+}CD8^{+} T cells among CD26$^{neg/low/high}$. **(g)** Representative plots of the frequency of CCR7^{+}CD8^{+} T cells among CD26$^{neg/low/high}$ subsets of CD8^{+} T cells. **(h)** The graphical illustrates the tendency of CCR7^{+}CD8^{+} T cells homing to lymph nodes in response to CCL19. **(i)** Cumulative data of the frequency of CCR7^{+}CD8^{+} T cells among CD26$^{neg/low/high}$ subsets. **(j)** Representative plots of the frequency of Cutaneous Lymphocyte Antigen (CLA)$^{+}$CD8^{+} T cells among CD26$^{neg/low/high}$ cells. **(k)** The graphical illustrates the tendency of CLA^{+}CD8^{+} T cells homing to skin following E/P Selectin binding on endothelial cells. **(l)** Cumulative data of the frequency of CLA^{+}CD8^{+} T cells among CD26$^{neg/low/high}$ cells. **(m)** Representative plots, and the frequency of migrated CD26^{neg}, CD26^{low}, and CD26^{high} CD8^{+} T cells at the baseline and after 18 hours in response to **(n)** Fetal Bovine Serum (FBS-10%), **(o)** RANTES (10 nM), and **(p)** IL-

18 (100ng/ml). Error bars represent the median with an interquartile range. Each dot represents an individual human sample.

3.3.8 $CD26^{low}CD8^{+}$ T cells are long-lived compared to $CD26^{neg/high}CD8^{+}$ T cells.

To evaluate the survival capacity of these three subpopulations of $CD8^{+}$ T cells, we subjected them to KLRG1 and CD127 (IL-7Rα) expression analysis because long-lived T cells express high levels of CD127 but low levels of KLRG1 [81]. Our studies show that $CD26^{low}CD8^{+}$ T cells have higher levels of CD127 but lower levels of KLRG1 expression in CLL patients (Fig. 6a, 6b). Given these observations, we next examined $CD26^{neg/low/high}$ cells in terms of apoptosis. This apoptotic assay confirmed the longevity of $CD26^{low}CD8^{+}$ T cells as they showed lesser Annexin-V expression than their $CD26^{neg}$ and $CD26^{high}$ counterparts (Fig. 6c, 6d). In parallel, we measured the frequency of T stem cell memory (TSCM) [63] ($CD45RA^{+}CCR7^{+}CD95^{+}$) in $CD26^{neg}$, $CD26^{low}$, and $CD26^{high}$ subsets of $CD8^{+}$ T cells. These analyses revealed that the $CD26^{low}$ subset had a significantly higher proportion of TSCM cells compared to their $CD26^{neg/high}$ counterparts, supporting a higher self-renewal propensity (Supplemental Fig. 5c). As $CD8^{+}$ T cells acquire terminal differentiation phenotype, they upregulate the expression of CD57 and/or CD16 along with GzmB and perforin. As a result, we observed a higher expression of CD57 and CD16 among $CD26^{neg}$ $CD8^{+}$ T cells (Fig. 6e, f, and Supplementary Fig. 5d). This is consistent with our previous observation of a higher GzmB and perforin expression in this subpopulation (Fig. 3a, b).

3.3.9 $CD26^{high}CD8^{+}$ T cells are sensitive to Galectin-9 (Gal-9) induced apoptosis.

To better investigate the mechanism associated with decreased CD26 frequency in CLL patients, we treated PBMCs from HCs with plasma (10%) obtained from CLL patients. After overnight culture, we found a significant reduction in the intensity of CD26 expression (Supplemental Fig. 5e, 5f). This observation suggested the presence of potential soluble mediator(s) in reducing CD26 expression in CLL patients. To identify the potential soluble factor, we performed multiplex ELISA and quantified 20 different cytokines/chemokines (Supplemental Fig. 5g, 5h). We tested the effects of some of the most abundant cytokines on CD26 expression, however, these cytokines exhibited no effects or increased the intensity of CD26 expression in CD8 T cells (Supplementary Fig. 5i-n). Next, we measured the levels of TGF-β as a potential contributing factor in the attenuation of CD26, as reported in human breast cancer [82]. Although total TGF-β levels were the same in HCs and CLL patients, we noted a significant decrease in the plasma levels of active form of TGF-β in CLL patients (Supplementary Fig. 5o, 5p). In agreement with the other report in breast cancer, we observed a significant decline in CD26 expression upon treatment with TGF-β *in vitro* (Supplementary Fig. 5q). Notably, we discovered a moderate but inverse correlation between the plasma free TGF-β level with the percentages of CD26-expressing $CD8^+$ T cells in CLL patients (Supplementary Fig. 5r). Although TGF-β may contribute to the reduction of CD26 levels, CLL patients had significantly lower levels of this cytokine in their plasma than HCs. Considering the apoptotic effects of Gal-9 on highly activated $CD8^+$ T cells [83,84] and our previous studies [53,85] we hypothesized that Gal-9 might contribute to the depletion of highly polyfunctional $CD26^{high}CD8^+$ T cells in CLL. Therefore, we found a significant elevation in the plasma Gal-9 levels in CLL patients versus HCs (Fig. 6g).

To test our hypothesis, we treated total $CD8^+$ T cells from HCs with recombinant human Gal-9 (0.02 $\mu g/ml$, physiologically relevant to the plasma levels) for 18 hours. We observed a significant decrease in the frequency of the $CD26^{high}$ subset (Fig. 6h, 6i), which was consistent with more robust apoptosis of $CD26^{high}CD8^+$ T cells (Fig. 6j, 6k). Overall, we discovered that Gal-9 exhibited a more pronounced apoptotic effect on the $CD26^{high}$ population than the $CD26^{low}$ and $CD26^{neg}$ subsets (Supplementary Fig. 5s). To identify the possible source of Gal-9, we cultured PBMCs from CLL and HCs overnight *in vitro* and subjected their culture supernatants to Gal-9 quantification. This study revealed a significantly higher Gal-9 shedding in PBMCs from CLL patients (Supplementary Fig. 5t). Our further analysis confirmed B-CLLs as the major source of Gal-9 when compared to their non-B-CLL counterparts (Fig. 6l). Moreover, we found significantly higher levels of intracytoplasmic Gal-9 in B-CLL compared to healthy B cells (Fig. 6m, 6n). These results suggest that malignant B cells are a significant source of increased Gal-9 in CLL. Also, due to the elevated levels of plasma IL-18, IL-12, and IL-15 in CLL patients (Supplementary Fig. 5u-w), we assessed the potential effects of these cytokines on CD26 expression. We found that this cytokine cocktail, at physiological concentration detected in the plasma, significantly enhanced apoptosis of $CD26^{high}CD8^+$ T cells (Fig. 6o, 6p, and Supplementary Fig. 6a). Overall, these observations suggest that B-CLL cells as a major source of elevated Gal-9 in CLL plasma could contribute to the depletion of $CD26^{high}CD8^+$ T cells. Alternatively, IL-18+IL-12+IL-15 may promote apoptosis of $CD26^{high}CD8^+$ T cells in CLL. However, these cytokines individually do not impact the expression of CD26.

3.3.10 $CD26^{+}$ T cells in mice have a different phenotype than their counterparts in humans.

To investigate further the role of CD26 cells in an animal model, we measured the frequency of CD26 in $CD8^{+}$ T cells of BALB/c mice. Surprisingly, we found that nearly 100% of $CD8^{+}$ T cells in mice regardless of their niche expressed CD26 (e.g., thymus, blood, and spleen) (Supplementary Fig. 6b, 6c). In addition, we did not see $CD26^{high/low}$ subpopulations in $CD8^{+}$ T cells of mice, therefore, the CD26 expression pattern is completely different in mice than humans. More importantly, our observations show that $CD26^{high}CD8^{+}$ T cells are enriched with MAIT cells in humans (Fig. 2); however, mice MAIT cells have a $CD44^{high}CD62^{LOW}$ phenotype [86]. These observations demonstrate that CD26-expressing cells have a different phenotype in humans than mice.

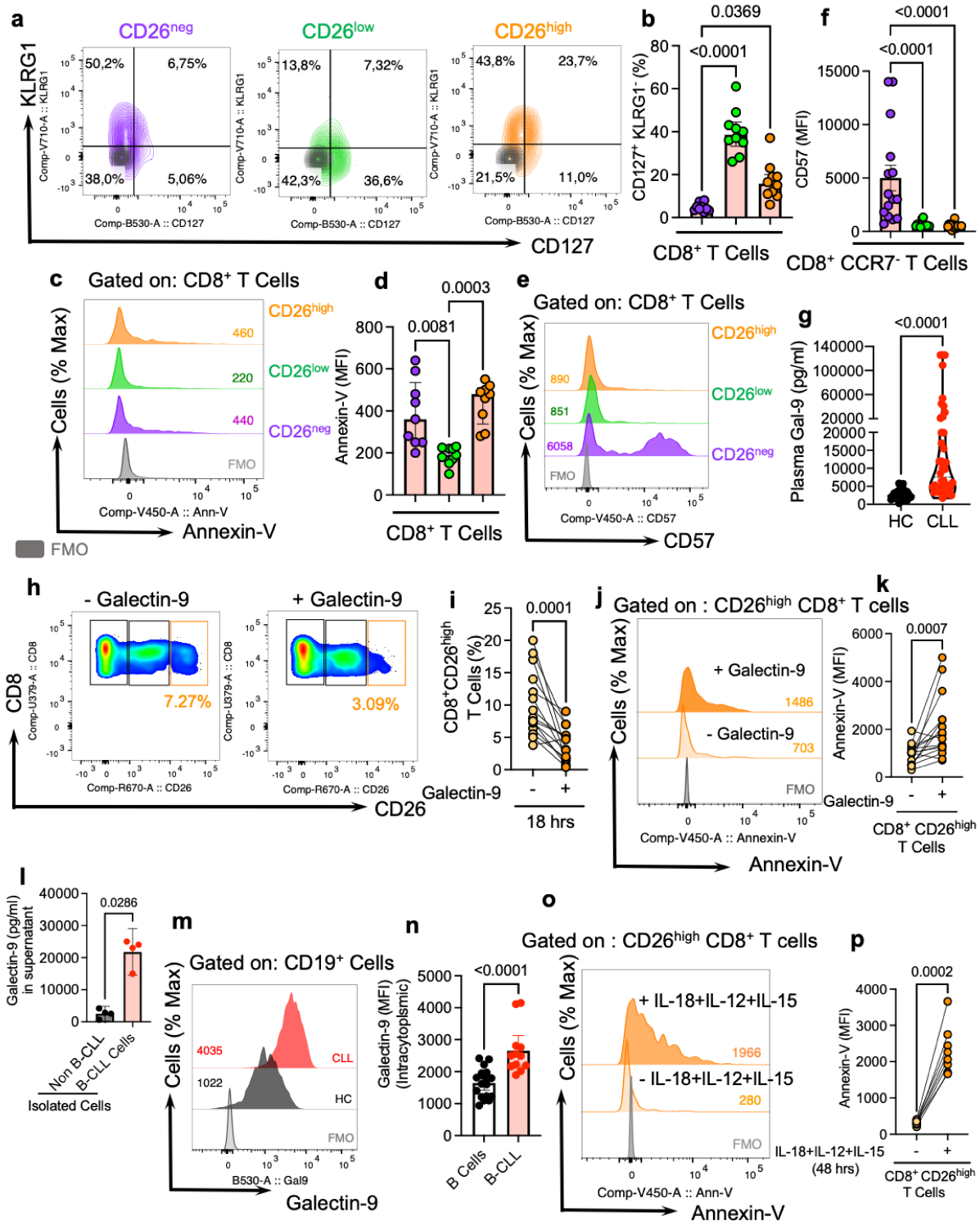


Fig. 6. Gal-9 preferentially promotes CD26^{high} CD8^{+} T cells apoptosis in CLL. **(a)** Representative plots, and **(b)** cumulative data of the frequency of

$CD127^+/KLRG1^-$ cells among $CD26^{neg/low/high}$ subsets of $CD8^+$ T cells. **(c)** Representative plots, and **(d)** cumulative data of the intensity of Annexin-V expression (MFI) among $CD26^{neg/low/high}$ $CD8^+$ T cells. **(e)** Representative plots, and **(f)** cumulative data of the MFI of $CD57^+$ among $CD26^{neg/low/high}$ $CD8^+$ T cells. **(g)** Cumulative data of the plasma Gal-9 concentrations in HCs and CLL patients. **(h)** Representative plots, and **(i)** cumulative data of the frequency of $CD26^{high}CD8^+$ T cells following stimulation with anti-CD3/CD28 in the presence or absence of recombinant human Gal-9 (0.02 μg/ml). **(j)** Representative plots, and **(k)** cumulative data of the intensity of Annexin-V expression in $CD26^{high}$ $CD8^+$ T cells following stimulation with anti-CD3/CD28 in the presence or absence of Gal-9. **(l)** Cumulative data of Gal-9 concentration in the supernatants of isolated non-B cells versus malignant B cells (B-CLL) after 12 hours culture. **(m)** Representative plots, and **(n)** cumulative data of the intracytoplasmic MFI of Gal-9 in B cells from HC versus B-CLLs. **(o)** Representative plots, and **(p)** cumulative data of the frequency of Annexin-V expressing $CD8^+$ T cells in $CD26^{high}$ cells at the baseline versus stimulation with a cytokine cocktail (IL-18+IL-12+IL-15) (100 ng/ml of each) for 18 hrs. Error bars represent the median with an interquartile range. Each dot represents an individual human sample.

3.4 Discussion

$CD8^+$ T cells become dysfunctional/exhausted during chronic conditions such as cancer [85,87]. Clinical approaches such as immune checkpoint blockers and adoptive immune cell therapies have shown promising outcomes in different cancer types. One advantage of adoptive cell therapy is identifying and infusing selected polyfunctional $CD8^+$ T cells with enhanced antitumor properties into cancer patients. Our study provides a novel insight into the immunological properties of $CD26^+CD8^+$ T cells in CLL patients. We observed significant decline of polyfunctional $CD26^+CD8^+$ T cells in CLL patients.

First, we stratified $CD26^+CD8^+$ T cells into $CD26^{low}/CD26^{high}$ and observed that the percentage and total number of these cells were declined in CLL patients. We discovered that $CD26^{high}CD8^+$ T cells were mainly transitional and effector memory cells, as reported elsewhere [28]. However, the $CD26^{low}$ subset was highly enriched with naïve, stem cell and central memory $CD8^+$ T cells. Our finding that both $CD26^{high}$

and $CD26^{low}$ were augmented with $CD27^{+}$ T cells suggests that such cells may have a selective advantage compared to $CD26^{neg}CD8^{+}$ T cells. The increased proliferative capacity and IL-2 production by $CD26^{high}$/$CD26^{low}$ cells likely reflect a costimulatory signal from CD27 vis NF-κB activation [88]. The presence of such signal from CD27 may contribute to the enhanced survival and persistence of antigen-specific $CD8^{+}$ T cells to protect the host from malignancy, as reported in HIV-infected individuals [89]. Moreover, we found that $CD26^{high}CD8^{+}$ T cells displayed a Tc1/Tc17 phenotype. This was illustrated by the abundance of CXCR3/CCR6/CCR4 expressing cells in this subpopulation [72]. Notably, we noted that $CD26^{high}CD8^{+}$ T cells had a prominent propensity to exhibit cytotoxic properties by high expression of GzmB, perforin, and IFN$-\gamma$ upon stimulation with a cytokine cocktail (IL-12+IL-18+IL-15) or TCR, as reported for MAIT cells [90]. Of note, $CD26^{+}CD8^{+}$ T cells exhibited a more robust response to the cytokine cocktail than TCR-induced stimulation. Furthermore, they displayed a greater IL-2 expression capacity. This intrinsic IL-2 production capacity of $CD26^{high}CD8^{+}$ T cells may enable them to have a stemness-like feature as reported in chronic viral infection [91]. In addition, elevated GzmK contents in $CD26^{high}CD8^{+}$ T cells in a quiescent state, along with stimulation-induced upregulation of GzmB, further support their polyfunctionality [92].

Moreover, we characterized $CD26^{+}CD8^{+}$ T cells based on the defined surrogate markers for MAIT cells ($CD161^{high}$ TVα7.2^{+}) [65]. Although $CD26^{high}CD8^{+}$ T cells were enriched with MAIT-like cells, they display a heterogenous subset of $CD8^{+}$ T cells in CLL patients. This is consistent with another reports that MAIT-like surrogate markers can be affected by the disease status [65] and are not definite markers for MAIT cell identification. To overcome this issue, the implication of MR-1 tetramers as a

confirmatory approach for the identification of MAIT cells within CD26^{high}CD8^{+} T cells has been suggested [65]. Also, we noted a higher ROR$\gamma\delta$ expression in CD26^{high} CD8^{+} T cells in favor of a Tc17 or MAIT17 phenotype [72]. The plasticity of Tc17 cells and their either protective or pathogenic role in the context of cancer has been the subject of controversy [93]. Therefore, further studies are required to appreciate better the role of CD26^{high}CD8^{+} T cells in CLL patients and other solid cancers.

Moreover, we found that CD26^{high}CD8^{+} T cells express elevated levels of IL-18Rα, which enables them to respond to cytokine-induced stimulation (e.g., IL-18) and migrate toward the inflammation site as reported for MAIT cells in bacterial infection [94].

In addition, we found it intriguing that CD26^{high}CD8^{+} T cells express chemokine receptors such as CCR5, CCR6, integrin-β7, and CD69, which possibly promotes the trafficking of CD26^{high}CD8^{+} T cells into inflamed tissues (mucosal sites) and tumors. Collectively, these capabilities signify the multifunctional and plasticity of CD26^{high} cells to protect against bacterial infections and tumors. Based on our observations, we posit that CD26^{high}CD8^{+} T cell deficiency might be one potential explanation for the increased susceptibility to recurrent bacterial infections and tumor progression in CLL patients. Therefore, CD26^{high}CD8^{+}T cells may employ key mechanisms such as polyfunctionality, migration, and stemness to survive and destroy cancer cells, as reported for MAIT cells [95].

In contrast to CD26^{high}T cells, we discovered that CD26^{low}CD8^{+} T cells mainly were naïve, stem cells memory, and central memory, but their frequency gradually decreased as they differentiated into transitional memory, effector memory, and effector T cells. Although CD26^{high} and CD26^{low}CD8^{+} T cells displayed some similar

characteristics, they were distinct in many aspects. For instance, $CD26^{low}CD8^{+}$ T cells acquired cytotoxic properties upon TCR and cytokine-triggered stimulation. In addition, we observed that $CD26^{low}CD8^{+}$ T cells were long-lived memory T cells ($CD127^{+}$ $KLRG1^{-}$) with higher proliferative capabilities and more stemness-cell- like features, as reported elsewhere [96]. These properties make $CD26^{low}CD8^{+}$ T cells a potential reservoir of long-lived memory cells with crucial roles in immune homeostasis and response to tumor cells.

To gain a better insight into different $CD8^{+}$ T cell subsets, we also studied $CD26^{neg}CD8^{+}$ T cells in CLL and HCs. These studies revealed that $CD26^{neg}CD8^{+}$ T cells mimicked antigen-experienced T cells. The main crowd of $CD26^{neg}CD8^{+}$ T cells closely resembled transitional, effector memory, and effector T cells. We found that $CD26^{neg}$ T cells enriched with CXCR3/CCR4 expression and displayed a Tc1/Tc2 profile. Furthermore, these cells contained high levels of GzmB and perforin content in the absence of reactivation, and TCR or cytokine-mediated stimulation did not impact their cytolytic molecules expression capabilities *in vitro*. This suggests that $CD26^{neg}CD8^{+}$ T cells are at their maximum functional potentials with minimal plasticity. In addition to cytolytic properties (high GzmB, perforin, and CD107 expression), $CD26^{neg}$ T cells had elevated levels of CD57 and CD16, which fulfills the criteria of terminal effector T cells [97,98]. Moreover, we found that $CD26^{neg}$ T cells express elevated levels of co-inhibitory receptors (e.g., CD160, TIGIT, 2B4, CD39) but lower levels of co-stimulatory receptors (e.g., CD28, CD27). These observations beg the question of whether $CD26^{neg}$ T cell display an exhausted phenotype in CLL patients. This hypothesis was supported by their lower cytokine expression (e.g.,

IFN-γ and IL-2), proliferative capacity, and minimal responsiveness to *in vitro* stimulation.

We found it intriguing that $CD26^{neg}$ T cells substantially had lower CD28 expression than their $CD26^{low/high}$ siblings. Although we were unable to delineate the underlying mechanism, gradual downregulation of CD28 expression in T cells in response to chronic antigenic stimulation and aging has been reported [99], as we observed in our cohort (Supplementary Fig. 6d). Also, the elevated expression of CD57 levels in the $CD26^{neg}$ T cell subpopulation supports the concept of chronic antigen-dependent differentiation and proliferation [100].

Although the role of $CD26^{+}CD8^{+}$ T cells in cancer models has not been investigated, polyfunctional $CD4^{+}CD26^{high}$ T cells display markers of stemness/migration and elicit anti-tumor activity in different malignancies [26]. On the contrary, the expression of $CD26^{+}$ on cancer cells is linked to stemness, invasiveness, and increased metastatic capability [101]. Moreover, the enzymatic activity of CD26 in cancer models has not been fully understood. For example, one group reported that CD26 inhibition was associated with improved anti-tumor immunity [102]. However, another group showed that CD26 inhibition promotes tumor progression/metastasis [103]. As such, further investigation on the immunological role of CD26 beyond T cells in cancer models is needed. Such studies will enable us to determine whether genetic or therapeutic manipulation of CD26 expression can promote anti-tumor immunity.

This is the first study, to our knowledge, that characterizes human $CD8^{+}$ T cell subsets by CD26 expression and analyses their effector functions in CLL patients versus HCs. Specifically, our major findings are fourfold. The major results of our findings are summarized in the table shown in Fig.7a. Firstly, we found that $CD8^{+}$ T cells

expressing either low or high levels of CD26 were decreased in CLL patients. Secondly, our observations revealed that $CD26^{low/high}$ T cells were not terminally differentiated compared to $CD26^{neg}$ T cells. The $CD26^{high}$ T cell subset had transitional/effector memory and $CD26^{low}$ naïve and stem cell/central memory phenotype. Thirdly, the higher migration capacity of $CD26^{+}$ T cells may support their trafficking to lymph nodes and inflamed organs or the tumor microenvironment (TME). Whether this migratory capacity explains their deficiency in blood circulation needs to be determined. Fourthly, our finding that $CD26^{high}$/$CD26^{low}$ $CD8^{+}$ T cells are polyfunctional and exhibit greater migratory capacity, stemness, longevity, and proliferation capability make them a potential candidate for adoptive T cell transfer or CAR T cell therapy in CLL. Moreover, we found elevated levels of Gal-9 in the plasma of CLL patients. Considering particular apoptotic properties of Gal-9 on $CD8^{+}$ T cells, we discovered that $CD26^{high}CD8^{+}$ T cells were susceptible to apoptosis following exposure to Gal-9 and IL18+IL12+IL-15 *in vitro*. Therefore, the inflammatory milieu of CLL with the elevated levels of IL-18, IL-12, IL-15, and Gal-9 that are released from CLL cells might explain a mechanism that results in the reduction of $CD26^{high}CD8^{+}$ T cells pool in CLL (Fig. 7b). More importantly, Gal-9 is strongly associated with the elevation of pro-inflammatory cytokines/chemokines [54]. As such, Gal-9 might be involved in the inflammatory cascade and indirectly compromises anti-tumor immunity by depleting polyfunctional $CD26^{+}CD8^{+}$ T cells in CLL. Whether targeting Gal-9 could prevent the elimination of $CD26^{+}$ T cells in CLL merits further investigations.

We are aware of multiple study limitations. The age factor might impact $CD26^{+}$ T cell frequency [104]; however, this was not the case in our cohort.

Notably, a decline in $CD26^{+}CD8^{+}$ T cells has been reported in chronic viral infections such as HIV, CMV, and EBV [33]. Therefore, there is a possibility that $CD26^{high}CD8^{+}$ T cells, due to their enhanced trafficking abilities, are attracted to the gut and inflamed tissues whereas $CD26^{low}$ T cells are more likely to home to skin and lymph nodes. From another perspective, decreased $CD26^{+}CD8^{+}$ T cells might be considered as a potential predisposing mechanism for increased infection rates in CLL patients. Another limitation of our study was a small/single-centered cohort. Therefore, we strongly recommend performing similar studies in larger multiple-centered cohorts and different hematological malignancies and solid tumors to appreciate better the role of $CD26^{+}CD8^{+}$ T cells in cancer. Although we know that T cells in the blood circulation differ strongly from lymph node-derived T cells in CLL, performing such an invasive procedure is uncommon in CLL patients.

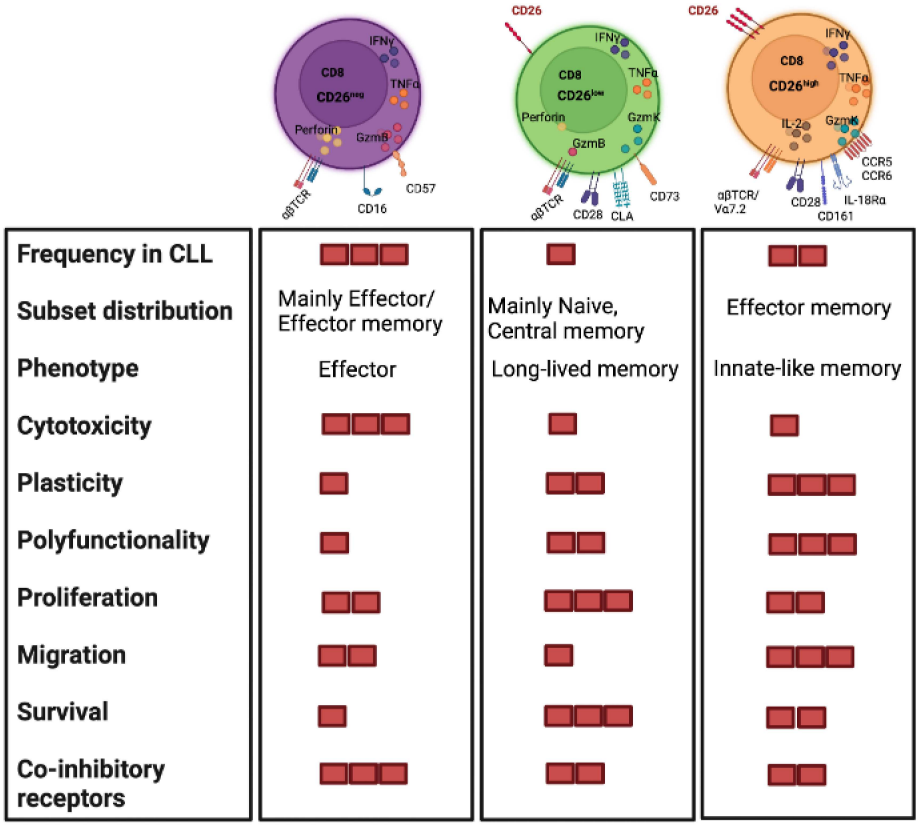
Finally, we could not obtain a larger blood volume for conducting more in-depth analysis such as RNAseq or single cell sequencing on different $CD26^{+}$ T cell subsets in CLL patients. Such studies will enable us to better characterize this T cell subset for therapeutic interventions.

3.5 Conclusions

In conclusion, our findings demonstrate the depletion of highly polyfunctional $CD26^{+}CD8^{+}$ T in CLL. These cells exhibit greater migratory capacity, stemness, longevity, and proliferation capability, which make them a potential candidate for adoptive T cell transfer or CAR T cell therapy in CLL. Considering the role of CD26: ADA in converting adenosine to inosine, $CD26^{+}$ T cells could bypass the immune suppressive effects of adenosine in the TME and periphery. Also, our results indicate the involvement of Gal-9 in the inflammatory cascade and indirectly compromises

anti-tumor immunity by depleting polyfunctional $CD26^{high}CD8^{+}$ T cells in CLL. Therefore, targeting Gal-9 to preserve polyfunctional T cells in CLL merits further investigations and should be considered.

a


CD26
IFNγ
CD8
CD26neg
TNFα
Perforin
GzmB
αβTCR
CD16
CD57
CD26
IFNγ
CD8
CD26low
TNFα
Perforin
GzmK
GzmB
αβTCR
CD28
CLA
CD73
CD26
IFNγ
CD8
CD26high
TNFα
IL-2
GzmK
αβTCR/
Vα7.2
CD28
IL-18Rα
CD161
CCR5
CCR6
Frequency in CLL
Subset distribution
Phenotype
Cytotoxicity
Plasticity
Polyfunctionality
Proliferation
Migration
Survival
Co-inhibitory receptors
Mainly Effector/ Effector memory
Effector
Mainly Naive, Central memory
Long-lived memory
Effector memory
Innate-like memory

b

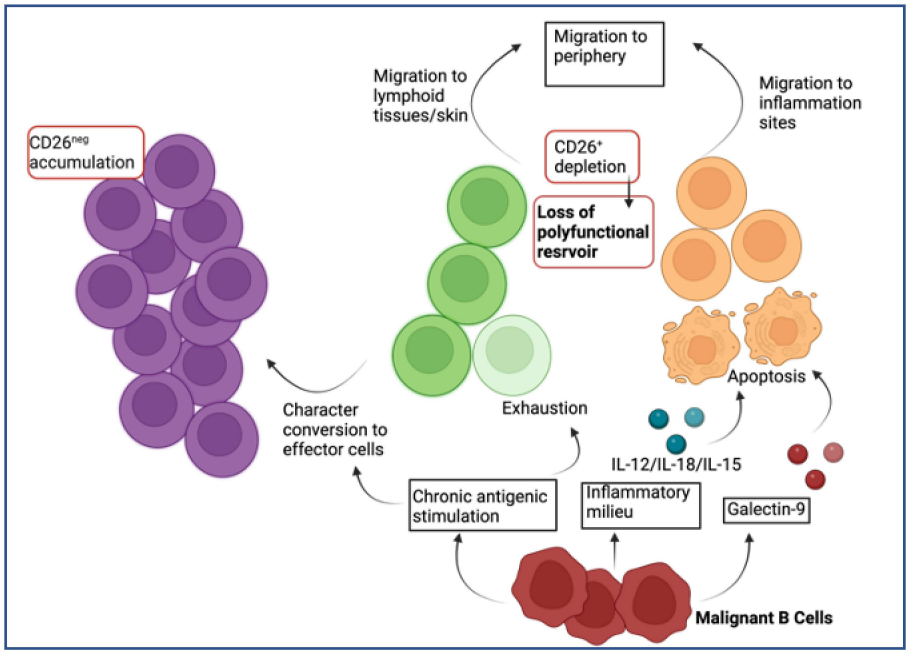

Migration to periphery
Migration to lymphoid tissues/skin
Migration to inflammation sites
CD26neg accumulation
CD26+ depletion
Loss of polyfunctional resrvoir
Apoptosis
Character conversion to effector cells
Exhaustion
IL-12/IL-18/IL-15
Chronic antigenic stimulation
Inflammatory milieu
Galectin-9
Malignant B Cells

Fig. 7. Visual summary. (a) CD26 expression defines three distinct populations of $CD8^+$ T cells in CLL with discrete properties. The table summarizes different properties of $CD26^{neg}$, $CD26^{low}$, and $CD26^{high}$ $CD8^+$ T cells ranked as high (■■■), moderate (■■), and low (■). **(b)** The illustration depicts the proposed mechanism of decreased frequency of $CD26^{low}$ and $CD26^{high}$ subsets and the expansion of $CD26^{neg}$ $CD8^+$ T cells in CLL. Migration towards inflamed tissues, elimination by apoptosis (Gal-9, Inflammatory cytokines), change of character/exhaustion due to chronic antigenic stimulation in CLL are proposed as potential mechanisms leading to the depletion of $CD26^+CD8^+$ in CLL.

3.6 Ethics and consent to participate.

This study was reviewed and approved by the Health Research Ethics Board of Alberta, Cancer Committee (HREBA#CC-17-0307). All participants provided informed written consent to participate in this study.

Moreover, animal studies were approved by the animal ethics board at the University of Alberta with the protocol number AUP0001021.

3.7 Acknowledgments

We thank all the volunteers who supported this study by donating their samples and dedicating their time, and the staff at the CLL clinic and medical laboratory at the Cross-Cancer Institute (CCI) for sample collection. We also would like to thank Faculty of Medicine and Dentistry's flow core facility at the University of Alberta.

3.8 Supplementary Tables

NO#	Age	Sex	Stage	IgHV mutation	FISH analysis	Treatment	Treatment Course
1	72	M	II	Unmutated	Trisomy 12	N	-
2	51	F	I	Not done	Not done	N	-
3	54	M	0	Unmutated	Del 13q	N	-
4	68	M	II	Not done	Del 13q	T	Chlorambucil+ Obinutuzumab
5	75	M	0	Not done	Del 13q	T	FCR 2 cycles
6	60	F	0	Not done	Not done	N	-
7	86	F	0	Not done	Not done	N	-
8	85	M	0	Not done	Not done	N	-
9	78	F	0	Not done	Not done	N	-
10	61	M	I	Not done	Not done	N	-
11	81	M	II	Not done	Not done	N	-
12	62	M	I	Not done	Del 17p Del 11q	T	FCR, RCVP, Allogenic stem cell transplant, Ibrutinib, Venetoclax, Rituximab
13	77	M	0	Not done	Not done	N	-
14	80	F	I	Not done	Not done	N	-
15	63	M	III	Mutated	Del 13q	N	-
16	65	M	II	Unmutated	Del 13q	N	-
17	73	M	II	Unmutated	Normal	N	-
18	82	M	III	Not done	Not done	T	Bendamustine+ Rituximab
19	71	F	IV	Not done	Not done	T	Bendamustine+ Rituximab Ibrutinib
20	78	F	I	Not done	Not done	N	-
21	71	F	0	Not done	Del 13q	T	Bendamustine+ Rituximab
22	62	M	I	Not done	Del 17p Del 11q	T	Ibrutinib+ Rituximab
23	68	M	IV	Unmutated	Del 11q Del 13q	T	FCR 2 cycles, Ibrutinib
24	53	M	II	Not done	Not done	N	-
25	54	M	II	Not done	Normal	N	_

26	71	M	II	Not done	Normal	T	Bendamustine+ Rituximab CHOP
27	91	F	I	Not done	Not done	T	Chlorambucil+ Obinutuzuma Ibrutinib
28	68	M	II	Not done	Not done	N	-
29	72	M	IV	Not done	Del 13q Trisomy 12	T	Chlorambucil+ Obinutuzumab
30	63	M	II	Not done	Del 13q	T	Ibrutinib+Venet oclax
31	65	F	0	Not done	Not done	N	-
32	55	F	0	Not done	Not done	N	-
33	74	F	I	Not done	Del 13q	N	-
34	79	F	I	Not done	Del 13q	T	Chlorambucil+ Obinutuzumab
35	70	M	III	Unmutate d	Trisomy 12	N	-
36	56	F	III	Mutated	Del 13q Del 11q	N	-
37	72	M	IV	Not done	Normal	N	-
38	45	F	II	Mutated	Del 13q	N	-
39	60	F	II	Indetermi nate	Trisomy 12	N	-
40	76	M	II	Unmutate d	Trisomy 12	N	-
42	77	M	I	Not done	Del 11q	T	Ibrutinib+Obnit uzumab Venetoclax+Rit uximab
43	57	M	IV	Not done	Del 13q Tp53 loss (22%)	T	FCR+Lenalidom ide Ibrutinib Venetoclax+Rit uximab
44	71	M	0	Not done	Not done	N	-
45	64	M	0	Not done	Del 13q	N	-
46	72	M	I	Indetermi nate	Trisomy 12	N	-
47	38	F	I	Unmutate d	Del 17p	T	Ibrutinib
48	73	M	I	Not done	Del 17p	T	FCR
49	54	M	I	Unmutate d	Trisomy 12	T	FCR

50	59	F	I	Mutated	Normal	N	-
51	77	M	0	Mutated	Del 13q	N	-
52	60	M	II	Mutated	Not done	N	-
53	65	F	I	Not done	Not done	N	-
54	49	M	I	Not done	Not done	N	-
55	87	F	I	Not done	Not done	N	-

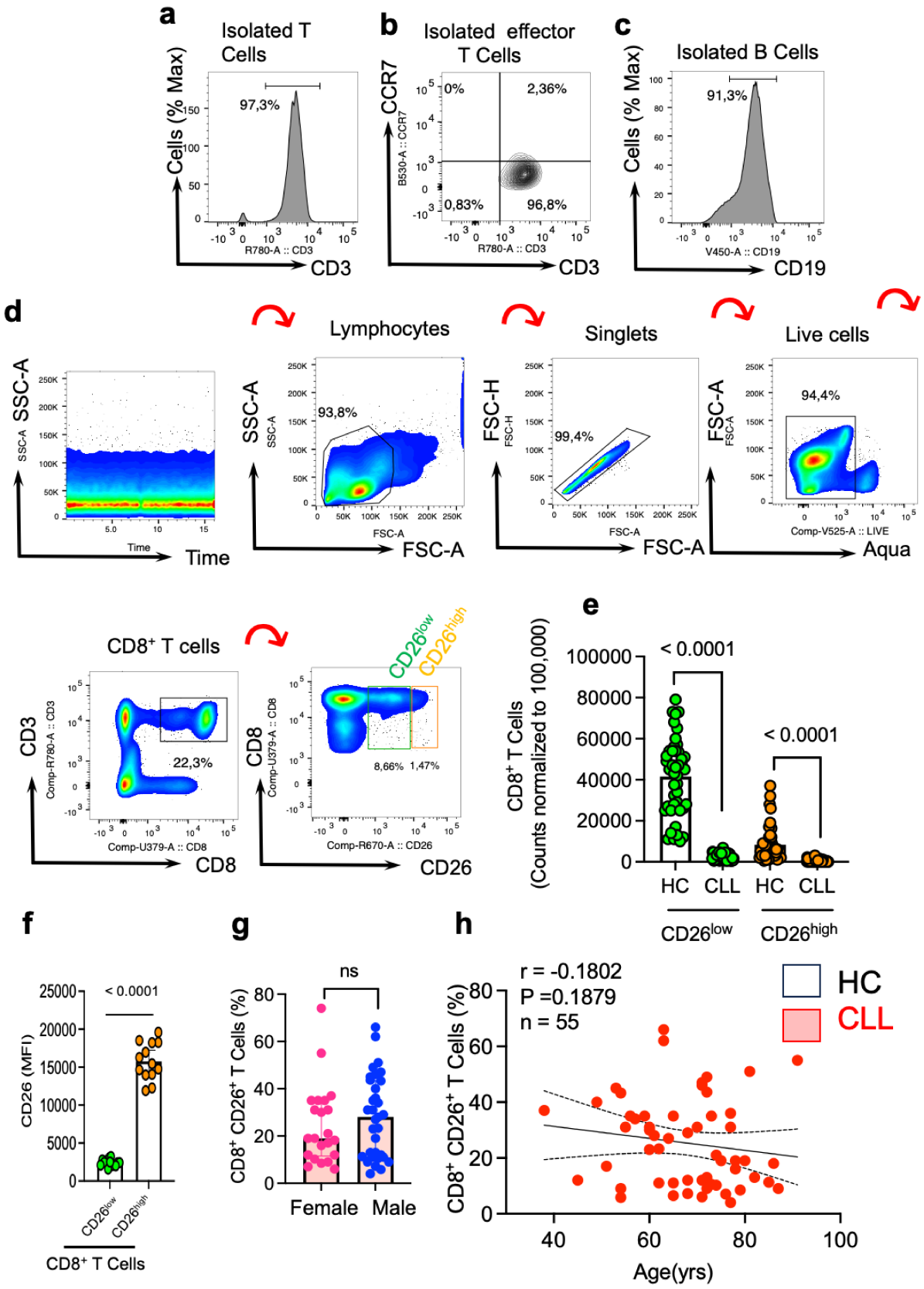
Supplementary Table 1. **Patients' characteristics.** Age (years old), Sex (F: Female, M: Male), Treatment (N: Naïve, T: Treated), Stage (Based on Rai staging system for CLL), All information is at the time when T cell repertoire was analyzed.

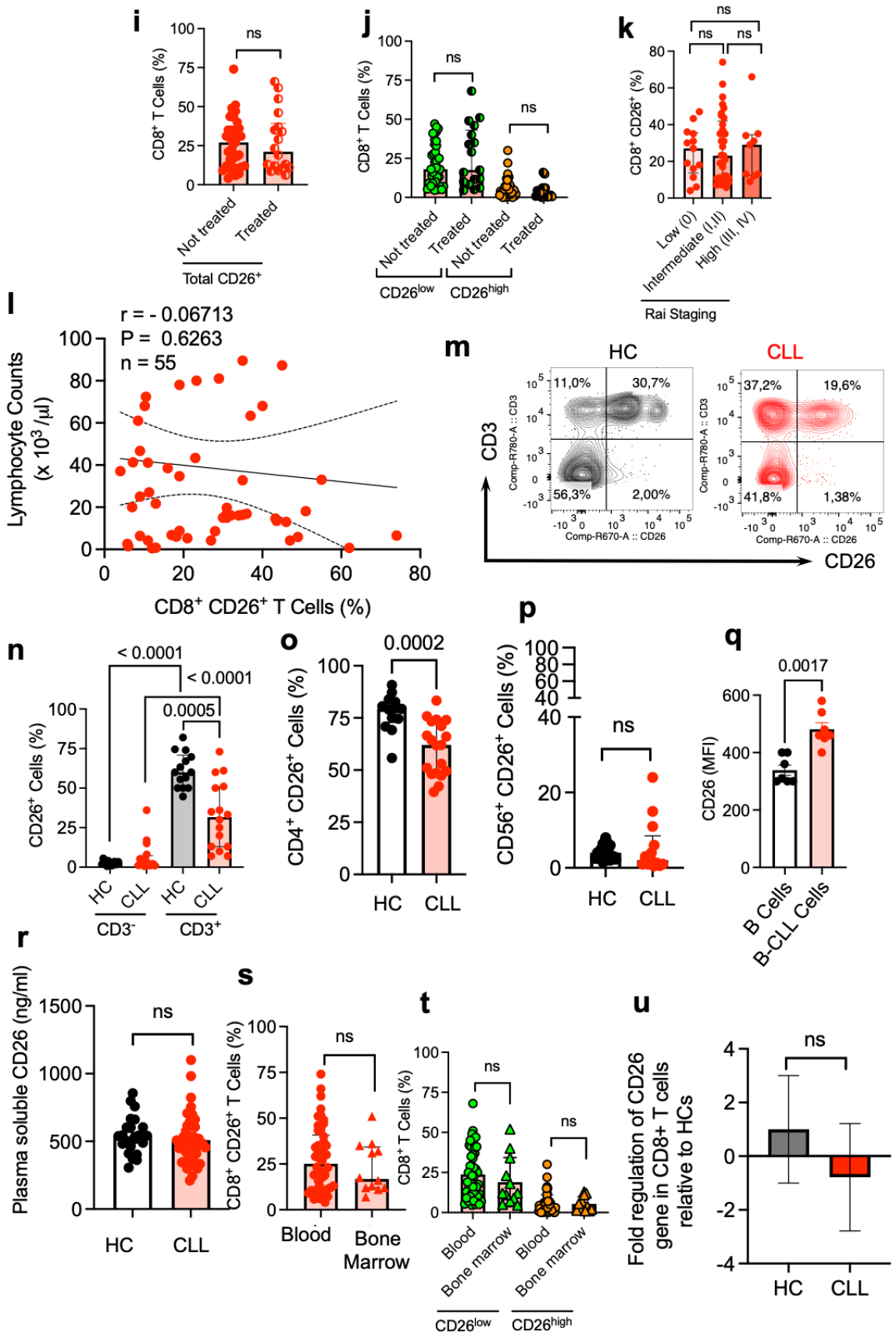
Characteristics	Values (Numbers)
Patients	
Total patients	55
Blood samples	48
Bone marrow samples	12
Age (yrs)	
Median	71
Range	38-91
Sex	
Female	22
Male	33
Clinical stage (Rai)	
Low (0)	13
Intermediate (I/II)	33
High (III/IV)	9
IGHV mutation	
Unmutated	10
Mutated	6
Not available/Indeterminate	37/2
FISH analysis	
Trisomy 12	7
Del 11q	5
Del 13q	15
Del 17p	4
TP53 loss+ Del 13q	1
Trisomy12+ Del 13q	1
Normal	4
Not available	18
Treatment	
Naïve	37
Treated	18

Supplementary Table 2. Summary of CLL cohort.

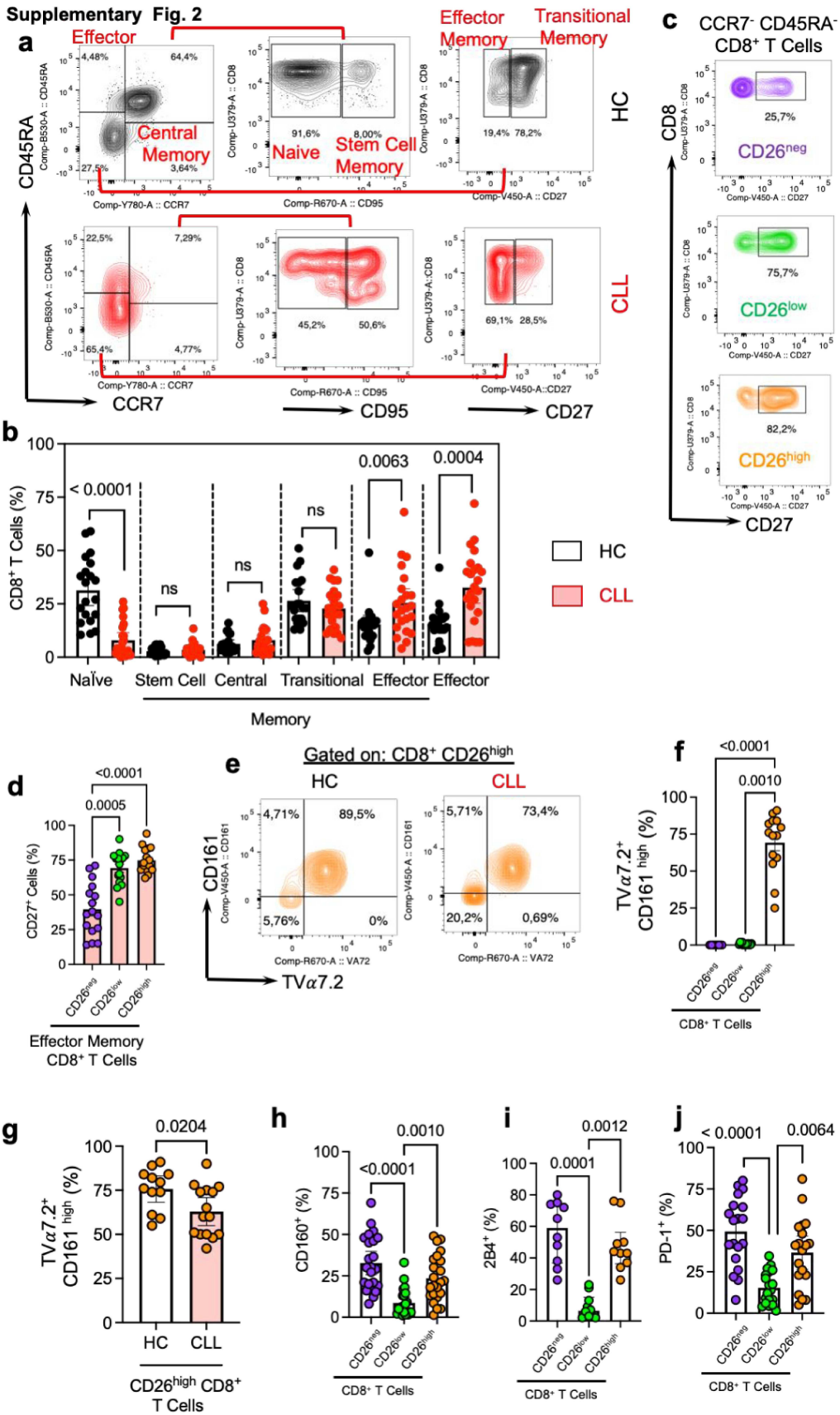
3.9 Supplementary Figures

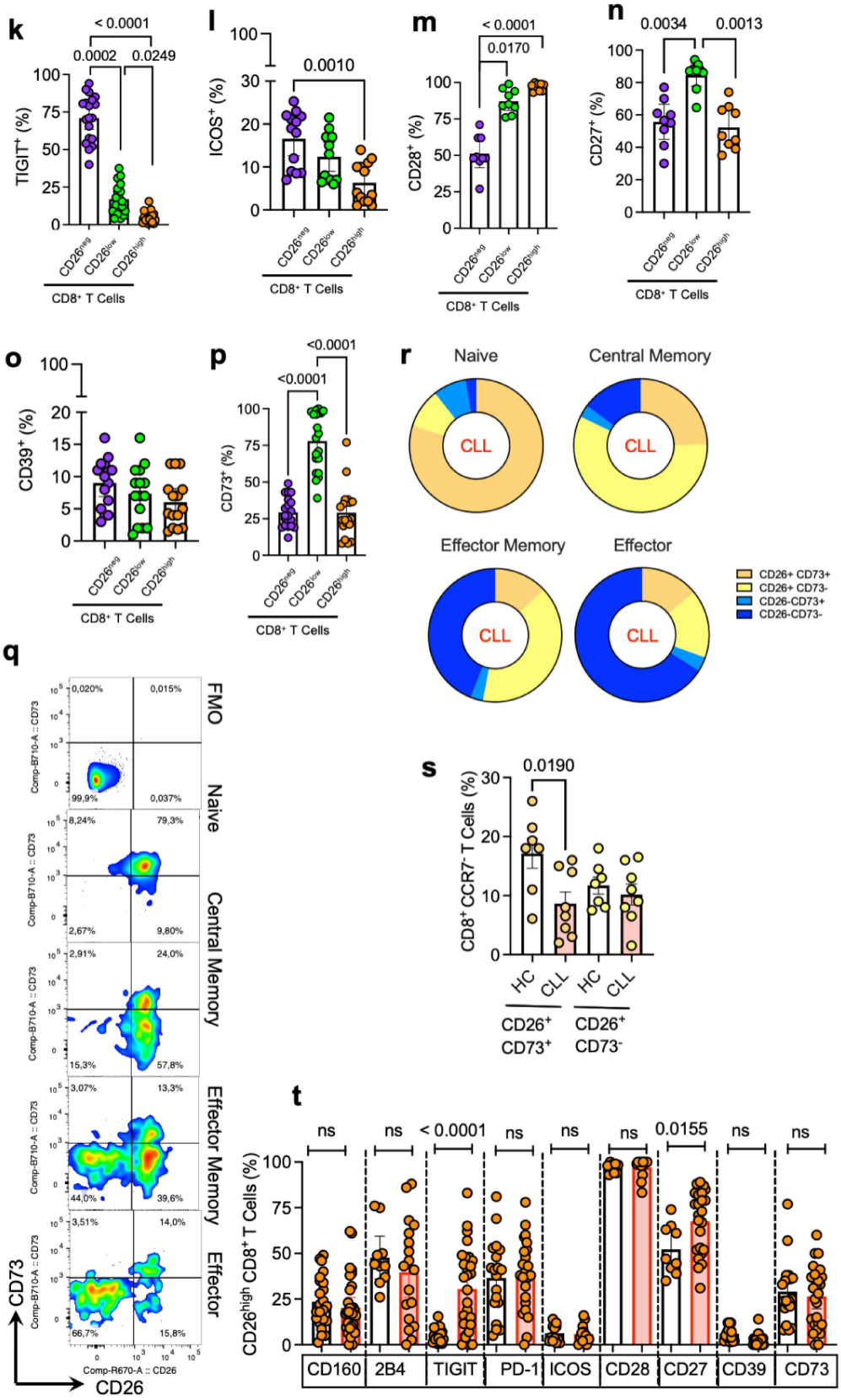
Supplementary Fig. 1



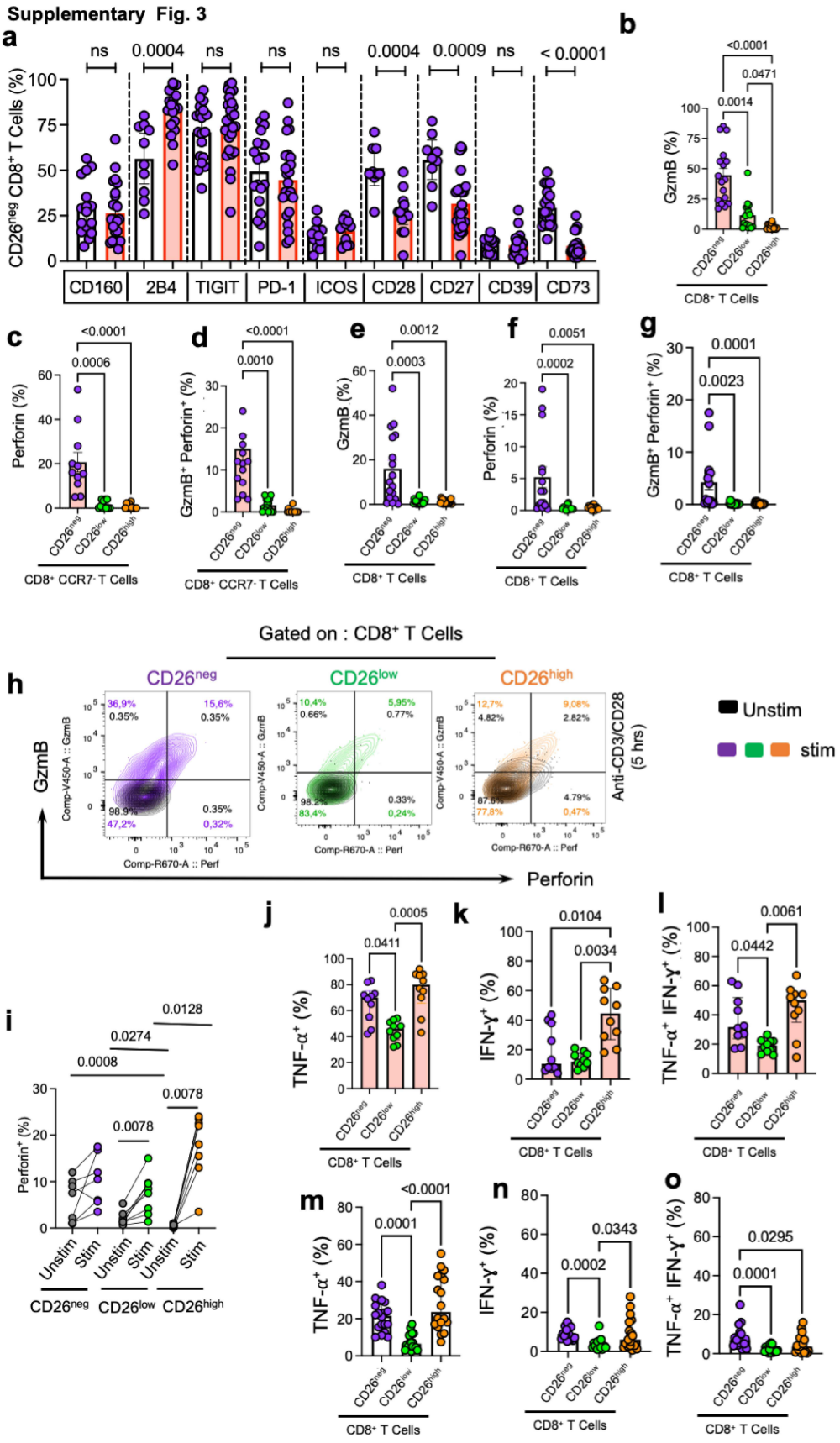

i
CD8+ T Cells (%)
ns
Not treated
Treated
Total CD26+
j
CD8+ T Cells (%)
ns
ns
Not treated
Treated
Not treated
Treated
CD26low
CD26high
k
CD8+ CD26+ (%)
ns
ns
ns
Low (0)
Intermediate (I,II)
High (III, IV)
Rai Staging
l
r = - 0.06713
P = 0.6263
n = 55
Lymphocyte Counts (x 10³ /µl)
CD8+ CD26+ T Cells (%)
m
HC
CLL
CD3
CD26
11,0%
30,7%
56,3%
2,00%
37,2%
19,6%
41,8%
1,38%
Comp-R780-A :: CD3
Comp-R670-A :: CD26
n
< 0.0001
< 0.0001
0.0005
CD26+ Cells (%)
HC
CLL
HC
CLL
CD3-
CD3+
o
0.0002
CD4+ CD26+ Cells (%)
HC
CLL
p
CD56+ CD26+ Cells (%)
ns
HC
CLL
q
0.0017
CD26 (MFI)
B Cells
B-CLL Cells
r
Plasma soluble CD26 (ng/ml)
ns
HC
CLL
s
CD8+ CD26+ T Cells (%)
ns
Blood
Bone Marrow
t
CD8+ T Cells (%)
ns
ns
Blood
Bone marrow
Blood
Bone marrow
CD26low
CD26high
u
Fold regulation of CD26 gene in CD8+ T cells relative to HCs
ns
HC
CLL

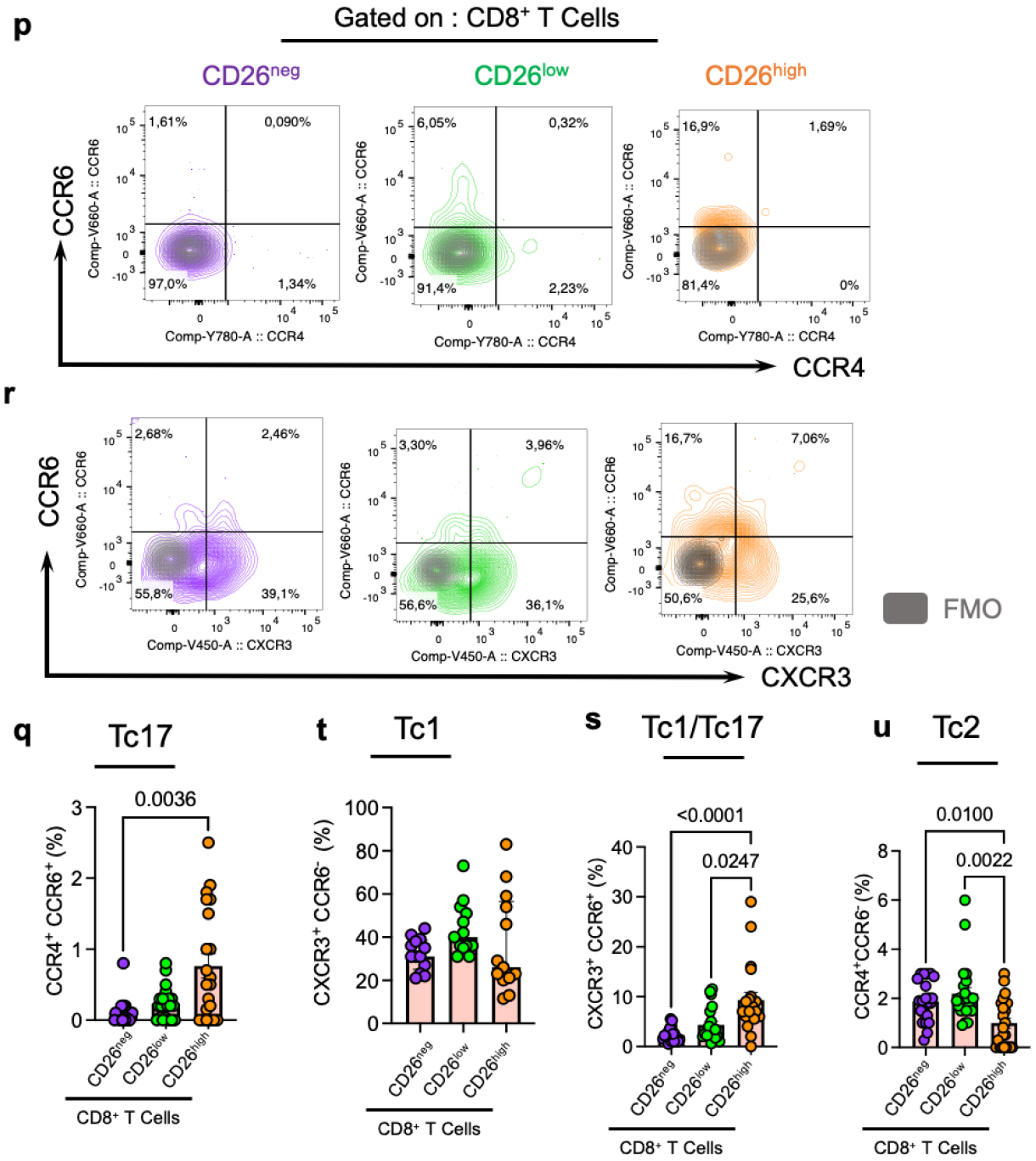
Supplementary Fig. 1. (a) Representative flow plot of the purity of isolated $CD3^+$ T cells, **(b)** $CD3^+CCR7^-$ cells, and **(c)** $CD19^+$ B cells. **(d)** Representative flow plots of the gating strategy for CD26 staining in $CD8^+$ T cells. **(e)** Cumulative data showing the number of $CD26^{low}$ and $CD26^{high}$ $CD8^+$ T cells as normalized in 100,000 $CD8^+$ T cells in HC and CLL patients. **(f)** Cumulative data comparing the Mean Fluorescence Intensity (MFI) of CD26 in $CD26^{low}$ and $CD26^{high}$ $CD8^+$ T cells. **(g)** Cumulative data comparing the frequency of $CD26^+CD8^+$ T cells in female versus male CLL patients. **(h)** Correlation between the age of CLL patients and the frequency of $CD26^+CD8^+$ T cells. **(i)** Cumulative data comparing the frequency of $CD26^+$ and, **(j)** $CD26^{low}$, $CD26^{high}$ $CD8^+$ T cells in treated versus non-treated CLL. **(k)** Cumulative data are comparing the proportion of $CD26^+CD8^+$ T cell in three clinical stages of CLL (Low/Intermediate/high) based on the Rai staging system. **(l)** Correlation between $CD26^+CD8^+$ T cell frequency and lymphocyte counts ($x10^3$ / μl) in CLL. **(m)** Representative flow plots, and **(n)** cumulative data of the frequency of CD26 among $CD3^-$ and $CD3^+$ T cells in HC and CLL. **(o)** Cumulative data showing the frequency of $CD26^+CD4^+$ T cells, and **(p)** $CD56^+$NK cells in HC versus CLL. **(q)** Cumulative data showing the MFI of CD26 in B cells from HCs and malignant B cells (B-CLL). **(r)** Cumulative data of the concentrations of soluble CD26 (ng/ml) in the plasma of HC and CLL. **(s)** Cumulative data of the frequency of $CD26^+$, and **(t)** $CD26^{low}$, $CD26^{high}$ $CD8^+$ T cell in the peripheral blood versus bone marrow of CLL. **(u)** Cumulative data of CD26 mRNA expression in $CD8^+$ T cells of HCs vs. CLL (n=15). Error bars represent the median with an interquartile range. Each dot represents an individual human sample.


Supplementary Fig. 2
a
Effector
Central Memory
Naive
Stem Cell Memory
Effector Memory
Transitional Memory
HC
CLL
CD45RA
CCR7
CD95
CD27
b
CD8⁺ T Cells (%)
< 0.0001
ns
ns
ns
0.0063
0.0004
Naïve
Stem Cell
Central
Transitional
Effector
Effector Memory
HC
CLL
c
CCR7⁻ CD45RA⁻ CD8⁺ T Cells
CD8
CD26neg
CD26low
CD26high
CD27
d
CD27⁺ Cells (%)
<0.0001
0.0005
CD26neg
CD26low
CD26high
Effector Memory CD8⁺ T Cells
e
Gated on: CD8⁺ CD26high
HC
CLL
CD161
TVα7.2
f
TVα7.2⁺ CD161 high (%)
<0.0001
0.0010
CD8⁺ T Cells
g
TVα7.2⁺ CD161 high (%)
0.0204
HC
CLL
CD26high CD8⁺ T Cells
h
CD160⁺ (%)
0.0010
<0.0001
CD8⁺ T Cells
i
2B4⁺ (%)
0.0012
0.0001
CD8⁺ T Cells
j
PD-1⁺ (%)
< 0.0001
0.0064
CD8⁺ T Cells


k
< 0.0001
0.0002
0.0249
TIGIT+ (%)
CD26neg
CD26low
CD26high
CD8+ T Cells
l
0.0010
ICOS+ (%)
m
< 0.0001
0.0170
CD28+ (%)
n
0.0034
0.0013
CD27+ (%)
o
CD39+ (%)
p
<0.0001
<0.0001
CD73+ (%)
r
Naive
Central Memory
Effector Memory
Effector
CLL
CD26+ CD73+
CD26+ CD73-
CD26-CD73+
CD26-CD73-
q
FMO
Naive
Central Memory
Effector Memory
Effector
0,020%
0,015%
99,9%
0,037%
8,24%
79,3%
2,67%
9,80%
2,91%
24,0%
15,3%
57,8%
3,07%
13,3%
44,0%
39,6%
3,51%
14,0%
66,7%
15,8%
Comp-B710-A :: CD73
Comp-R670-A :: CD26
CD73
CD26
s
0.0190
CD8+ CCR7- T Cells (%)
HC
CLL
CD26+ CD73+
CD26+ CD73-
t
ns
< 0.0001
0.0155
CD26high CD8+ T Cells (%)
CD160
2B4
TIGIT
PD-1
ICOS
CD28
CD27
CD39
CD73

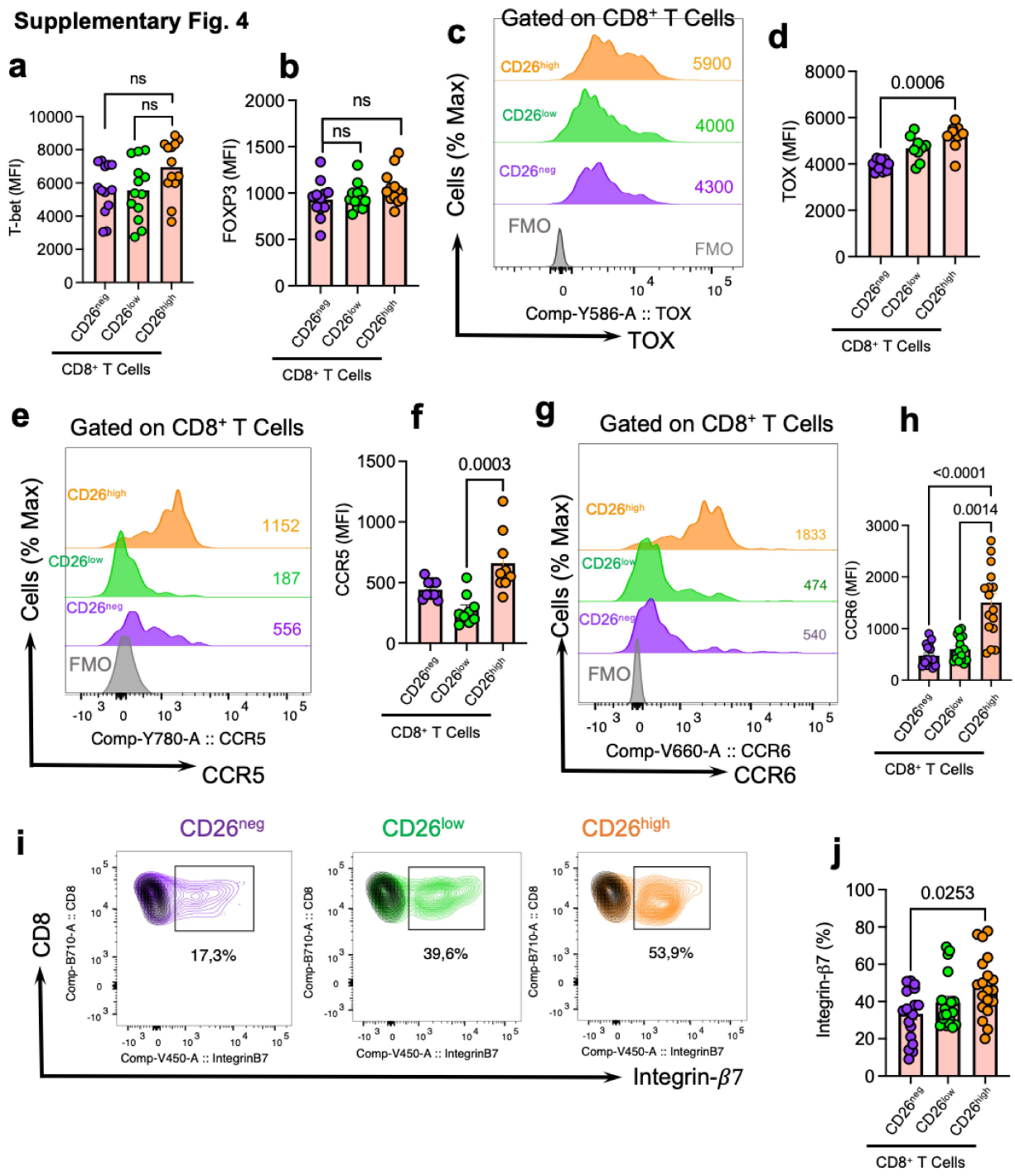
Supplementary Fig. 2. (a) Representative plots, and **(b)** cumulative data of the frequency of $CD8^+$ T cell subsets (e.g., naïve, stem cell memory, central memory, transitional memory, effector memory, and effectors). **(c)** Representative flow plots, and **(d)** cumulative data showing the frequency of CD27 expressing cells among $CD26^{neg/low/high}$ effector memory subsets of $CD8^+$ T cells in CLL. **(e)** Representative plots, and **(f)** Cumulative data of the frequency of $TV\alpha7.2^+CD161^{high}$ in $CD26^{high}$ $CD8^+$ T cells in HC. **(g)** Cumulative data of the frequency of $TV\alpha7.2^+$ $CD161^{high}$ in $CD26^{high}CD8^+$ T cells in HC versus CLL. **(h)** Cumulative data of the frequency of $CD160^+$, **(i)** $2B4^+$, **(j)** $PD\text{-}1^+$, **(k)** $TIGIT^+$, **(l)** $ICOS^{+,}$ **(m)** $CD28^+$, **(n)** $CD27^+$, **(o)** $CD39^+$, and **(p)** $CD73^+$ among $CD26^{neg}$, $CD26^{low}$, and $CD26^{high}$ $CD8^+$ T cells in HC. **(q)** Representative plots of the co-expression of CD26 and CD73 in different $CD8^+$ T cell subsets (naïve, central memory, effector memory, and effector) in CLL. **(r)** The pie charts show the pattern of CD26 and CD73 co-expression in different $CD8^+$ T cell subsets in CLL. **(s)** Cumulative data of the frequency of $CD26^+CD73^+$ versus $CD26^+CD73^-$ in $CD8^+$ T cells of HC versus CLL. **(t)** Cumulative data of the frequency of co-inhibitory/co-stimulatory receptors in $CD26^{high}CD8^+$ T cell subset in HC and CLL. Error bars represent the median with an interquartile range. Each dot represents an individual human sample.

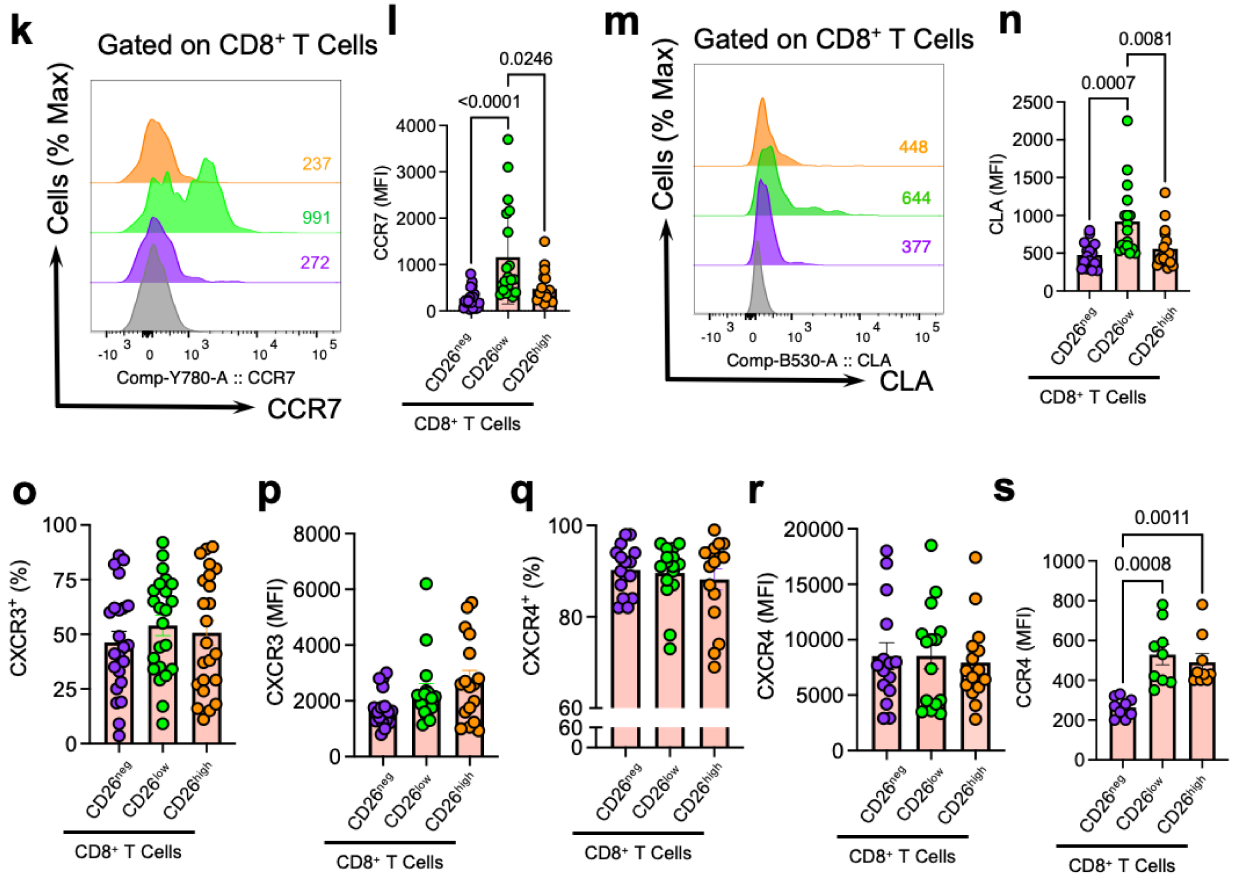

Supplementary Fig. 3
a
ns
0.0004
ns
ns
ns
0.0004
0.0009
ns
< 0.0001
CD26neg CD8+ T Cells (%)
CD160
2B4
TIGIT
PD-1
ICOS
CD28
CD27
CD39
CD73
b
<0.0001
0.0471
0.0014
GzmB (%)
CD8+ T Cells
c
<0.0001
0.0006
Perforin (%)
CD8+ CCR7- T Cells
d
<0.0001
0.0010
GzmB+ Perforin+ (%)
CD8+ CCR7- T Cells
e
0.0012
0.0003
GzmB (%)
CD8+ T Cells
f
0.0051
0.0002
Perforin (%)
CD8+ T Cells
g
0.0001
0.0023
GzmB+ Perforin+ (%)
CD8+ T Cells
CD26neg
CD26low
CD26high
Gated on : CD8+ T Cells
h
CD26neg
CD26low
CD26high
GzmB
Perforin
Anti-CD3/CD28 (5 hrs)
Unstim
stim
36,9%
0.35%
15,6%
0.35%
98.9%
47,2%
0.35%
0,32%
10,4%
0.66%
5,95%
0.77%
98.2%
83,4%
0.33%
0,24%
12,7%
4.82%
9,08%
2.82%
87.6%
77,8%
4.79%
0,47%
i
0.0128
0.0274
0.0008
0.0078
0.0078
Perforin+ (%)
Unstim
Stim
CD26neg
CD26low
CD26high
j
0.0005
0.0411
TNF-α+ (%)
CD8+ T Cells
k
0.0104
0.0034
IFN-γ+ (%)
CD8+ T Cells
l
0.0061
0.0442
TNF-α+ IFN-γ+ (%)
CD8+ T Cells
m
<0.0001
0.0001
TNF-α+ (%)
CD8+ T Cells
n
0.0343
0.0002
IFN-γ+ (%)
CD8+ T Cells
o
0.0295
0.0001
TNF-α+ IFN-γ+ (%)
CD8+ T Cells



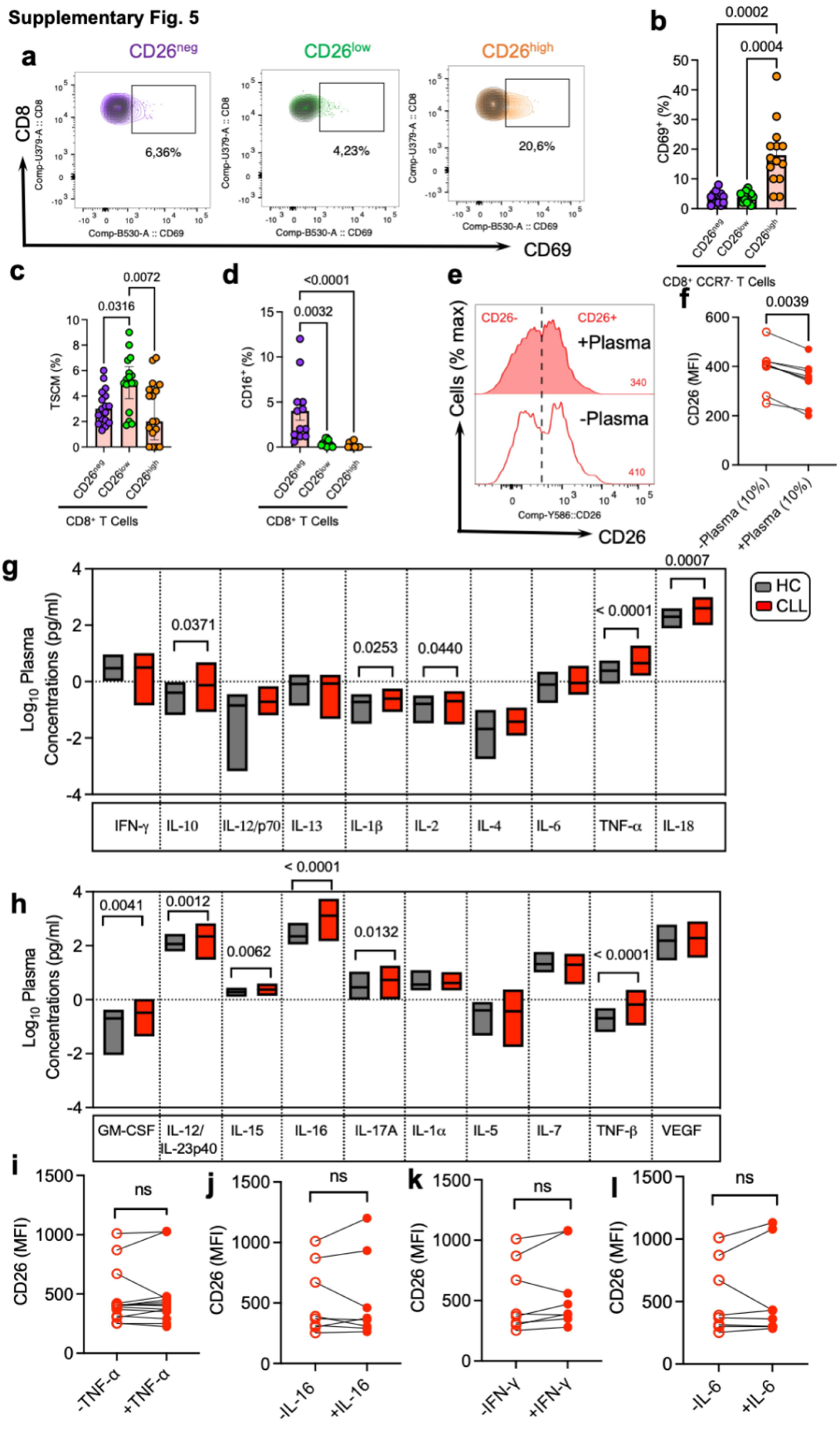
Supplementary Fig. 3. (a) Cumulative data of the frequency of co-inhibitory/co-stimulatory receptors in CD26^{neg} CD8^{+} T cell subset in HC and CLL. **(b)** Cumulative data of the frequency of GzmB^{+}, **(c)** Perforin^{+}, and **(d)** GzmB^{+}Perforin^{+} among CD26^{neg}, CD26^{low}, and CD26^{high} CD8^{+}CCR7^{-} T cells in CLL. **(e)** Cumulative data of the frequency of GzmB^{+}, **(f)** Perforin^{+}, and **(g)** GzmB^{+}Perforin^{+} CD8^{+} T cells among CD26^{neg}, CD26^{low}, and CD26^{high} subsets in HC. **(h)** Representative plots, and **(i)**

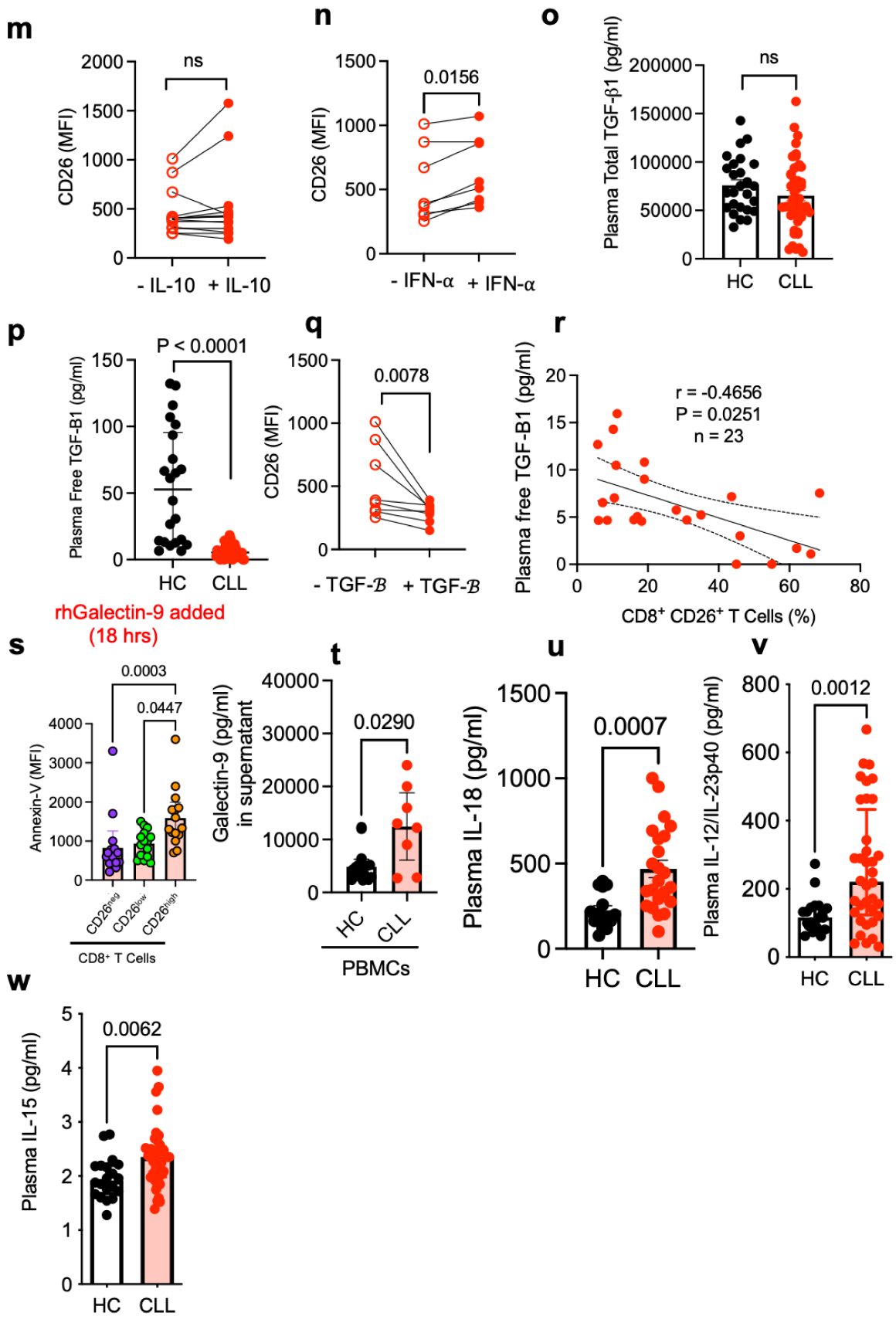
cumulative data of the frequency of GzmB and perforin expressing cells among $CD26^{neg}$, $CD26^{low}$, and $CD26^{high}$ $CD8^+$ T cells in CLL either unstimulated (black color) or stimulated for following 5 hours with anti-CD3/CD28 (3μg/ml, 1μg/ml). **(j)** Cumulative data of the frequency of TNF-α^+, **(k)** IFN-γ^+, and **(l)** TNF-α^+IFN-γ^+ cells among $CD26^{neg}$, $CD26^{low}$, and $CD26^{high}$ $CD8^+$ T cells in CLL. **(m)** Cumulative data of the frequency of TNF-α^+, **(n)** IFN-γ^+, and **(o)** TNF-α^+IFN-γ^+ among $CD26^{neg}$, $CD26^{low}$, and $CD26^{high}$ $CD8^+$ T cells in HC. **(p)** Representative plots, and cumulative data of the frequency of **(q)** $CCR4^+CCR6^+$ cells among $CD26^{neg}$, $CD26^{low}$, and $CD26^{high}$ $CD8^+$ T cells in CLL, considered as Tc17 cells. **(r)** Representative plots, and **(s)** cumulative data of the frequency of $CXCR3^+CCR6^+$ expressing cells among $CD26^{neg}$, $CD26^{low}$, and $CD26^{high}$ $CD8^+$ T cells in CLL, considered as Tc1/Tc17 cells. **(t)** Cumulative data of the frequency of $CXCR3^+CCR6^-$ among $CD26^{neg}$, $CD26^{low}$, and $CD26^{high}$ $CD8^+$ T cells in CLL, considered as Tc1 cells. **(u)** Cumulative data showing the frequency of $CCR4^+CCR6^-$ among $CD26^{neg}$, $CD26^{low}$, and $CD26^{high}$ $CD8^+$ T cells in CLL, considered as Tc2 cells. Error bars represent the median with an interquartile range. Each dot represents an individual human sample. Florescence minus one (FMO).


Supplementary Fig. 4
a
T-bet (MFI)
ns
ns
CD26neg
CD26low
CD26high
CD8+ T Cells
b
FOXP3 (MFI)
ns
ns
CD8+ T Cells
c
Gated on CD8+ T Cells
Cells (% Max)
CD26high
5900
CD26low
4000
CD26neg
4300
FMO
FMO
Comp-Y586-A :: TOX
TOX
d
TOX (MFI)
0.0006
CD8+ T Cells
e
Gated on CD8+ T Cells
Cells (% Max)
CD26high
1152
CD26low
187
CD26neg
556
FMO
Comp-Y780-A :: CCR5
CCR5
f
CCR5 (MFI)
0.0003
CD8+ T Cells
g
Gated on CD8+ T Cells
Cells (% Max)
CD26high
1833
CD26low
474
CD26neg
540
FMO
Comp-V660-A :: CCR6
CCR6
h
CCR6 (MFI)
<0.0001
0.0014
CD8+ T Cells
i
CD26neg
CD26low
CD26high
CD8
Comp-B710-A :: CD8
17,3%
39,6%
53,9%
Comp-V450-A :: IntegrinB7
Integrin-β7
j
Integrin-β7 (%)
0.0253
CD8+ T Cells

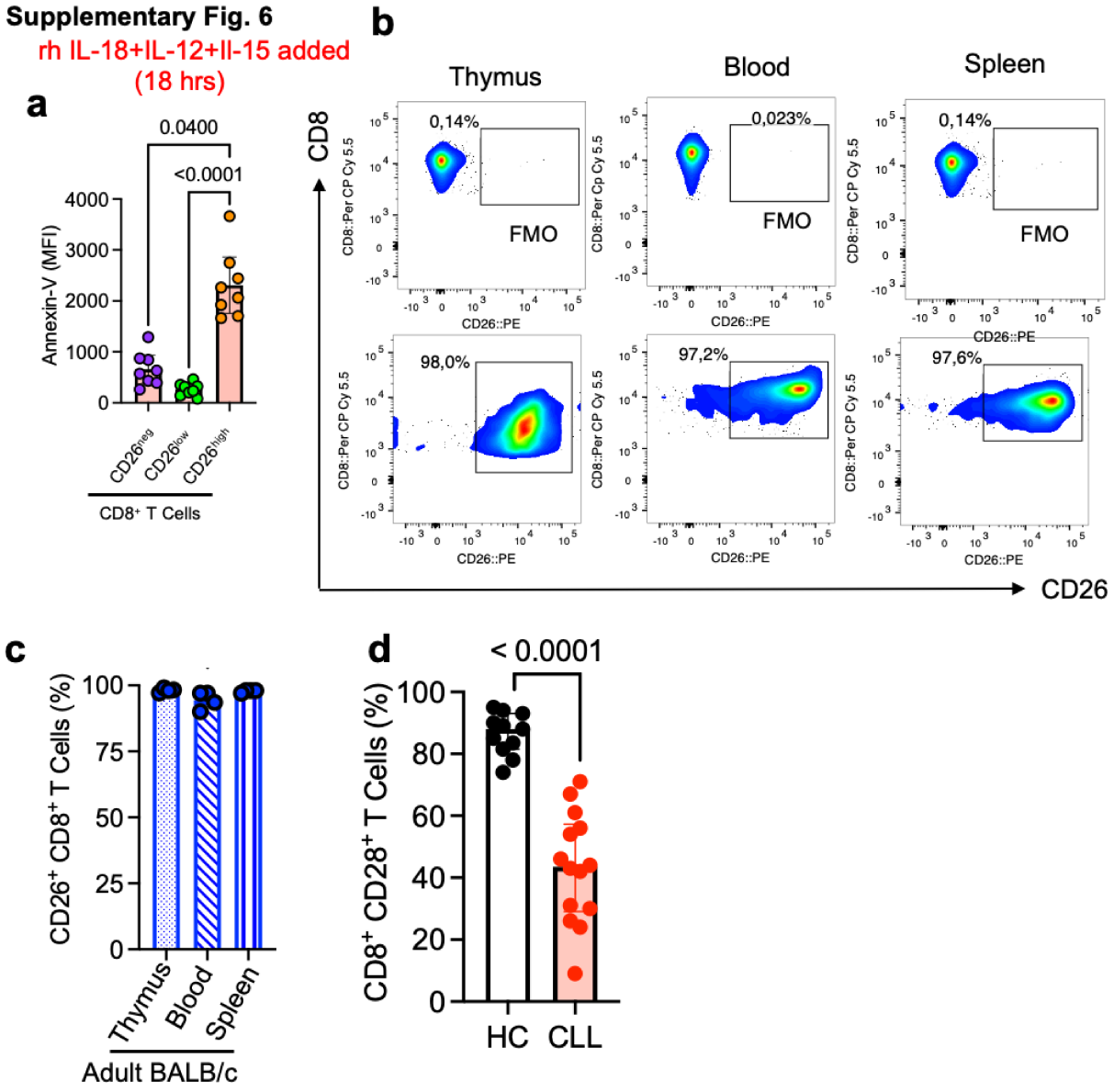


Supplementary Fig. 4. (a) Cumulative data of the expression of T-bet in different CD8$^+$ T cells subsets in CLL. **(b)** Cumulative data of the expression of FOXP3 in different CD8$^+$ T cells subsets in CLL. **(c)** Representative plots, and **(d)** cumulative data showing the intensity (MFI) of TOX expression among CD26^{neg}, CD26^{low}, and CD26^{high} CD8$^+$ T cells in CLL. **(e)** Representative plots, and **(f)** cumulative data showing the intensity (MFI) of CCR5 expression among CD26^{neg}, CD26^{low}, and CD26^{high} CD8$^+$ T cells in CLL. **(g)** Representative plots, and **(h)** cumulative data showing the intensity of CCR6 expression in CD26^{neg}, CD26^{low}, and CD26^{high} CD8$^+$ T cells in CLL. **(i)** Representative plots, and **(j)** cumulative data showing the intensity of Integrin-β7 expression in CD26^{neg}, CD26^{low}, and CD26^{high} CD8$^+$ T cells in CLL. **(k)** Representative plots, and **(l)** cumulative data of the intensity of CCR7 expression in CD26^{neg}, CD26^{low}, and CD26^{high} CD8$^+$ T cells in CLL. **(m)** Representative plots, and **(n)** cumulative data of the intensity of CLA (Cutaneous Lymphocyte Antigen) expression in CD26^{neg}, CD26^{low}, and CD26^{high} CD8$^+$ T cells in CLL. **(o)** Cumulative data of the frequency, and **(p)** the intensity of CXCR3$^+$ expression in CD26^{neg}, CD26^{low}, and CD26^{high} CD8$^+$ T cells in CLL. **(q)** Cumulative data of the frequency, and **(r)** the intensity of CXCR4$^+$ expression in CD26^{neg}, CD26^{low}, and CD26^{high} CD8$^+$ T cells in CLL. **(s)** Cumulative data of the intensity of CCR4 expression in CD26^{neg}, CD26^{low}, and CD26^{high} CD8$^+$ T cells in CLL. Error bars represent the median with an interquartile range. Each dot represents an individual human sample.


Supplementary Fig. 5
a
CD26neg
CD26low
CD26high
CD8
CD69
6,36%
4,23%
20,6%
b
0.0002
0.0004
CD69+ (%)
CD8+ CCR7- T Cells
c
0.0072
0.0316
TSCM (%)
CD8+ T Cells
d
<0.0001
0.0032
CD16+ (%)
CD8+ T Cells
e
CD26-
CD26+
+Plasma
-Plasma
Cells (% max)
CD26
f
0.0039
CD26 (MFI)
-Plasma (10%)
+Plasma (10%)
g
0.0007
HC
CLL
Log10 Plasma Concentrations (pg/ml)
0.0371
0.0253
0.0440
< 0.0001
IFN-γ
IL-10
IL-12/p70
IL-13
IL-1β
IL-2
IL-4
IL-6
TNF-α
IL-18
h
< 0.0001
0.0041
0.0012
0.0062
0.0132
< 0.0001
GM-CSF
IL-12/ IL-23p40
IL-15
IL-16
IL-17A
IL-1α
IL-5
IL-7
TNF-β
VEGF
i
ns
CD26 (MFI)
-TNF-α
+TNF-α
j
ns
-IL-16
+IL-16
k
ns
-IFN-γ
+IFN-γ
l
ns
-IL-6
+IL-6


m
CD26 (MFI)
ns
- IL-10
+ IL-10
n
CD26 (MFI)
0.0156
- IFN-α
+ IFN-α
o
Plasma Total TGF-β1 (pg/ml)
ns
HC
CLL
p
Plasma Free TGF-B1 (pg/ml)
P < 0.0001
HC
CLL
q
CD26 (MFI)
0.0078
- TGF-B
+ TGF-B
r
Plasma free TGF-B1 (pg/ml)
r = -0.4656
P = 0.0251
n = 23
CD8+ CD26+ T Cells (%)
rhGalectin-9 added
(18 hrs)
s
Annexin-V (MFI)
0.0003
0.0447
CD26neg
CD26low
CD26high
CD8+ T Cells
t
Galectin-9 (pg/ml)
in supernatant
0.0290
HC
CLL
PBMCs
u
Plasma IL-18 (pg/ml)
0.0007
HC
CLL
v
Plasma IL-12/IL-23p40 (pg/ml)
0.0012
HC
CLL
w
Plasma IL-15 (pg/ml)
0.0062
HC
CLL

Supplementary Fig. 5. (a) Representative plots, and **(b)** cumulative data of the frequency of CD69 expressing cells among $CD26^{neg}$, $CD26^{low}$, and $CD26^{high}$ $CD8^+$ T cells in CLL. **(c)** Cumulative data showing the frequency of TSCM (T Stem Cell Memory: $CCR7^+$ $CD45RA^+CD95^+$) among $CD26^{neg}$, $CD26^{low}$, and $CD26^{high}$ $CD8^+$ T cells in CLL. **(d)** Cumulative data showing the frequency of $CD16^+$ in $CD26^{neg}$, $CD26^{low}$, and $CD26^{high}$ $CD8^+$ T cells in CLL. **(e)** Representative histogram plots, and **(f)** cumulative data of CD26 expression in $CD8^+$ T cells upon culture with 10% plasma. **(g, h)** showing detected plasma concentrations of different cytokines and chemokines in CLL versus HCs. Cumulative data showing the intensity of CD26 in the presence/absence of **(i)** TNF-α, **(j)** IL-16, **(k)** IFN-γ, **(l)** IL-6, **(m)** IL-10, and **(n)** IFN-α. **(o)** Detected total plasma TGF-β and **(p)** free TGF-β in CLL versus HCs. **(q)** Cumulative data showing the intensity of CD26 expression in $CD8^+$ T cells in the presence/absence of free TGF-β. **(r)** The correlation of plasma free TGF-β with the frequency of $CD26^+CD8^+$ T cells in CLL patients. **(s)** Cumulative data showing the intensity of Annexin-V expression in $CD26^{neg}$, $CD26^{low}$, and $CD26^{high}$ $CD8^+$ T cells from CLL patients following treatment with recombinant human Gal-9 (0.02 μg/ml) *in-vitro*. **(t)** Concentrations of Gal-9 (pg/ml) in supernatants of PBMCs ($1x\ 10^6$ cells/well) from HC and CLL were collected 18 hours post culture. **(u)** Concentrations of IL-18 (pg/ml) **(v)** IL-12/IL-23p40 (pg/ml), and **(w)** IL-15 (pg/ml) in the plasma of HC versus CLL. Error bars represent the median with an interquartile range. Each dot represents an individual human sample.



Supplementary Fig. 6. (a) Cumulative data showing the intensity of Annexin-V expression in $CD26^{neg}$, $CD26^{low}$, and $CD26^{high}$ $CD8^+$ T cells from CLL patients following 8 hours of culture in the presence of a cytokine cocktail (IL-18+IL-12+IL-15) (100ng/ml of each). **(b)** Representative flow cytometry plots, and **(c)** cumulative data of CD26-expressing cells among $CD8^+$ T cells in the thymus, blood, and spleen of BALB/c mice. **(d)** Cumulative data showing the frequency of $CD28^+CD8^+$ T cells in HC versus CLL. Error bars represent the median with an interquartile range. Each dot represents an individual human sample or a mouse. Florescence minus one (FMO).

3.10 References

1. Engel M, Hoffmann T, Wagner L, Wermann M, Heiser U, Kiefersauer R, et al. The crystal structure of dipeptidyl peptidase IV (CD26) reveals its functional regulation and enzymatic mechanism. Proc Natl Acad Sci U S A. 2003;100:5063–8.

2. Yaron A, Naider F, Scharpe S. Proline-Dependent Structural and Biological Properties of Peptides and Proteins. Crit Rev Biochem Mol Biol [Internet]. Taylor & Francis; 1993 [cited 2022 Jun 6];28:31–81. Available from: https://doi.org/10.3109/10409239309082572

3. Ahrén B, Hughes TE. Inhibition of dipeptidyl peptidase-4 augments insulin secretion in response to exogenously administered glucagon-like peptide-1, glucose-dependent insulinotropic polypeptide, pituitary adenylate cyclase-activating polypeptide, and gastrin-releasing peptide in mice. Endocrinology. 2005;146:2055–9.

4. Chitadze G, Wehkamp U, Janssen O, Brüggemann M, Lettau M. The Serine Protease CD26/DPP4 in Non-Transformed and Malignant T Cells. Cancers [Internet]. 2021 [cited 2022 Jun 8];13:5947. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8657226/

5. Abbott CA, Baker E, Sutherland GR, McCaughan GW. Genomic organization, exact localization, and tissue expression of the human CD26 (dipeptidyl peptidase IV) gene. Immunogenetics. 1994;40:331–8.

6. Morimoto C, Torimoto Y, Levinson G, Rudd CE, Schrieber M, Dang NH, et al. 1F7, a novel cell surface molecule, involved in helper function of CD4 cells. J Immunol Baltim Md 1950. 1989;143:3430–9.

7. Bühling F, Junker U, Reinhold D, Neubert K, Jäger L, Ansorge S. Functional role of CD26 on human B lymphocytes. Immunol Lett. 1995;45:47–51.

8. Bühling F, Kunz D, Reinhold D, Ulmer AJ, Ernst M, Flad HD, et al. Expression and functional role of dipeptidyl peptidase IV (CD26) on human natural killer cells. Nat Immun. 1994;13:270–9.

9. Gliddon DR, Howard CJ. CD26 is expressed on a restricted subpopulation of dendritic cells in vivo. Eur J Immunol. 2002;32:1472–81.

10. Shah Z, Kampfrath T, Deiuliis JA, Zhong J, Pineda C, Ying Z, et al. Long-term dipeptidyl-peptidase 4 inhibition reduces atherosclerosis and inflammation via effects on monocyte recruitment and chemotaxis. Circulation. 2011;124:2338–49.

11. Shahbaz S, Xu L, Osman M, Sligl W, Shields J, Joyce M, et al. Erythroid precursors and progenitors suppress adaptive immunity and get invaded by SARS-CoV-2. Stem Cell Rep. 2021;16:1165–81.

12. Dang NH, Torimoto Y, Shimamura K, Tanaka T, Daley JF, Schlossman SF, et al. 1F7 (CD26): a marker of thymic maturation involved in the differential regulation of the CD3 and CD2 pathways of human thymocyte activation. J Immunol Baltim Md 1950. 1991;147:2825–32.

13. Yan S, Marguet D, Dobers J, Reutter W, Fan H. Deficiency of CD26 results in a change of cytokine and immunoglobulin secretion after stimulation by pokeweed mitogen. Eur J Immunol. 2003;33:1519–27.

14. Pinheiro MM, Stoppa CL, Valduga CJ, Okuyama CE, Gorjão R, Pereira RMS, et al. Sitagliptin inhibit human lymphocytes proliferation and Th1/Th17 differentiation in vitro. Eur J Pharm Sci Off J Eur Fed Pharm Sci. 2017;100:17–24.

15. Yegutkin GG. Nucleotide- and nucleoside-converting ectoenzymes: Important modulators of purinergic signalling cascade. Biochim Biophys Acta. 2008;1783:673–94.

16. Dong RP, Kameoka J, Hegen M, Tanaka T, Xu Y, Schlossman SF, et al. Characterization of adenosine deaminase binding to human CD26 on T cells and its biologic role in immune response. J Immunol [Internet]. American Association of Immunologists; 1996 [cited 2022 Jun 6];156:1349–55. Available from: https://www.jimmunol.org/content/156/4/1349

17. Kameoka J, Tanaka T, Nojima Y, Schlossman SF, Morimoto C. Direct association of adenosine deaminase with a T cell activation antigen, CD26. Science. 1993;261:466–9.

18. Martín M, Huguet J, Centelles JJ, Franco R. Expression of ecto-adenosine deaminase and CD26 in human T cells triggered by the TCR-CD3 complex. Possible role of adenosine deaminase as costimulatory molecule. J Immunol Baltim Md 1950. 1995;155:4630–43.

19. Ohnuma K, Uchiyama M, Yamochi T, Nishibashi K, Hosono O, Takahashi N, et al. Caveolin-1 triggers T-cell activation via CD26 in association with CARMA1. J Biol Chem. 2007;282:10117–31.

20. Rai AK, Thakur CP, Kumar P, Mitra DK. Impaired expression of CD26 compromises T-cell recruitment in human visceral leishmaniasis. Eur J Immunol [Internet]. 2012 [cited 2022 Jun 8];42:2782–91. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/eji.201141912

21. Am L, P P, C D, G B, K S, K A, et al. Kinetic investigation of chemokine truncation by CD26/dipeptidyl peptidase IV reveals a striking selectivity within the chemokine family. J Biol Chem [Internet]. J Biol Chem; 2001 [cited 2022 Jun 6];276. Available from: https://pubmed.ncbi.nlm.nih.gov/11390394/

22. Mortier A, Gouwy M, Van Damme J, Proost P, Struyf S. CD26/dipeptidylpeptidase IV—chemokine interactions: double-edged regulation of inflammation and tumor

biology. J Leukoc Biol [Internet]. 2016 [cited 2022 Jun 29];99:955–69. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7166560/

23. Cheng H-C, Abdel-Ghany M, Pauli BU. A novel consensus motif in fibronectin mediates dipeptidyl peptidase IV adhesion and metastasis. J Biol Chem. 2003;278:24600–7.

24. Brezinschek RI, Lipsky PE, Galea P, Vita R, Oppenheimer-Marks N. Phenotypic characterization of CD4+ T cells that exhibit a transendothelial migratory capacity. J Immunol Baltim Md 1950. 1995;154:3062–77.

25. Bengsch B, Seigel B, Flecken T, Wolanski J, Blum HE, Thimme R. Human Th17 cells express high levels of enzymatically active dipeptidylpeptidase IV (CD26). J Immunol Baltim Md 1950. 2012;188:5438–47.

26. Bailey SR, Nelson MH, Majchrzak K, Bowers JS, Wyatt MM, Smith AS, et al. Human CD26high T cells elicit tumor immunity against multiple malignancies via enhanced migration and persistence. Nat Commun [Internet]. Nature Publishing Group; 2017 [cited 2022 Jun 15];8:1961. Available from: https://www.nature.com/articles/s41467-017-01867-9

27. Nelson MH, Knochelmann HM, Bailey SR, Huff LW, Bowers JS, Majchrzak-Kuligowska K, et al. Identification of human CD4+ T cell populations with distinct antitumor activity. Sci Adv [Internet]. 2020 [cited 2022 Jun 8];6:eaba7443. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7458458/

28. Hatano R, Ohnuma K, Yamamoto J, Dang NH, Morimoto C. CD26-mediated co-stimulation in human CD8(+) T cells provokes effector function via pro-inflammatory cytokine production. Immunology. 2013;138:165–72.

29. Yamada Y, Jang J, Meester ID, Inci I, Weder W, Jungraithmayr W. Acute Allograft Rejection Is Attenuated By CD26-Inhibition Through IL-17 Suppression in Mouse Lung Transplants. J Heart Lung Transplant [Internet]. Elsevier; 2015 [cited 2022 Jun 8];34:S52. Available from: https://www.jhltonline.org/article/S1053-2498(15)00157-6/fulltext

30. Yamada Y, Jang J-H, Meester ID, Baerts L, Vliegen G, Inci I, et al. CD26 costimulatory blockade improves lung allograft rejection and is associated with enhanced interleukin-10 expression. J Heart Lung Transplant [Internet]. Elsevier; 2016 [cited 2022 Jun 8];35:508–17. Available from: https://www.jhltonline.org/article/S1053-2498(15)01519-3/fulltext

31. Zhao X, Zhang K, Daniel P, Wisbrun N, Fuchs H, Fan H. Delayed allogeneic skin graft rejection in CD26-deficient mice. Cell Mol Immunol [Internet]. 2019 [cited 2022 Jun 8];16:557–67. Available from: https://europepmc.org/articles/PMC6804736

32. Jungraithmayr W, Enz N. CD26 – The emerging role of a costimulatory molecule in allograft rejection. Cell Mol Immunol [Internet]. Nature Publishing Group; 2020

[cited 2022 Jun 8];17:1208–9. Available from: https://www.nature.com/articles/s41423-018-0028-9

33. Ibegbu CC, Xu Y-X, Fillos D, Radziewicz H, Grakoui A, Kourtis AP. Differential expression of CD26 on virus-specific CD8(+) T cells during active, latent and resolved infection. Immunology. 2009;126:346–53.

34. Vanham G, Kestens L, De Meester I, Vingerhoets J, Penne G, Vanhoof G, et al. Decreased expression of the memory marker CD26 on both CD4+ and CD8+ T lymphocytes of HIV-infected subjects. J Acquir Immune Defic Syndr. 1993;6:749–57.

35. Morrison ME, Vijayasaradhi S, Engelstein D, Albino AP, Houghton AN. A marker for neoplastic progression of human melanocytes is a cell surface ectopeptidase. J Exp Med. 1993;177:1135–43.

36. Sharma PK, Wong EB, Napier RJ, Bishai WR, Ndung'u T, Kasprowicz VO, et al. High expression of CD26 accurately identifies human bacteria-reactive MR1-restricted MAIT cells. Immunology. 2015;145:443–53.

37. Treiner E, Duban L, Bahram S, Radosavljevic M, Wanner V, Tilloy F, et al. Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. Nature. 2003;422:164–9.

38. Porcelli S, Yockey CE, Brenner MB, Balk SP. Analysis of T cell antigen receptor (TCR) expression by human peripheral blood CD4-8- alpha/beta T cells demonstrates preferential use of several V beta genes and an invariant TCR alpha chain. J Exp Med. 1993;178:1–16.

39. Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, Liu L, et al. MR1 presents microbial vitamin B metabolites to MAIT cells. Nature. 2012;491:717–23.

40. Dusseaux M, Martin E, Serriari N, Péguillet I, Premel V, Louis D, et al. Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting T cells. Blood. 2011;117:1250–9.

41. Fergusson JR, Smith KE, Fleming VM, Rajoriya N, Newell EW, Simmons R, et al. CD161 Defines a Transcriptional and Functional Phenotype across Distinct Human T Cell Lineages. Cell Rep [Internet]. 2014 [cited 2022 Jun 1];9:1075–88. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2211124714008286

42. Seifert M, Sellmann L, Bloehdorn J, Wein F, Stilgenbauer S, Dürig J, et al. Cellular origin and pathophysiology of chronic lymphocytic leukemia. J Exp Med. 2012;209:2183–98.

43. Allegra A, Tonacci A, Musolino C, Pioggia G, Gangemi S. Secondary Immunodeficiency in Hematological Malignancies: Focus on Multiple Myeloma and Chronic Lymphocytic Leukemia. Front Immunol [Internet]. 2021 [cited 2022 Jun

17];12. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2021.738915

44. Riches JC, Davies JK, McClanahan F, Fatah R, Iqbal S, Agrawal S, et al. T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. Blood [Internet]. 2013 [cited 2022 Jun 15];121:1612–21. Available from: https://doi.org/10.1182/blood-2012-09-457531

45. Bozorgmehr N, Okoye I, Oyegbami O, Xu L, Fontaine A, Cox-Kennett N, et al. Expanded antigen-experienced CD160+CD8+effector T cells exhibit impaired effector functions in chronic lymphocytic leukemia. J Immunother Cancer. 2021;9:e002189.

46. Wang Y, Achenbach SJ, Rabe KG, Shanafelt TD, Call TG, Ding W, et al. Cause of death in patients with newly diagnosed chronic lymphocytic leukemia (CLL) stratified by the CLL-International Prognostic Index. Blood Cancer J [Internet]. Nature Publishing Group; 2021 [cited 2022 Jun 10];11:1–4. Available from: https://www.nature.com/articles/s41408-021-00532-1

47. Molica S. Second neoplasms in chronic lymphocytic leukemia: incidence and pathogenesis with emphasis on the role of different therapies. Leuk Lymphoma. 2005;46:49–54.

48. Christopoulos P, Pfeifer D, Bartholomé K, Follo M, Timmer J, Fisch P, et al. Definition and characterization of the systemic T-cell dysregulation in untreated indolent B-cell lymphoma and very early CLL. Blood. 2011;117:3836–46.

49. Landego I, Hewitt D, Hibbert I, Dhaliwal D, Pieterse W, Grenier D, et al. PD-1 inhibition in malignant melanoma and lack of clinical response in chronic lymphocytic leukemia in the same patients: a case series. Curr Oncol [Internet]. 2020 [cited 2022 Jun 29];27:169–72. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7339846/

50. Mancikova V, Smida M. Current State of CAR T-Cell Therapy in Chronic Lymphocytic Leukemia. Int J Mol Sci [Internet]. 2021 [cited 2022 Jun 11];22:5536. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8197365/

51. Lee S. Stages of chronic lymphocytic leukemia (CLL) [Internet]. Can. Cancer Soc. [cited 2022 Jun 1]. Available from: https://cancer.ca/en/cancer-information/cancer-types/chronic-lymphocytic-leukemia-cll/staging

52. Bozorgmehr N, Mashhouri S, Perez Rosero E, Xu L, Shahbaz S, Sligl W, et al. Galectin-9, a Player in Cytokine Release Syndrome and a Surrogate Diagnostic Biomarker in SARS-CoV-2 Infection. mBio [Internet]. 2021 [cited 2023 Jan 6];12:e00384-21. Available from: https://journals.asm.org/doi/10.1128/mBio.00384-21

53. Shahbaz S, Dunsmore G, Koleva P, Xu L, Houston S, Elahi S. Galectin-9 and VISTA Expression Define Terminally Exhausted T Cells in HIV-1 Infection. J Immunol [Internet]. American Association of Immunologists; 2020 [cited 2022 Jun 29];

Available from: https://www.jimmunol.org/content/early/2020/03/20/jimmunol.1901481

54. Elahi S, Shahbaz S, Houston S. Selective Upregulation of CTLA-4 on CD8+ T Cells Restricted by HLA-B*35Px Renders them to an Exhausted Phenotype in HIV-1 infection. PLOS Pathog [Internet]. Public Library of Science; 2020 [cited 2022 Jul 29];16:e1008696. Available from: https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1008696

55. Shahbaz S, Okoye I, Blevins G, Elahi S. Elevated ATP via enhanced miRNA-30b, 30c, and 30e downregulates the expression of CD73 in CD8+ T cells of HIV-infected individuals. PLOS Pathog [Internet]. Public Library of Science; 2022 [cited 2022 Jun 29];18:e1010378. Available from: https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1010378

56. Dunsmore G, Rosero EP, Shahbaz S, Santer DM, Jovel J, Lacy P, et al. Neutrophils promote T-cell activation through the regulated release of CD44-bound Galectin-9 from the cell surface during HIV infection. PLOS Biol [Internet]. Public Library of Science; 2021 [cited 2022 Jun 29];19:e3001387. Available from: https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.3001387

57. Li M, Yao D, Zeng X, Kasakovski D, Zhang Y, Chen S, et al. Age related human T cell subset evolution and senescence. Immun Ageing [Internet]. 2019 [cited 2022 Jun 1];16:24. Available from: https://doi.org/10.1186/s12979-019-0165-8

58. Sulda ML, Abbott CA, Macardle PJ, Hall RK, Kuss BJ. Expression and prognostic assessment of dipeptidyl peptidase IV and related enzymes in B-cell chronic lymphocytic leukemia. Cancer Biol Ther. 2010;10:180–9.

59. Bauvois B, De Meester I, Dumont J, Rouillard D, Zhao HX, Bosmans E. Constitutive expression of CD26/dipeptidylpeptidase IV on peripheral blood B lymphocytes of patients with B chronic lymphocytic leukaemia. Br J Cancer. 1999;79:1042–8.

60. Sallusto F, Lenig D, Förster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature. 1999;401:708–12.

61. Shahbaz S, Xu L, Sligl W, Osman M, Bozorgmehr N, Mashhouri S, et al. The Quality of SARS-CoV-2-Specific T Cell Functions Differs in Patients with Mild/Moderate versus Severe Disease, and T Cells Expressing Coinhibitory Receptors Are Highly Activated. J Immunol Baltim Md 1950. 2021;207:1099–111.

62. Lugli E, Gattinoni L. Harnessing Stem Cell-Like Memory T Cells for Adoptive Cell Transfer Therapy of Cancer. In: Ascierto PA, Stroncek DF, Wang E, editors. Dev T Cell Based Cancer Immunother [Internet]. Cham: Springer International Publishing; 2015 [cited 2022 Oct 11]. p. 183–209. Available from: https://doi.org/10.1007/978-3-319-21167-1_8

63. Mahnke YD, Brodie TM, Sallusto F, Roederer M, Lugli E. The who's who of T-cell differentiation: Human memory T-cell subsets. Eur J Immunol [Internet]. 2013 [cited 2022 Jun 1];43:2797–809. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/eji.201343751

64. Song D-G, Ye Q, Poussin M, Harms GM, Figini M, Powell DJ. CD27 costimulation augments the survival and antitumor activity of redirected human T cells in vivo. Blood. 2012;119:696–706.

65. Gherardin NA, Souter MN, Koay H-F, Mangas KM, Seemann T, Stinear TP, et al. Human blood MAIT cell subsets defined using MR1 tetramers. Immunol Cell Biol. 2018;96:507–25.

66. Allard B, Allard D, Buisseret L, Stagg J. The adenosine pathway in immuno-oncology. Nat Rev Clin Oncol [Internet]. Nature Publishing Group; 2020 [cited 2022 Jun 1];17:611–29. Available from: https://www.nature.com/articles/s41571-020-0382-2

67. Raskov H, Orhan A, Christensen JP, Gögenur I. Cytotoxic CD8+ T cells in cancer and cancer immunotherapy. Br J Cancer [Internet]. Nature Publishing Group; 2021 [cited 2022 Jun 1];124:359–67. Available from: https://www.nature.com/articles/s41416-020-01048-4

68. Elahi S, Dinges WL, Lejarcegui N, Laing KJ, Collier AC, Koelle DM, et al. Protective HIV-specific CD8+ T cells evade Treg cell suppression. Nat Med. 2011;17:989–95.

69. Trapani JA, Smyth MJ. Functional significance of the perforin/granzyme cell death pathway. Nat Rev Immunol. 2002;2:735–47.

70. Lopez JA, Susanto O, Jenkins MR, Lukoyanova N, Sutton VR, Law RHP, et al. Perforin forms transient pores on the target cell plasma membrane to facilitate rapid access of granzymes during killer cell attack. Blood. 2013;121:2659–68.

71. Peters PJ, Borst J, Oorschot V, Fukuda M, Krähenbühl O, Tschopp J, et al. Cytotoxic T lymphocyte granules are secretory lysosomes, containing both perforin and granzymes. J Exp Med. 1991;173:1099–109.

72. Mousset CM, Hobo W, Woestenenk R, Preijers F, Dolstra H, van der Waart AB. Comprehensive Phenotyping of T Cells Using Flow Cytometry. Cytom Part J Int Soc Anal Cytol. 2019;95:647–54.

73. Sekine T, Perez-Potti A, Nguyen S, Gorin J-B, Wu VH, Gostick E, et al. TOX is expressed by exhausted and polyfunctional human effector memory CD8+ T cells. Sci Immunol. 2020;5:eaba7918.

74. Slaney CY, Kershaw MH, Darcy PK. Trafficking of T cells into tumors. Cancer Res. 2014;74:7168–74.

75. Stein JV, Nombela-Arrieta C. Chemokine control of lymphocyte trafficking: a general overview. Immunology [Internet]. 2005 [cited 2022 Jun 2];116:1–12. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1802414/

76. Wang C, Kang SG, Lee J, Sun Z, Kim CH. The roles of CCR6 in migration of Th17 cells and regulation of effector T-cell balance in the gut. Mucosal Immunol [Internet]. Nature Publishing Group; 2009 [cited 2022 Jun 2];2:173–83. Available from: https://www.nature.com/articles/mi200884

77. Hamann A, Andrew DP, Jablonski-Westrich D, Holzmann B, Butcher EC. Role of alpha 4-integrins in lymphocyte homing to mucosal tissues in vivo. J Immunol [Internet]. American Association of Immunologists; 1994 [cited 2022 Jun 2];152:3282–93. Available from: https://www.jimmunol.org/content/152/7/3282

78. Baekkevold ES, Yamanaka T, Palframan RT, Carlsen HS, Reinholt FP, von Andrian UH, et al. The CCR7 ligand elc (CCL19) is transcytosed in high endothelial venules and mediates T cell recruitment. J Exp Med. 2001;193:1105–12.

79. Picker LJ, Michie SA, Rott LS, Butcher EC. A unique phenotype of skin-associated lymphocytes in humans. Preferential expression of the HECA-452 epitope by benign and malignant T cells at cutaneous sites. Am J Pathol. 1990;136:1053–68.

80. Mackay LK, Braun A, Macleod BL, Collins N, Tebartz C, Bedoui S, et al. Cutting edge: CD69 interference with sphingosine-1-phosphate receptor function regulates peripheral T cell retention. J Immunol Baltim Md 1950. 2015;194:2059–63.

81. Remmerswaal EBM, Hombrink P, Nota B, Pircher H, Ten Berge IJM, van Lier RAW, et al. Expression of IL-7Rα and KLRG1 defines functionally distinct CD8+ T-cell populations in humans. Eur J Immunol. 2019;49:694–708.

82. Mezawa Y, Daigo Y, Takano A, Miyagi Y, Yokose T, Yamashita T, et al. CD26 expression is attenuated by TGF-β and SDF-1 autocrine signaling on stromal myofibroblasts in human breast cancers. Cancer Med. 2019;8:3936–48.

83. Kashio Y, Nakamura K, Abedin MJ, Seki M, Nishi N, Yoshida N, et al. Galectin-9 Induces Apoptosis Through the Calcium-Calpain-Caspase-1 Pathway. J Immunol [Internet]. American Association of Immunologists; 2003 [cited 2022 Jul 20];170:3631–6. Available from: https://www.jimmunol.org/content/170/7/3631

84. Lhuillier C, Barjon C, Niki T, Gelin A, Praz F, Morales O, et al. Impact of Exogenous Galectin-9 on Human T Cells. J Biol Chem [Internet]. 2015 [cited 2022 Jul 20];290:16797–811. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4505427/

85. Okoye I, Xu L, Motamedi M, Parashar P, Walker JW, Elahi S. Galectin-9 expression defines exhausted T cells and impaired cytotoxic NK cells in patients with virus-associated solid tumors. J Immunother Cancer. 2020;8:e001849.

86. Rahimpour A, Koay HF, Enders A, Clanchy R, Eckle SBG, Meehan B, et al. Identification of phenotypically and functionally heterogeneous mouse mucosal-associated invariant T cells using MR1 tetramers. J Exp Med. 2015;212:1095–108.

87. Okoye IS, Houghton M, Tyrrell L, Barakat K, Elahi S. Coinhibitory Receptor Expression and Immune Checkpoint Blockade: Maintaining a Balance in CD8+ T Cell Responses to Chronic Viral Infections and Cancer. Front Immunol. 2017;8:1215.

88. Hendriks J, Xiao Y, Borst J. CD27 promotes survival of activated T cells and complements CD28 in generation and establishment of the effector T cell pool. J Exp Med. 2003;198:1369–80.

89. Ochsenbein AF, Riddell SR, Brown M, Corey L, Baerlocher GM, Lansdorp PM, et al. CD27 Expression Promotes Long-Term Survival of Functional Effector–Memory CD8+Cytotoxic T Lymphocytes in HIV-infected Patients. J Exp Med [Internet]. 2004 [cited 2022 Jun 29];200:1407–17. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2211945/

90. Leng T, Akther HD, Hackstein C-P, Powell K, King T, Friedrich M, et al. TCR and Inflammatory Signals Tune Human MAIT Cells to Exert Specific Tissue Repair and Effector Functions. Cell Rep. 2019;28:3077-3091.e5.

91. Kahan SM, Bakshi RK, Ingram JT, Hendrickson RC, Lefkowitz EJ, Crossman DK, et al. Intrinsic IL-2 production by effector CD8 T cells affects IL-2 signaling and promotes fate decisions, stemness, and protection. Sci Immunol [Internet]. American Association for the Advancement of Science; 2022 [cited 2022 Jun 14];7:eabl6322. Available from: https://www.science.org/doi/10.1126/sciimmunol.abl6322

92. Bouwman AC, van Daalen KR, Crnko S, ten Broeke T, Bovenschen N. Intracellular and Extracellular Roles of Granzyme K. Front Immunol [Internet]. 2021 [cited 2022 Jun 14];12. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2021.677707

93. Lückel C, Picard FelixSR, Huber M. Tc17 biology and function: Novel concepts. Eur J Immunol [Internet]. 2020 [cited 2022 Jun 17];50:1257–67. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/eji.202048627

94. Leeansyah E, Boulouis C, Kwa ALH, Sandberg JK. Emerging Role for MAIT Cells in Control of Antimicrobial Resistance. Trends Microbiol [Internet]. 2021 [cited 2022 Jun 15];29:504–16. Available from: https://www.sciencedirect.com/science/article/pii/S0966842X20303140

95. Godfrey DI, Koay H-F, McCluskey J, Gherardin NA. The biology and functional importance of MAIT cells. Nat Immunol [Internet]. Nature Publishing Group; 2019 [cited 2022 Jun 17];20:1110–28. Available from: https://www.nature.com/articles/s41590-019-0444-8

96. Murata K, Tsukahara T, Emori M, Shibayama Y, Mizushima E, Matsumiya H, et al. Identification of a novel human memory T-cell population with the characteristics of

stem-like chemo-resistance. OncoImmunology [Internet]. Taylor & Francis; 2016 [cited 2022 Jun 17];5:e1165376. Available from: https://doi.org/10.1080/2162402X.2016.1165376

97. Walling BL, Kim M. LFA-1 in T Cell Migration and Differentiation. Front Immunol [Internet]. 2018 [cited 2022 Jun 29];9. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2018.00952

98. Pangrazzi L, Reidla J, Carmona Arana JA, Naismith E, Miggitsch C, Meryk A, et al. CD28 and CD57 define four populations with distinct phenotypic properties within human CD8+ T cells. Eur J Immunol [Internet]. 2020 [cited 2022 Jun 29];50:363–79. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7079235/

99. Mou D, Espinosa J, Lo DJ, Kirk AD. CD28 Negative T cells: is their loss our gain? Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg [Internet]. 2014 [cited 2022 Jun 17];14:2460–6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4886707/

100. Strioga M, Pasukoniene V, Characiejus D. CD8+ CD28− and CD8+ CD57+ T cells and their role in health and disease. Immunology [Internet]. 2011 [cited 2022 Jun 17];134:17–32. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3173691/

101. Pang R, Law WL, Chu ACY, Poon JT, Lam CSC, Chow AKM, et al. A subpopulation of CD26+ cancer stem cells with metastatic capacity in human colorectal cancer. Cell Stem Cell. 2010;6:603–15.

102. Barreira da Silva R, Laird ME, Yatim N, Fiette L, Ingersoll MA, Albert ML. Dipeptidylpeptidase 4 inhibition enhances lymphocyte trafficking, improving both naturally occurring tumor immunity and immunotherapy. Nat Immunol. 2015;16:850–8.

103. Wang H, Liu X, Long M, Huang Y, Zhang L, Zhang R, et al. NRF2 activation by antioxidant antidiabetic agents accelerates tumor metastasis. Sci Transl Med. 2016;8:334ra51.

104. Detel D, Baticic L, Varljen J. The influence of age on intestinal dipeptidyl peptidase IV (DPP IV/CD26), disaccharidases, and alkaline phosphatase enzyme activity in C57BL/6 mice. Exp Aging Res. 2008;34:49–62.

4 Chapter 4: Transcriptomic profiling of peripheral blood cells in HPV-associated carcinoma patients receiving the combined valproic acid and avelumab therapy.

A version of this chapter is under review: **Bozorgmehr N**, Syed H, Mashhouri S, Walker J, and Elahi S. Transcriptomic profiling of peripheral blood cells in HPV-associated carcinoma patients receiving the combined Valproic acid and Avelumab therapy. Molecular oncology. Under review.

4.1 Background

Tumor cells exploit diverse mechanisms to suppress or evade immune surveillance to facilitate tumor progression and metastasis [1]. Tumor cells recruit and educate different suppressor cells to build an immunosuppressive tumor microenvironment (TME); consisting of myeloid-derived suppressor cells (MDSCs), neutrophils, tumor-associated macrophages (TAMs), regulatory T cells (Tregs), and $CD71^+$ erythroid cells (CECs) [2–5]. Moreover, tumor-associated soluble mediators (e.g., cytokines), altered metabolism, aberrant vessels, and curbed stromal cells can influence the immune response against the tumor [6]. Although immune checkpoint inhibitors (ICIs) targeting PD-1, PD-L1, and CTLA-4 have revolutionized the cancer field, only some patients exhibit durable clinical outcomes [7]. Other patients either do not respond (non-responders) or initially respond but eventually acquire resistance [8,9]. Hence, this remains a prevailing clinical need in the ICIs field to discriminate non-responders from responders at the baseline or during treatment. Such studies to identify potential responders from non-responders will save lives and facilitate clinical decision-making.

There is growing evidence that tumor recruits and manipulates myeloid cells to transform them into immunosuppressive cells as an anti-tumor immune response evasion mechanism [10,11]. Myeloid cells constitute the majority of peripheral blood immune cells and include different subsets with distinct functional properties [1]. Granulocytes, monocytes, and dendritic cells are the major innate immune components in the periphery. The TME of solid tumors is a fertile niche to recruit and educate MDSCs, divided into monocytic (M-MDSC) and granulocytic (PMN-MDSCs) suppressor cells [12]. Given their immunosuppressive nature, it is evident that these

myeloid cells suppress T cell effector functions and interfere with the efficacy of ICIs [13]. Accordingly, targeting myeloid cells to improve the clinical outcomes of ICIs has been considered [14]. Although ICIs in solid tumors have been widely investigated, their application in virus-associated tumors merits further investigation. Human papilloma Virus (HPV) is dsDNA virus. Carcinogenic variants (HPV-16, -18) integrate into the host's DNA and cause squamous cell carcinoma of the cervix, anus, vulva, penis, and oropharynx [15]. Generally, HPV-associated carcinomas are genetically less complex and induce a potent antitumoral response leading to improved overall survival [15]. Nevertheless, often the diagnosis of these tumors occurs very late when tumors are locally advanced or become metastatic [16]. Immunotherapy appears to be a promising approach for treating HPV- associated tumors due to the complex virus and host interactions [17]. Mounting evidence has shown that HPV-associated carcinomas are potential candidates for ICIs since they modulate the expression of co-inhibitory receptors. For example, the E7 oncoprotein generated by HPV-16 augments PD-L1 expression [18], and similarly, E6 and E7 oncoproteins induce hypermethylation of DNA repair genes and prompt CTLA-4 upregulation [19]. Alongside FDA approved pembrolizumab (anti-PD-1) therapy for recurrent or metastatic cervical cancer in 2018 [20]. However, failure to respond to such treatments has raised the necessity of combined treatment options. For instance, ICIs combined with histone deacetylases (HDACs) inhibitors have shown promising results by enhancing anti-tumor immunity [21].

Valproic acid is an HDAC (Histone deacetylase) inhibitor and is a common medication for treating neurologic disorders. Recently its promising therapeutic effects against HIV and cancer have been revealed [22]. Genetic modifications in DNA are linked to

cancer progression, and HDACs are essential regulators of gene expression [23]. Given HDACs' dysregulation in different cancers, they could be potential therapeutic targets. Almost all cancer cells exhibit increased HDAC activity that impacts gene expression and cell differentiation in many aspects [24]. These gene transcriptional changes impact DNA repair, cell cycle control, apoptosis, differentiation, nuclear import, metabolism, and vascular function [22]. Valproic acid is widely studied in different cancers due to its apoptotic effects on malignant cells [25,26]. Reports have shown that Valproic acid halts the proliferation of HPV-associated cancer cell lines [27] and increases cytotoxic T lymphocyte response to HPV-associated cervical cancers [28]. It is reported that valproic acid has various immunological properties and modulates innate and adaptive immune responses [29].

One of the significant challenges in the ICIs field is potential predictive biomarkers to identify patients who will benefit or respond positively to ICIs. Due to the complexity of genetic host factors, tumor types, tumor mutations, and differential immune responses following ICIs treatment [8], identifying potentially predictive biomarkers is highly valuable. In this study, we utilized bulk RNA sequencing (RNAseq) of PBMCs from HPV-associated carcinoma patients to identify responders from non-responders. Patients were enrolled in a non-randomized, single-arm, phase II clinical trial investigating the effects of oral valproate combined with Avelumab (anti-PD-L1) [30]. Given the impracticality of tumor tissue biopsies, our results support the value of bulk RNAseq of the peripheral blood as a non-invasive approach to predict ICI response. We anticipate our results will improve clinical decision-making in ICIs and inform future interventions.

4.2 Materials and methods

4.2.1 Study population

Peripheral blood samples were collected in EDTA tubes from patients with advanced HPV-associated carcinoma (39 samples from n=11) who enrolled in the LATENT trial study (Lytic Activation To Enhance Neoantigen-directed Therapy) the Cross Cancer Institute, University of Alberta. This study was a non-randomized, single-arm, basket phase II clinical trial investigating the effects of oral valproate combined with Avelumab (anti-PD-L1) in patients with virus-associated solid tumors [30]. All tumors were p16-positive squamous cell carcinoma from different sites (e.g., genitourinary, anal, head, and neck). Clinical data and evaluated responses to treatment based on iRECIST criteria [31] were collected for further analysis. Patient characteristics are summarized in Supplementary Table 4.6.1.

4.2.2 Ethics statement

This study was approved by the Health Research Ethics Board of Alberta (HREBA protocol # HREBA CC-17-0374) at the University of Calgary. Written informed consent to participate in the study was obtained from all participants.

4.2.3 Cell isolation

Peripheral blood mononuclear cells (PBMCs) were isolated from fresh blood using Ficoll-Paque gradients (GE Healthcare). RPMI 1640 (Sigma-Aldrich) supplemented with 10% FBS (Sigma-Aldrich) and 1% penicillin/streptomycin (Sigma-Aldrich) was

used for PBMC culture. Isolated PBMCs were cryopreserved in TRIzol (Invitrogen) for further RNA isolation.

4.2.4 Library construction and sequencing

Total RNA (39 samples from 11 patients) was extracted from cryopreserved PBMCs in TRIzol reagent (Direct-zol RNA kit) based on the manufacturer's protocol, as reported elsewhere [32]. Samples' characteristics are summarized in Supplementary Table 4.6.2. Isolated RNA was quantified, and the quality of RNA samples was assessed by Agilent Bioanalyzer. Samples with RNA Integrity Numbers (RIN) of more than 6.5 were selected and purified (Supplementary Fig. 1a, Supplementary Table 4.6.2) using Poly A selection with oligo dTs conjugated to paramagnetic beads. Per the kit's instruction, the first and second strands of cDNA libraries were constructed from 100 ng of extracted RNA using the TruSeq RNA Library Prep kit v2 (Illumina). T-A ligation was added to the blunted and A-tailed cDNA before doing 12 cycles of PCR to incorporate illumine adapters containing multiplexing barcodes. A HiSeq 2500 Illumina instrument sequenced samples on a paired-end 150 cycles protocol. Demultiplexed data generated are available from the SRA portal on NCBI under Accession Number GSE229014.

4.2.5 Bioinformatic analyses

Kallisto software aligned fragments to the human cDNA database (GRCh38). One hundred permutations were selected during pseudo-alignments and bias corrections. For statistical analysis, patients were divided into two groups responders (R, n=4) and non-responders (NR, n=7). Differential Expression analyses were extracted from

the count data using the DESeq2 R package version 4.2.0 (2022-04-22). Transcript abundance differences were considered as differentially expressed with an absolute Log_2 fold change > 1 and *Padj* value less than 0.05 (*False Discovery Rate (FDR)* < 0.05). A list of differentially expressed transcripts was obtained for each pairwise comparison: cross-sectional group comparisons (between R and NR at baseline and endpoint) and longitudinal (R at cycle1, 2, and endpoint vs. cycle 0, NR at cycle1,2, and endpoint vs. cycle 0). QIAGEN IPA software (2023) was used for enrichment and functional analysis. As proof, results were also compared to Metascape (v3.5.20230101) [33] software. The R scripts were used for data visualization.

4.2.6 Digital cytometry

The CIBERSORTx from Newman lab (https://cibersortx.stanford.edu/) [34] is used to deconvolute the immune cell fractions and abundances from the isolated PBMCs' bulk RNA seq data [35]. The TPM (Transcripts per million) files, representative of the gene length-normalized expression data, were uploaded as the mixture gene files to the CIBERSORTx website and compared to software incorporated LM22 signature matrix file. The type and abundance of immune cells were merged into six cell types, including B cells, $CD8^+$ T cells, $CD4^+$ T cells, Natural Killer (NK) cells, Monocytes, and granulocytes. The *p-values* < 0.05 were considered statistically significant.

4.2.7 Flow cytometry

Conjugated fluorophore monoclonal antibodies were purchased from BD Bioscience, Thermo Fischer Scientific, and Biolegend. The list of antibodies used for this study is as follows: anti-CD3 (SK7), anti-CD14 (M5E2), anti-CD15 (HI98), anti-CD66b

(G10F5), anti-CD11b (ICRF44). As reported previously, surface staining was carried out ex-vivo using fresh PBMCs[30,36]. LSR-Fortessa SORP flow cytometry machine and Flow Jo software (V.10.8.1) were used for data acquirement and analysis.

4.2.8 ELISA and Multiplex assays

Multiplex analyses of plasma cytokines/chemokines were conducted by Mesoscale Discovery (MSD) kits, as we have reported before [36,37]. Data were obtained on a V-plex® Sector Imager 2400 plate reader and analyzed on the MSD Workbench 3.0 software. Moreover, plasma soluble Galectin-9, PD-L1, IL-18, and TGF-β were detected by DuoSet ELISA kit (R&D) following the manufacturer's protocols. Synergy H1 Biotek microplate reader was used to acquire the ELISA data and analyzed by Gen5 V.2.07 software. Also, plasma samples from 10 patients enrolled in this clinical trial were used to validate soluble mediator measurement as an ancillary cohort (Supplementary Table 4.6.3).

4.2.9 Statistical analysis

We used Graph Pad Prism software (Version 9.5.0) for the statistical analysis. The Mann-Whitney U or Wilcoxon signed-rank test was used appropriately for non-paired or paired comparisons. P-values less than 0.05 were counted as statistically significant. Data showed as Mean ± SEM.

4.3 Results

4.3.1 Clinical trial outlines and patient characteristics

The LATENT Clinical Trial study (ClinicalTrials.gov Identifier: NCT03357757) [38] was an open-label, non-randomized, single-arm phase II to investigate the effects of combined Valproic acid and Avelumab (anti-PD-L1) in a cohort of 40 virus associated solid tumors (VASTs) [30]. The treatment started with oral daily Valproic acid (VA) followed by Avelumab (10 mg/kg, i.v., every two weeks) (Fig.1a). Every two weeks counted as cycles. We analyzed blood samples throughout this trial at cycle 0 (C0) reflects the baseline before the initiation of treatment, two weeks after the initiation of oral Valproic acid as cycle 1 (C1), two weeks after Avelumab treatment (C2), and cycles 6/7 considered as the Endpoint (EP). The response to treatment was evaluated by the iRECIST criteria [31] (based on RECIST 1.1) at the EP, which was 12 weeks after the trial initiation. Although our cohort consisted of EBV and HPV-associated carcinoma patients, this study was focused on HPV-associated carcinoma. Based on sample availability for described cycles (C0, C1, C2, and EP), we collected 39 samples from 11 patients (Supplementary Tables 1, 2). Also, ten patients, five non-responders and five responders, from the same clinical trial were used to validate plasma analytes (Supplementary table 3). The workflow of the study is illustrated in Figures 1b and 1c. For analysis purposes, we grouped them based on their best overall clinical response assessment (iRECIST criteria) to either non-responders (NR) or responders (R). The NR group consisted of iCPD (immune confirmed progressive disease), clinical progression, and iUPDs (immune unconfirmed progressive disease); however, the R group included iCCR (confirmed complete response), iCPR (confirmed partial response), and iSD (stable disease) (Supplementary Table 1). The NR group was composed of 24 samples from 7 patients, including C0 (n=7), C1 (n=5), C2 (n=6), and EP (n=6). The R group consisted of 15 samples from 4 patients, including C0

(n=4), C1 (n=3), C2 (n=4), and EP (n=4) (Supplementary Table 2). To investigate the longitudinal changes and group differences, we also compared the differentially expressed transcripts in each group to their baseline (C0) and the R to the NR group at the baseline and EP, accordingly (Fig. 1d).

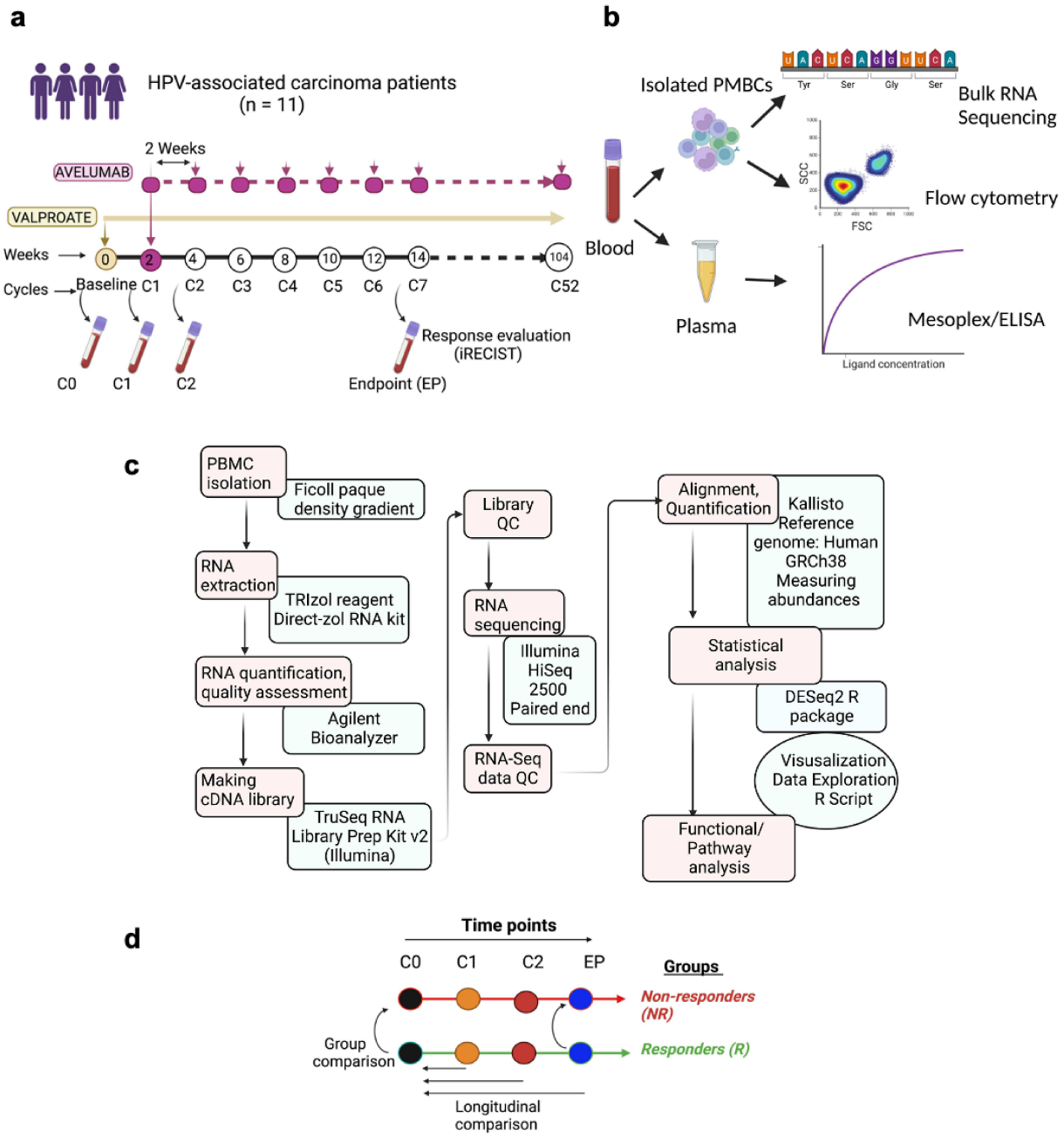


Fig. 1. Overview of the clinical trial, the study, and experimental design. (a) Metastatic HPV-associated carcinoma patients received daily Valproic acid (VA) at the initiation of the trial (C0) for 2 weeks, from week 2 (cycle 1) they received Avelumab (anti-PD-L1) every 2 weeks combined with daily VA throughout the study. At the baseline (Cycle 0 = C0), cycle 1 (C1), cycle 2 (C2), and cycle 6/7 (Endpoint = EP) blood was collected. **(b)** PBMCs were isolated for bulk RNA sequencing and flowcytometry, Plasma was separated for Mesoplex/ELISA study. **(c)** Workflow showing sequential stages of RNA extraction, RNA Sequencing, and data extraction. **(d)** Analysis flowchart showing group comparison between responders and non-responders and comparing timepoints in each group to the baseline.

4.3.2 Differential transcript expression profiles of peripheral blood immune cells in non-responders and responders.

To investigate the differences in transcriptional profiles through the cycles, the RNAseq was conducted on total RNA extracted from PBMCs (Supplementary Table 2). Euclidean distances from the regularized-logarithmic transformation (rlog) of the count data were calculated in all samples and shown as a heatmap (Supplementary Fig.1b).

To evaluate transcriptional changes following VA, Avelumab, and combo treatment, each sample was compared to their C0 pairwise manner (e.g., C1 to C0, C2 to C0, and EP to C0). In brief, each group showed differential changes in their transcriptome following treatment. When C1 was compared to C0 in the NR group, we found 540 transcripts were upregulated, and 854 transcripts were downregulated, significantly (Fig. 2a and Supplementary Fig. 1c). These changes appeared to be more significant at C2 compared to C0 with having 941 transcripts upregulated and 790 transcripts downregulated (Fig. 2b and Supplementary Fig. 1d). When the EP was compared to C0, we noted only 136 and 159 transcripts were up and downregulated in the NR

group, respectively (Fig. 2c and Supplementary Fig. 1e). To better visualize the dispersion of whole transcripts in different cycles in the NR group, we used PCA plots. These analyses showed a minimal overlap of transcripts between patients (Fig. 2d). Moreover, the upset plots illustrate the differentially expressed transcripts, down or upregulated in different cycles in the NR (Fig. 2e, 2f). Interestingly, in the R group, we found only a small portion of transcripts were changed after the VA treatment; 16 were upregulated vs. 11 were downregulated (Fig. 2g and Supplementary Fig. 1f). However, once the R group was placed on Avelumab, we observed substantial changes at the transcriptional level in their PBMCs compared to the baseline (Fig. 2h and Supplementary Fig. 1g). Finally, we noted a moderate change in transcripts of the R group at the EP compared to the baseline (~200 transcripts were up or downregulated) (Fig. 2i and Supplementary Fig. 1h). By deploying the PCA plots, we found negligible overlap between patients (Fig. 2j). Finally, by using the upset plots we compared transcriptional changes between C1, C2, and EP in the R group (Fig. 2k, 2l).

Collectively, comparing both groups to the baseline, while NRs showed a substantial change at the transcriptional levels at C1 and C2, responders revealed slight changes at C1.

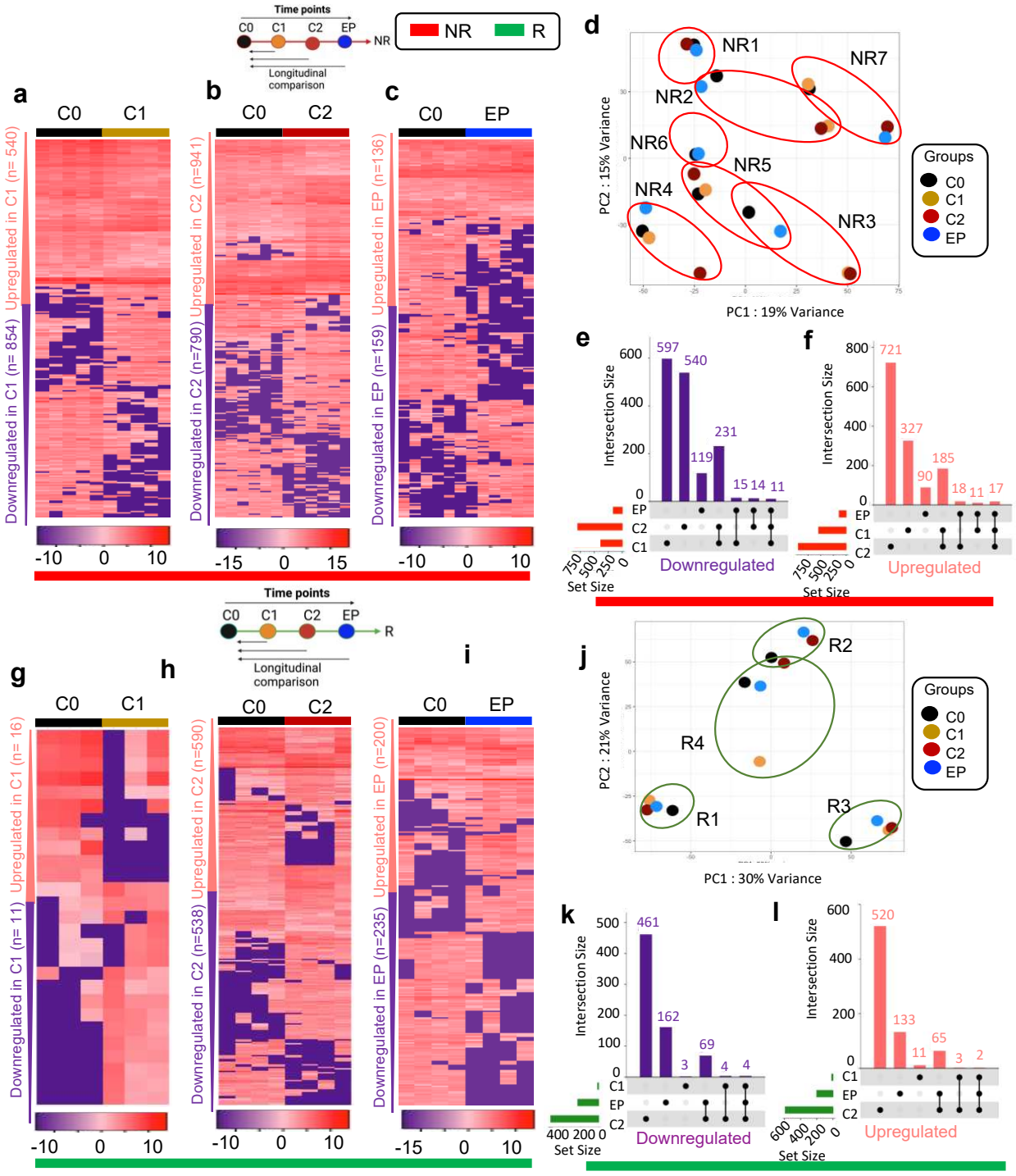


Fig. 2. Plots depicting normalized abundance of differentially expressed transcripts. (a) Heatmap showing differentially expressed transcripts in non-responders (NR) at cycle1 (C1) vs the baseline (C0), and **(b)** Cycle2 **(**C2) vs C0, and **(c) The** Endpoint **(**EP) vs C0. **(d) The** principal component analysis (PCA) plots on the Euclidian distances between NRs in different timepoints, Black dot represents (C0), tan dot (C1), dark red (C2), and blue (EP). **(e)** UpSet plots describing differentially expressed either downregulated (Purple bars) or **(f)** Upregulated

(Indian red bars) transcripts in NRs in one or more comparisons. Each timepoint compared to C0. Vertical bars represent intersection size which is the number of differentially expressed transcripts in one or more comparisons. Red sidebars show the number of transcripts that were found differentially expressed in each timepoint compared to C0. Black dots represent one timepoint comparisons, and black dots connected with a line indicate differentially expressed transcripts in more than one timepoint comparison. **(g)** Heatmap showing differentially expressed transcripts in responders (R) in C1 vs C0, and **(h)** C2 vs C0, and **(i)** EP vs C0. **(j)** Principal component analysis (PCA) plot on the Euclidian distances between Rs in different timepoints, Black dot represents (C0), tan dot (C1), dark red (C2), and blue (EP). **(k)** UpSet plots describing differentially expressed transcripts either downregulated (Purple bars) or **(l)** Upregulated (Indian red bars) in Rs in one or more comparisons. For the heatmap plotting regularized logarithmic transformation (rlog) was priorly applied to the raw counts. Only transcripts with a False Discovery rate (FDR or p_{adj}) > 0.05, and an absolute $\log_2$ (fold change) > 1 are included. The number of downregulated or upregulated transcripts in each comparison is indicated with purple or Indian red vertical arrows on the right of each heatmap. The magnitude of the $\log_2$ fold change is depicted in color key bar beneath each heatmap.

4.3.3 Valproic acid downregulated JAK/STAT and Glycolysis canonical pathways in non-responders.

As an HDAC inhibitor, VA exerts epigenetic effects by modulating non-histone and histone proteins[22], and it targets various cell types based on their HDAC activity[39]. To investigate the biological effects of VA on immune cells, the transcriptome profile of peripheral blood mononuclear cells of NR and R groups in C1 (2 weeks post-VA treatment) was compared to the baseline (C0). The enriched signaling analysis was generated using IPA canonical pathways (QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis) version (01-21-02). A log p-value < 1.3 and an absolute Z-score > 1.5 were considered significant for canonical pathway analysis. Differentially expressed transcripts of each cycle were analyzed for metabolic, apoptosis, cellular immune responses, humoral immune responses, cytokine signaling, and canonical

transcription pathways. The results of comparative analysis comparing the differential enrichment in NRs and Rs in different cycles versus C0 analyzed with IPA and Metascape are shown in (Fig. 3a, 3b, Supplementary Fig. 2a, 2b). We found 20 pathways were enriched as indicated in the NR group at C1 versus the baseline Fig. 3a. Given a small subset of differentially expressed transcripts in the R group at C1 vs. the C0 (Fig. 2g), we did not detect any enriched signaling pathway in this group following treatment with VA (Fig. 3b). However, a wide range of pathways was significantly downregulated in the NR group such as B cell receptor, T cells receptor, NK cell signaling, and IL-2 signaling (Fig. 3a). Moreover, the Janus-Kinase/Signal Transducer and activator of transcription (JAK/STAT) signaling pathway was downregulated and subsequently cytokines/chemokines-associated with this pathway (e.g., IL-2, IL-17, IL-23, IL-3, GM-CSF, and FLT-3) were downregulated [40]. Notably, IL-8 signaling was downregulated as it binds to CXCR1/CXCR2 on the surface of neutrophils and transduces STAT3 activation through PI3K and JAK2 [41]. Most transcripts of genes involved in JAK/STAT/STAT3 pathways were downregulated in C1 compared to C0 except MAP2K4, HGF, MTOR, and PTPN6 (Fig. 3c). Moreover, we found metabolic pathway including glycolysis and gluconeogenesis were inhibited in the NR group at C1 (Fig. 3a) as illustrated by the downregulation of transcripts of genes related to ENO1, ENO2, ALDO, and HIF-1α transcripts as key regulators of these pathways [42](Fig. 3d). We also assessed differentially expressed transcripts related to T and B cells, which confirmed the downregulation of several transcripts in C1 compared to the baseline in the NR group as shown in the volcano plot (Supplementary Fig. 2c). However, transcripts related to PLCG1, MAP2K4, MAP2K6, MAP3K8, MTOR, IGLV9-49, IGHV3-73, and PTPN6 genes were upregulated in C1

compared to C0 in the NR group (Supplementary Fig. 2c). We also noted the downregulation and upregulation of a variety of transcripts associated with myeloid cells in C1 compared to the baseline in this group (Supplementary Fig. 3a). This is in agreement with reported effects of VA on myeloid cells and MDSCs [43]. In summary, our observations reveal the suppressive effects of VA on different signaling pathways associated with immune cell function.

4.3.4 The activation of myeloid cells following the first dose of anti-PD-L1 therapy.

In responders to PD-L1 blockade has been associated with enhanced inflammatory properties of myeloid cells and a greater antigen presentation in dendritic cells (DCs) [44]. To investigate the immediate effects of Avelumab on PBMCs, we analyzed the differential expression of transcripts at C2 compared to the baseline. Pathway enrichment analysis using the IPA demonstrated the significant upregulation of several pathways associated with myeloid cell activation and inflammation, including TREM1, Toll-Like receptor (TLR), PKR in interferon induction and antiviral response, JAK-2 in hormone-like cytokine signaling, and S100 family signaling (Fig. 3b). Transcripts of TLR1, TLR2, TLR10, ITGB1, S100A9, S100A12, NLRP3, STAT1, and MYD88 were upregulated and IFNGR1, MMP19, MMP2, and NFKBI were downregulated in C2 compared to baseline, which shows modifications in the innate immune response. (Supplementary Fig. 3b). Importantly, we observed the upregulation of metabolic signaling pathways, including the pyrimidine ribonucleotide de-novo biosynthesis, salvage pathway of pyridine ribonucleotides, and PFKFB4 (6-phosphofructokinase/fructose-2,6-biphosphotase 4) signaling pathway at C2 in the R

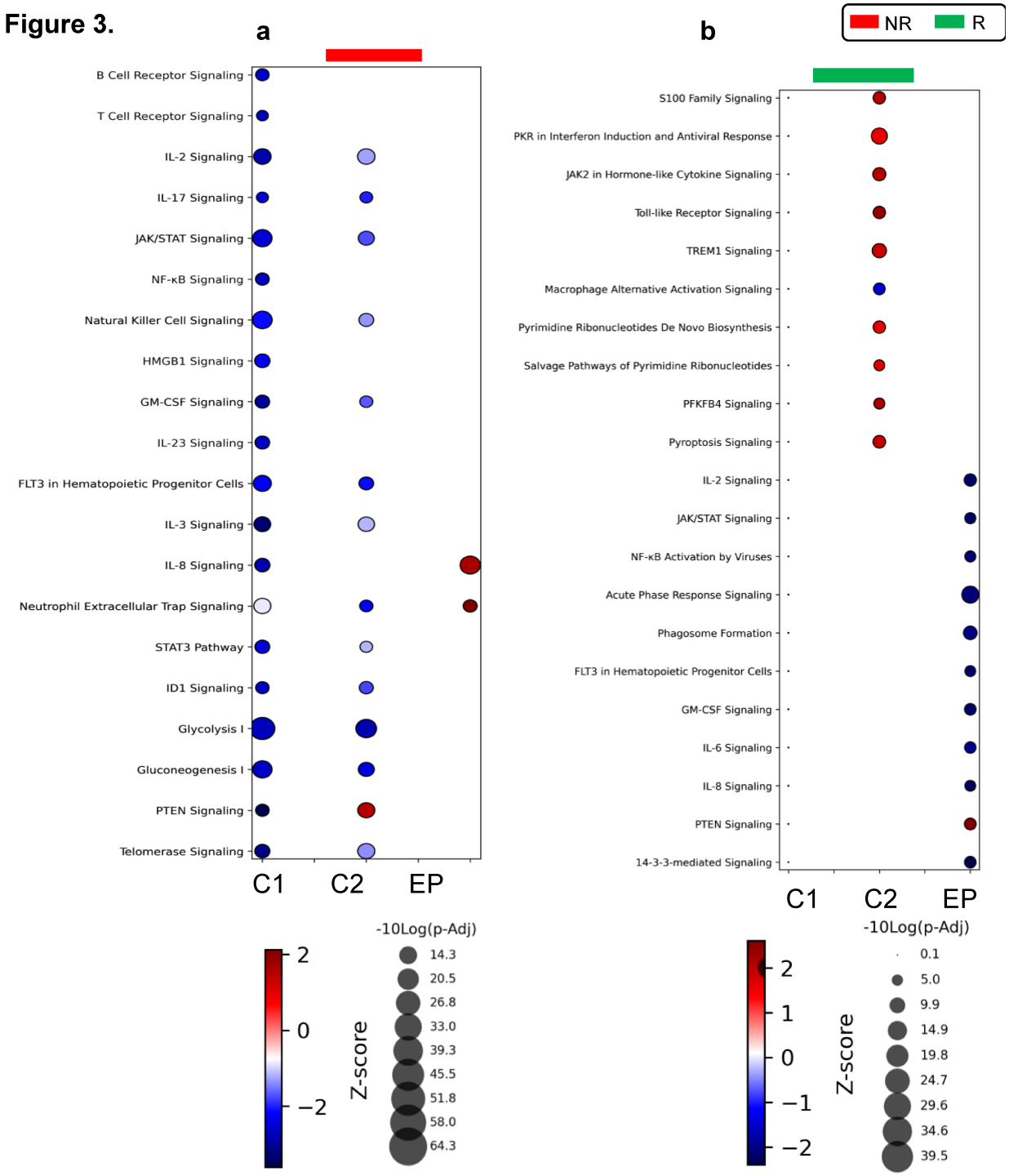
group (Fig. 3b). The related transcript changes are shown in Supplementary fig. 3c. These pathways may provide metabolic intermediates as essential resources for the RNA, DNA synthesis as well as the plasma membrane elements for the activated myeloid cells [45]. We also noted the upregulation of the Pyroptosis pathway at C2 in the R group, which indicates programmed cell death in macrophages, DC, neutrophils, and the release of inflammatory cytokines [46]. Collectively, these observations support the activation of the innate immune response, mainly myeloid cells, following the anti-PD-L1 therapy in the R group.

4.3.5 IL-8 signaling pathway is activated in non-responders at the endpoint.

Comparing EP to the baseline**,** we found enrichment of pathways associated with IL-8 signaling and neutrophil extracellular trap formation (NET) in the NR group (Fig. 3a). In contrast, IL-8 signaling was significantly inhibited in the R group at the EP (Fig. 3b). The main transcripts associated with the NET and IL-8 signaling pathways are illustrated in NR and R groups (Fig. 3e-g). These results demonstrate the upregulation of various transcripts associated with NET formation and IL-8 signaling in the NR group at C1, C2, and the EP (Fig. 3e, 3f). For instance, transcripts of NLRP3, RAF1, MAP2K6, and ITGB2 related to NET signaling, and BCL2L1, MRAS, MPO, and IKBKB related to IL-8 signaling were upregulated. YES1, COL23A1, PIK3CB, and KRAS were downregulated at the EP. However, a limited number of transcripts associated with IL-8 signaling were significantly upregulated in the R group at C2; however, at the EP, just CXCR1 was upregulated (Fig. 3g). Furthermore, our functional analysis using the IPA revealed an enriched neutrophil movement in the

NR group (Supplementary Fig. 4a) but T cell proliferation and migration was enriched in the R group at the EP (Supplementary Fig. 4b).

Figure 3.


a
B Cell Receptor Signaling
T Cell Receptor Signaling
IL-2 Signaling
IL-17 Signaling
JAK/STAT Signaling
NF-κB Signaling
Natural Killer Cell Signaling
HMGB1 Signaling
GM-CSF Signaling
IL-23 Signaling
FLT3 in Hematopoietic Progenitor Cells
IL-3 Signaling
IL-8 Signaling
Neutrophil Extracellular Trap Signaling
STAT3 Pathway
ID1 Signaling
Glycolysis I
Gluconeogenesis I
PTEN Signaling
Telomerase Signaling
C1
C2
EP
2
0
−2
Z-score
-10Log(p-Adj)
14.3
20.5
26.8
33.0
39.3
45.5
51.8
58.0
64.3
b
NR
R
S100 Family Signaling
PKR in Interferon Induction and Antiviral Response
JAK2 in Hormone-like Cytokine Signaling
Toll-like Receptor Signaling
TREM1 Signaling
Macrophage Alternative Activation Signaling
Pyrimidine Ribonucleotides De Novo Biosynthesis
Salvage Pathways of Pyrimidine Ribonucleotides
PFKFB4 Signaling
Pyroptosis Signaling
IL-2 Signaling
JAK/STAT Signaling
NF-κB Activation by Viruses
Acute Phase Response Signaling
Phagosome Formation
FLT3 in Hematopoietic Progenitor Cells
GM-CSF Signaling
IL-6 Signaling
IL-8 Signaling
PTEN Signaling
14-3-3-mediated Signaling
C1
C2
EP
2
1
0
−1
−2
Z-score
-10Log(p-Adj)
0.1
5.0
9.9
14.9
19.8
24.7
29.6
34.6
39.5

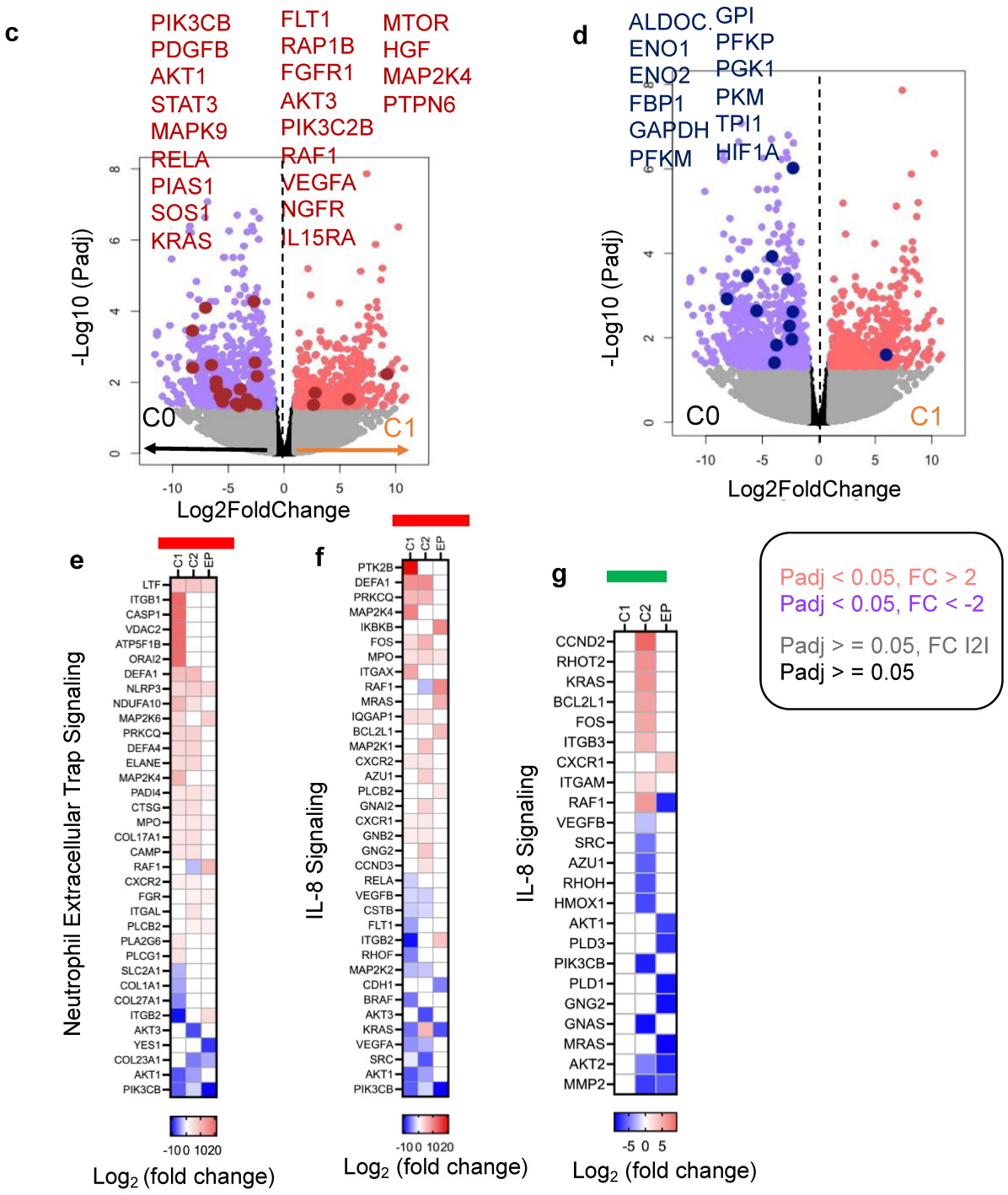


Fig. 3. Longitudinal comparison analysis of different timepoints compared to the baseline. (a) Enriched signaling pathway analysis comparing C1, C2, and EP to C0 in NRs. **(b)** Enriched signaling pathway analysis comparing C1, C2, and EP to C0 in Rs. **(c)** Volcano plot showing differentially expressed transcripts in C1 compared to C0 related to JAK/STAT and STAT3 pathways, and **(d)** Glycolysis, Gluconeogenesis I pathways in NR group. **(e)** Heatmap comparing differentially expressed transcripts in C1, C2, and EP compared to C0, related to Neutrophil extracellular trap formation,

and **(f)** IL-8 signaling pathway in NRs. **(g)** Heatmap comparing differentially expressed transcripts in C1, C2, and EP compared to C0, related to IL-8 signaling pathways in Rs. Enriched canonical (a, b) were analysed by IPA comparison analysis and analysed for Apoptosis, cellular and humoral immune signaling, cytokine signaling and transcription signaling. Threshold of Z-Score which predicts the direction of change for the function was set to an absolute value of Z-score > 1.5 (Blue represents downregulation and red represents upregulation). In the bubble plots for the purpose of presentation Padj values multiplied by 10. -log (Padj) > 1.3 were considered significant. For Volcano plots, -log (Padj) > 1.3 and absolute log_2 fold change >2 considered as significant. For transcript heatmaps (3e-g) log (Padj) < 0.05 were considered as significant and absolute log_2 fold change > +1.5 and < -1.5 were considered as upregulated (red) or downregulated (blue).

4.3.6 Enriched myeloid immune signature in non-responders.

We compared differentially expressed transcripts in R and NR at the baseline. PCA plots showed 3 out of 4 responders clustered together (Supplementary Fig. 4c). Then, we compared differentially expressed transcripts at the baseline. These analyses revealed 25 transcripts were upregulated and 113 transcripts were downregulated in Rs vs. NRs (Fig. 4a). When the same comparison was performed at the EP, we found 79 transcripts were upregulated, and 372 transcripts were downregulated in Rs vs. NRs (Fig. 4b). Moreover, the total number of upregulated and downregulated transcripts at C0, EP, and C0/EP are provided in Fig. 4c, 4d. Similar analyses were performed for cycles 1 and 2, which showed a differential pattern of transcripts in Rs and NRs, as shown in heatmaps and upset plots (Supplementary Fig. 4d-g). Given the enrichment of NETs and neutrophil degranulation pathways in PBMCs of NRs (Fig. 3a, 3e, 3h), we suggest this could be related to the abundance of low-density poly morphonuclear cells such as MDSCs in this group [47]. Hence, we compared transcripts of myeloid gene subsets in different cycles between groups. These analyses revealed a lower enrichment for myeloid subsets in Rs, mainly noticeable at

the EP (Fig. 4e-g). Among these downregulated transcripts of genes related to suppressor functions of MDSC, we can see CSF1, MMP7, and PTGES2 (Fig. 4f, 4g). Moreover, transcripts related to PMN degranulation (e.g., CD63, CTSB, CTSL, CTSZ, DNASE2B, FCAR, ITGAM) and neutrophil extracellular trap genes (e.g., ITGB2, PLCB2, S100A16, TRIM26) were also downregulated in Rs (Fig. 4e-g). In contrast, we observed transcripts related to T cell functions such as FCRL6, FGFBP2, NKG7, SAMD3, TRGC2, ZNF683, RUNX3, MX1, and GZMH were unregulated in Rs (Fig. 4h). However, transcripts of PRDM1 that code BLIMP-3 and LGALS9 that codes Galectin-9 (Gal-9), T cell exhaustion markers, were decreased in Rs (Fig. 4h). Of note, the IFN-signalling was downregulated in Rs both at the C0 and EP. For example, IFNGR1, TRIM26, IRF8, and OAS2 transcripts were downregulated at the EP in Rs (Fig 4i). In summary, NRs exhibited a phenotype enriched with PMN-derived transcripts and genes associated with activated MDSCs.

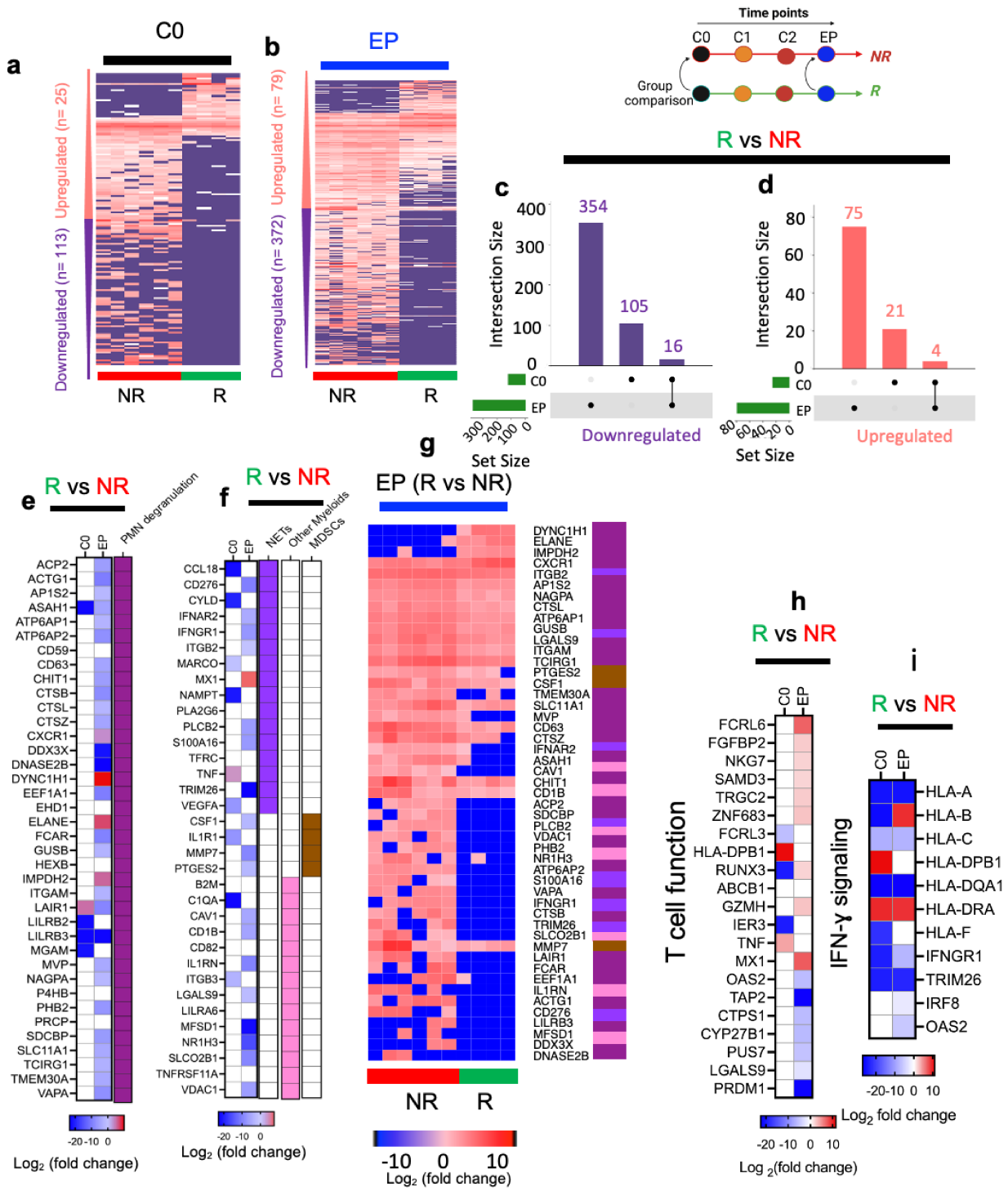


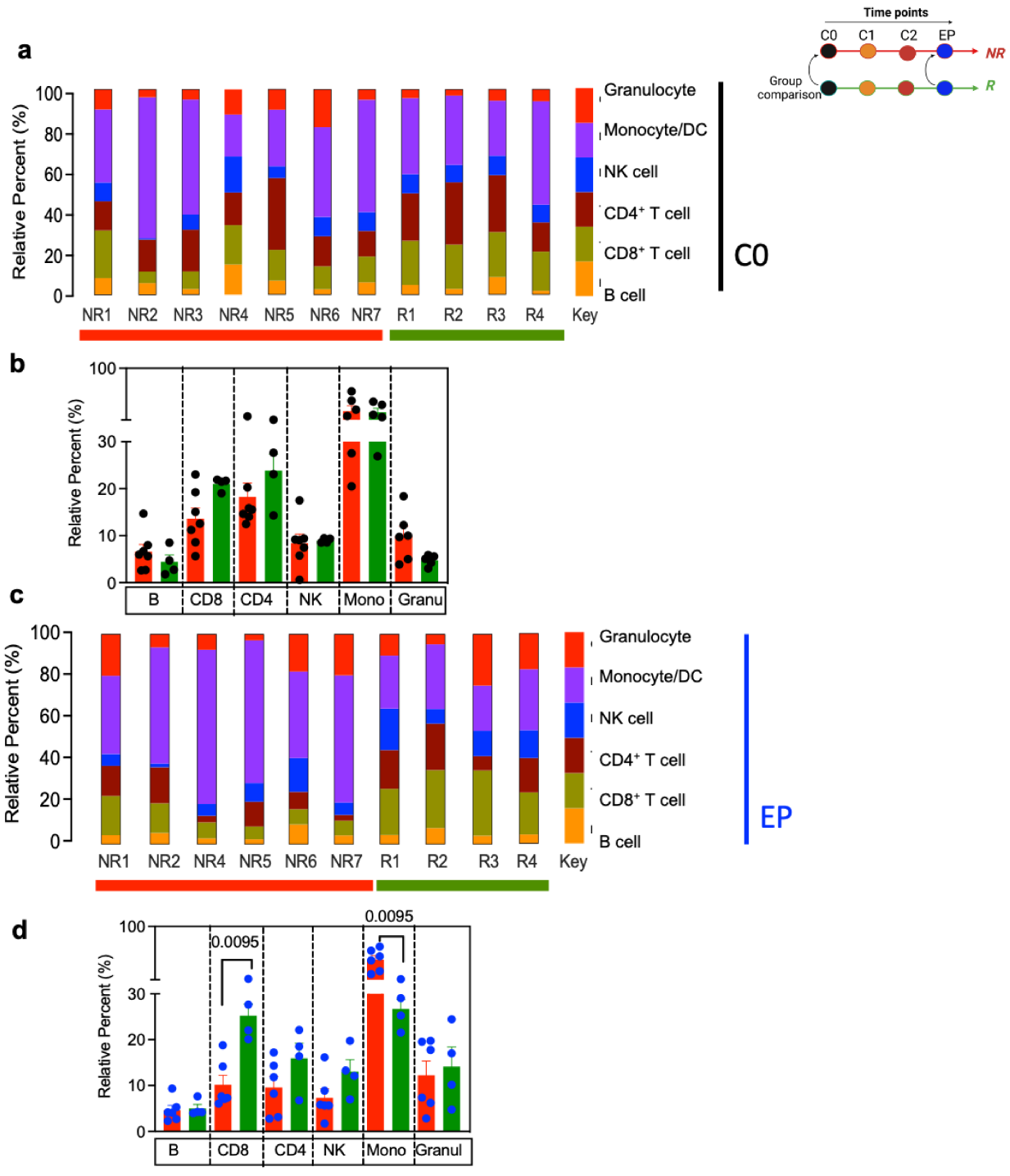
Fig. 4. Group comparison of responder vs non-responders at the baseline and endpoint. (a) Heatmap showing differentially expressed transcripts in Rs vs

NRs at the baseline (C0). **(b)** Heatmap showing differentially expressed transcripts in Rs vs NRs at the endpoint (EP). **(c)** UpSet plots describing downregulated differentially expressed transcripts (Purple bars), and **(d)** Upregulated (Indian red bars) in Rs vs NRs at C0 and EP. **(e)** Heatmaps of differentially expressed transcripts comparing Rs vs NRs at C0 and EP related to PMN degranulation, and **(f)** NETs, MDSCs and other myeloid cells. **(g)** Heatmap depicting differentially expressed transcripts related to genes of myeloid cells in Rs vs NRs at the EP. **(h)** Heatmap of differentially expressed transcripts related to T cells in Rs vs NRs at C0 and EP. **(i)** Heatmap of differentially expressed transcripts related to IFN-γ signaling in Rs vs NRs at C0 and EP.

4.3.7 Immune cell populations and abundances estimated using digital cytometry.

To determine the relative frequency of different immune cells in the bulk RNA seq, we carried out the CIBERSORTx analysis to deconvolute the estimated 22 types of immune cells (LM22) [34]. To simplify our data analysis, 22 cell subsets were merged into six main immune cell lineages: B cells, $CD8^+$ T cells, $CD4^+$ T cells, Natural Killer (NK) cells, Monocytes, and granulocytes. Comparing the relative percentages of immune cell subsets between groups at the baseline, we did not notice any significant difference between Rs and NRs despite a higher trend of enriched $CD8^+$ and $CD4^+$ T cells in Rs (Fig. 5a, 5b). Similarly, there was no significant difference between the groups at C1 and C2 (Supplementary fig. 5a, 5b). Although we observed significantly higher percentages of $CD8^+$ T cells in PBMCs of Rs, the monocyte fraction was significantly enriched in the NR group (Fig. 5c, 5d). In agreement, we found that the myeloid (Monocytes and Granulocytes) to lymphoid (B, $CD8^+$ T, $CD4^+$ T, and NK cells) ratio was significantly higher in NRs at the EP (Fig. 5e). Immune cell subset variation over time obtained by CIBERSORTx are presented in Supplementary fig. 5c, 5d. Moreover, we validated our analyzed results using the CIBERSORTx by flow

cytometry. These observations confirmed the expansion of myeloid cells, possibly at the expense of reduced $CD8^+$ T cells in NRs at the EP (Fig. 5f-i). Of note, the myeloid cell phenotype changes over time. At the baseline, myeloid cells were mainly $CD11b^+CD14^+$ (Fig. 5k-n), but this population appeared to express $CD11b^+$ $CD14^-$ $CD15^+$ $CD66b^+$ at the EP, which is consistent with an enriched PMN-MDSC phenotype [12] (Fig. 5o-r). Collectively, the estimation of immune cell fractions distinguishes Rs from NRs at the endpoint, as the R group had a lower myeloid-to-lymphoid ratio. The myeloid-to-lymphoid ratio agrees with reports that a higher myeloid cell/MDSCs to lymphoid ratio is accompanied by a worse prognosis in cancer [48].


a
Time points
C0
C1
C2
EP
NR
Group comparison
R
Relative Percent (%)
100
80
60
40
20
0
NR1
NR2
NR3
NR4
NR5
NR6
NR7
R1
R2
R3
R4
Key
Granulocyte
Monocyte/DC
NK cell
CD4+ T cell
CD8+ T cell
B cell
C0
b
Relative Percent (%)
100
30
20
10
0
B
CD8
CD4
NK
Mono
Granu
c
Relative Percent (%)
100
80
60
40
20
0
NR1
NR2
NR4
NR5
NR6
NR7
R1
R2
R3
R4
Key
Granulocyte
Monocyte/DC
NK cell
CD4+ T cell
CD8+ T cell
B cell
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B
CD8
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Mono
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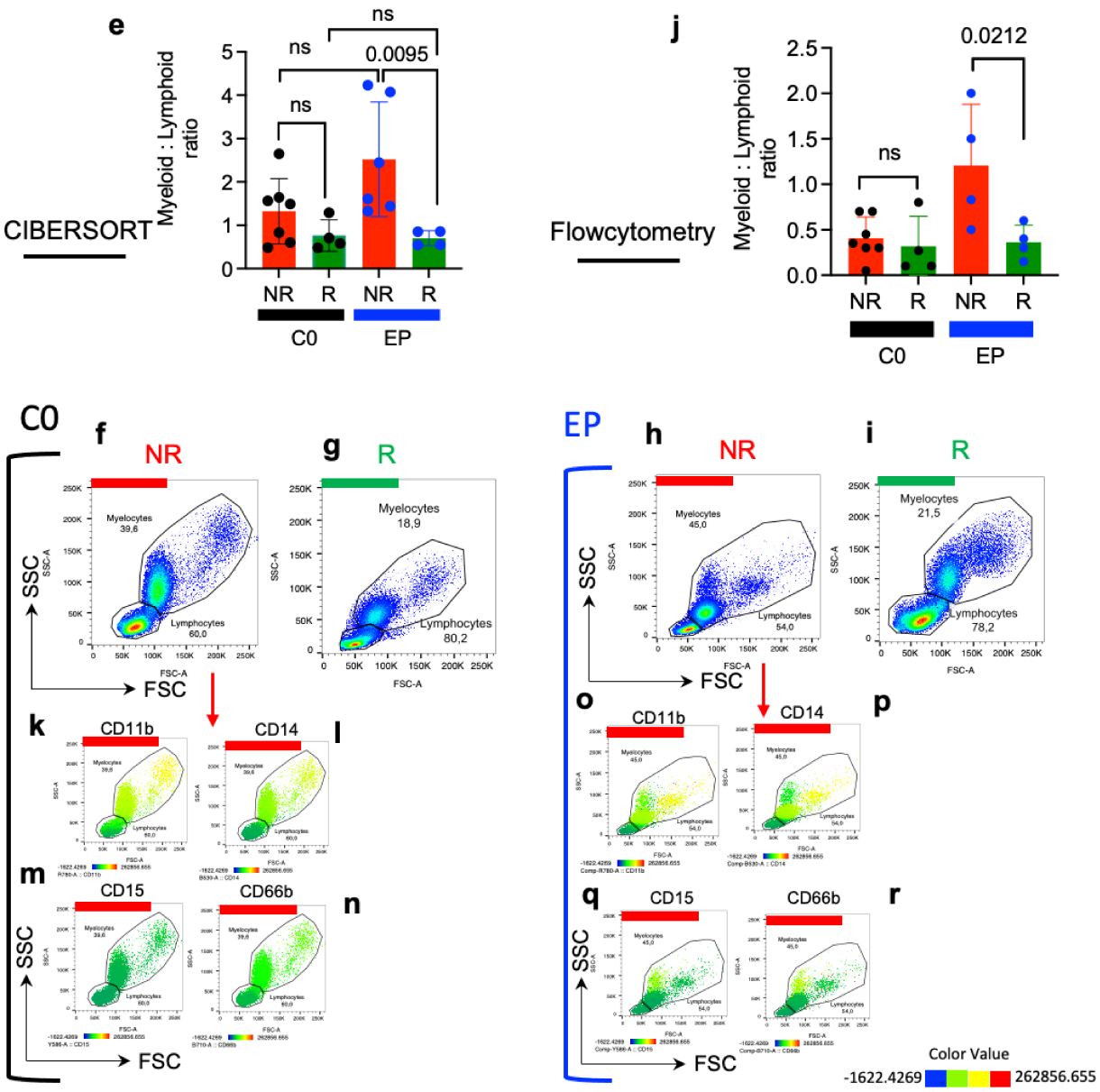


Fig. 5. Immune cell composition in responders and non-responders at the baseline and endpoint. (a) Bar charts representing relative percentages of immune cell composition in NRs and Rs at C0. **(b)** Cumulative data comparing the frequency of 6 types of immune cells in Rs (green) and NRs (red) at C0. **(c)** Bar charts representing relative percentages of immune cell compositions in NRs and Rs at the EP. **(d)** Bar charts comparing the frequency of 6 types of immune cells in Rs (green) and NRs (red) at the EP. **(e)** Cumulative data of myeloid to lymphoid ratios in Rs and NRs at the C0 and EP acquired by CIBERSORTx. The immune cell compositions acquired by importing TPM values of RNA sequencing data to CIBERSORTx and deconvoluted to LM22 reference signature matrix. **(f)** Representative flow plots showing lymphocyte and myelocyte populations in NR, and **(g)** R at C0. **(h)**

Representative flow plots showing lymphocyte and myelocyte populations in NR, and **(i)** R at the EP. **(j)** Cumulative data showing myeloid to lymphoid ratios in Rs and NRs at C0 and EP obtained by flowcytometry. **(k-n)** Representative flow plots showing CD11b, CD14, CD15, and CD66b positive cells in the population of myelocytes in NR at C0. **(o-r)** and at the EP. P-value < 0.05 was considered as significant.

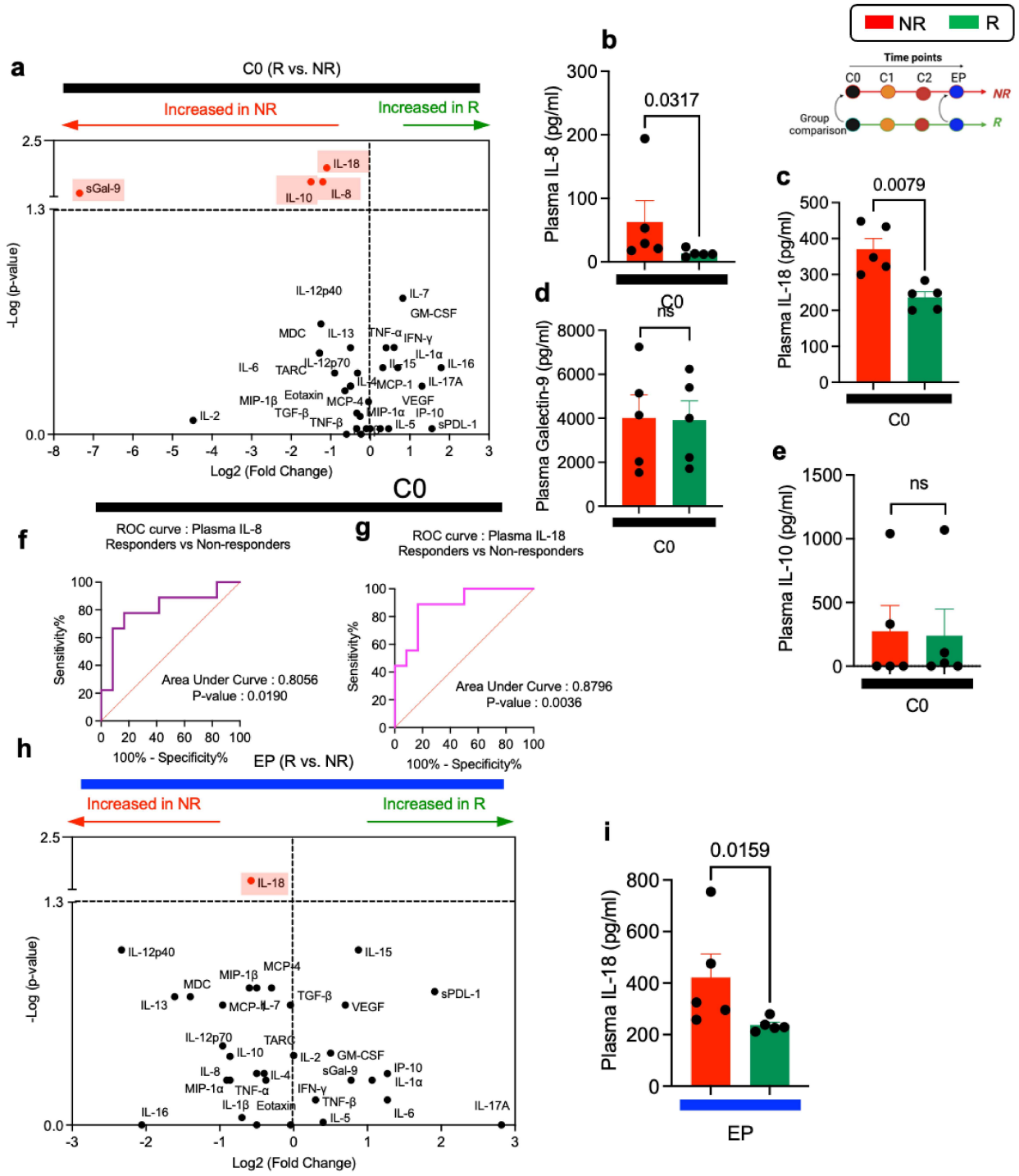
4.3.8 Plasma IL-8/IL-18 concentrations and IL-8/IL-18 signaling pathways discriminate non-responders from responders at the baseline.

Cytokines and chemokines secreted by tumor, stromal, and immune cells play crucial roles in shaping and orchestrating immune responses against tumors[49]. To investigate the cytokine and chemokine profiles in the plasma, we measured 28 analytes in the plasma of patients at C0, C1, and EP (Mesoplex), including IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12p70, IL-13, TNF-α, GM-CSF, IL-1α, IL-5, IL-7, IL-12p40, IL-15, IL-16, IL-17A, TNF-β, VEGF, Eotaxin, MIP-1α, TARC, IP-10, MIP-1β, IL-8, MCP-1, MDC, MCP-4. In addition, the plasma was subjected to Gal-9, IL-18, soluble PDL-1, and TGF-β ELISAs. At the baseline, IL-8, IL-18, Gal-9, and IL-10, were highly elevated in NRs compared to Rs (Fig. 6a). However, the concentration of other measured analytes remained comparable (Fig. 6a). The concentration of these four analytes was measured in a second cohort consisted of ten patients including five NRs and five Rs (Supplementary Table 3). In agreement, IL-8 and IL-18 concentrations were significantly higher in NRs (Fig. 6b, 6c), but this was not the case for Gal-9 and IL-10 (Fig. 6d, 6e). Then, we hypothesized that plasma IL-8/IL-18 levels could be used as biomarkers to stratify potential NRs from Rs as a predictive strategy. Therefore, we used the receiver operating characteristic (ROC) curve to

estimate the best cut-off values of the plasma IL-8/IL-18 at the baseline, as we have reported elsewhere [50]. The area under the curve to discriminate NRs from Rs for IL-8 and IL-18 were 0.8056 and 0.8796, respectively (Fig. 6f, 6g). The cut-off value of 20.77 pg/ml for IL-8 and 311.2 pg/ml for IL-18 showed a test sensitivity of (75% and 88.89%) and test specificity of (76.92% and 76.92%), respectively. However, following VA treatment, the concentrations of soluble markers between Rs and NRs were similar (Supplementary Fig. 5e). IL-18 was elevated in NRs vs. Rs at the EP (Fig. 6h) and was validated by ELISA (Fig. 6i). Finally, we compared the concentration of soluble analytes at the EP versus the C0 in each group. These observations showed a significant increase in the plasma concentration of IL-1β, IL-10, and VEGF in NRs but not in the R group over time (Supplementary fig. 6a-c). These markers are best known to be associated with poor prognostic in cancer patients [51–53]. Moreover, these cytokines are related to MDSCs development and expansion [54]. Therefore, our data indicate the elevation of plasma IL-8 and IL-18 at the baseline in NRs. Hence, these biomarkers could be used to discriminate NRs from Rs. Likewise, a positive link between these cytokines with poor prognostic in various cancer types has been shown [55,56].

Recently it has been shown that biomarkers relying on signaling pathways are better predictors of response to immunotherapy [57]. Therefore, we compared the enriched signaling pathways by IPA and Metascape analysis in Rs vs. NRs at the baseline (Supplementary fig. 6e, 6d). Transcripts related to IL-8 (analyzed by IPA), IL-18, and VEGFR (analyzed by Metascape, Reactome, Wikipathway) pathways were downregulated in Rs but highly increased in NRs (Fig. 6j). Also, we noted enriched

IL-8 (analyzed by IPA) and VEFG/VEGFR (Analysed by Metascape, Reactome) signaling pathway in NRs at the endpoint (Fig. 6k).



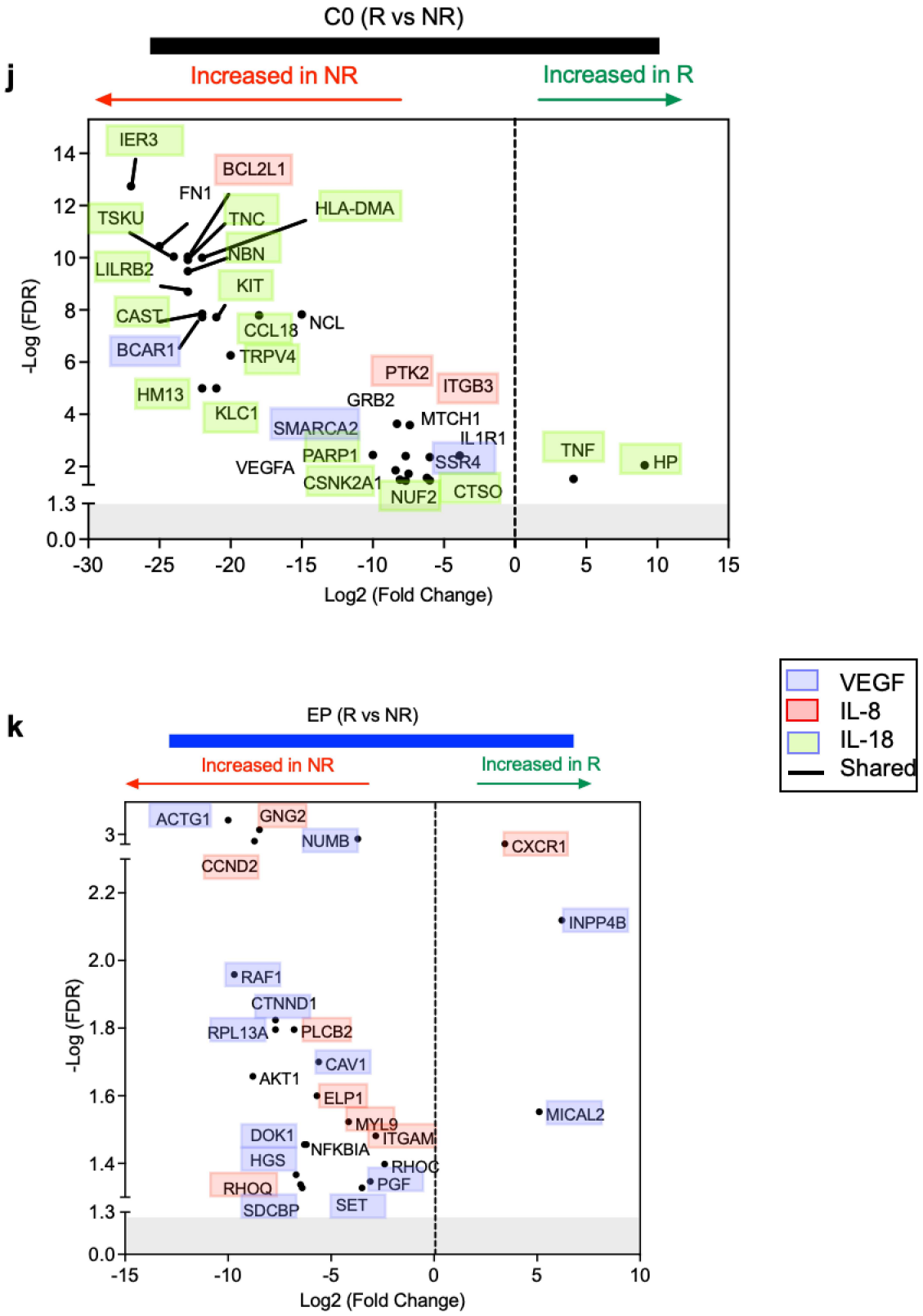

C0 (R vs NR)
j
Increased in NR
Increased in R
IER3
BCL2L1
FN1
TSKU
TNC
HLA-DMA
NBN
LILRB2
KIT
CAST
CCL18
NCL
BCAR1
TRPV4
PTK2
ITGB3
HM13
GRB2
KLC1
MTCH1
SMARCA2
IL1R1
PARP1
SSR4
VEGFA
CSNK2A1
NUF2
CTSO
TNF
HP
-Log (FDR)
Log2 (Fold Change)
k
EP (R vs NR)
Increased in NR
Increased in R
ACTG1
GNG2
NUMB
CXCR1
CCND2
INPP4B
RAF1
CTNND1
RPL13A
PLCB2
CAV1
AKT1
ELP1
MICAL2
MYL9
ITGAM
DOK1
NFKBIA
HGS
RHOC
RHOQ
PGF
SDCBP
SET
-Log (FDR)
Log2 (Fold Change)
VEGF
IL-8
IL-18
Shared

Fig. 6. Soluble mediator analysis between responders and non-responders at the baseline and endpoint. (a) Volcano plot illustrating the significance and magnitude of differences in soluble mediators in Rs vs NRs at C0. **(b)** Cumulative data of the plasma concentration of IL-8 obtained from 10 patients of the validation cohort at C0. **(c)** Cumulative data of the plasma concentration of IL-18 obtained from 10 patients of the validation cohort at C0. **(d)** Cumulative data of the plasma concentration of Gal-9 obtained from 10 patients of the validation cohort at C0. **(e)** Cumulative data of the plasma concentration of IL-10 obtained from 10 patients of the validation cohort at C0. **(f)** The receiver operating characteristic (ROC) curve of comparison between plasma IL-8 in Rs and NRs. **(g)** The ROC curve of comparison between plasma IL-18 in Rs and NRs. **(h)** Volcano plot illustrating the significance and magnitude of the differences in soluble mediators in Rs vs NRs at the EP. **(i)** Cumulative data of the plasma concentration of IL-18 obtained from 10 patients of the validation cohort at the EP. **(j)** Volcano plot showing differentially expressed transcripts in Rs compared to NRs related to VEGF, IL-8, and IL-18 signaling pathways at C0. **(k)** Volcano plot showing differentially expressed transcripts in Rs compared to NRs related to VEGF, and IL-8 signaling pathways at the EP. In Volcano plots -log (p-value/FDR) > 1.3 was considered as significant. Absolute Log2 fold change > 1 was considered as threshold for up or downregulation. P-value < 0.05 was considered as significant.

4.4 Discussion

This study demonstrates the circulating immune transcriptome and plasma cytokine levels of HPV-associated squamous cell carcinoma patients grouped into Rs and NRs at the baseline and throughout the treatment. Non-responders had elevated basal plasma IL-8/IL-18 levels and activated IL-8/IL-18 signaling pathways in peripheral blood immune cells. Following treatment at the endpoint, the NR group showed further upregulation of IL-8 signaling accompanied by the NET formation pathway activation and elevated plasma IL-18 (Fig. 7a, 7b). The IL-8 signaling pathway is linked to the chemoattraction of MDSCs, neutrophils, and other myeloid cell populations to the tumor microenvironment. Similarly, IL-18 is associated with M-MDSC induction [58,59]. As such, our observations imply that the enhanced IL-8/IL-

18 signaling by the accumulation of MDSCs contributes to an immune non-responsiveness to ICIs (e.g., Avelumab).

In line with this hypothesis, deconvoluted data with digital cytometry (CIBERSORTx) [34] and flow cytometry analysis revealed a higher myeloid-to-lymphoid ratio in NRs compared to Rs in peripheral blood at the endpoint. Extracted myeloid transcriptome in NR and Rs revealed prohibitive myeloid expression in NRs with predominant neutrophil-related transcripts such as granule and NET formation. CSF1, IL1R1, MMP7, and PTGES2 related to myeloid suppressive functions were upregulated in NRs, as reported elsewhere [60]. Myeloid compartment enrichment following Avelumab therapy might be a resistance strategy mechanism in NRs. In agreement with our results, previous studies have shown the capacity of myeloid cells/MDSCs to arbitrate resistance and poor prognosis in gastric cancer [61]. Although human neutrophils and PMN-MDSCs possess similar phenotypes, neutrophils are located in the high-density gradient portion, whereas PMN-MDSCs are present with PBMCs as light density when subjected to cell isolation [12]. Given our methodology of subjecting PBMCs to RANseq, the saturated neutrophil signature in NRs reflects PMN-MDSCs. However, further studies are required to determine the effector functions of these cells [12].

The myeloid signature in NRs was associated with an enhanced NET formation pathway and elevated IL-8/IL-18 in NRs at the endpoint. IL-8 (CXCL8) is a chemokine released by tumor, stromal, and myeloid cells and attracts myeloid cells to tumor tissue [62]. Conversely, IL-8 is involved in angiogenesis and tumor progression [63]. Conversely, IL-18 is linked to poor prognosis as an immunosuppressor cytokine in

cancer [58]. Our data support the sensitivity and specificity of plasma IL-8 and IL-18 as predictive biomarkers in NRs. A similar correlation between plasma IL-8 levels and poor immune response to anti-PD-L1 has been reported [63]. However, the implication of plasma IL-18 as a prognostic biomarker in cancer has been debated and controversial [55] and merits further investigations.

Moreover, other cytokines such as IL-6, IL-1β, VEGF, and IL-10 have been correlated with poor prognosis in cancer patients due to their role in regulating myeloid cell (e.g., MDSCs) differentiation and recruitment to the tumor microenvironment [64,65]. Likewise, we observed an increase in plasma, IL-1β, IL-10, and VEGF in our NRs cohort, which was associated with an enhanced VEGF/VEGFR signaling pathway [66]. Although plasma IL-10 and Gal-9 levels were elevated at the baseline in the NR group, our validating cohort did not verify these observations. Therefore, further studies in larger cohorts are needed to clarify the role of soluble Gal-9 in virus-associated cancers considering its role in T-cell exhaustion [30].

Recent evidence unveils a role for NET formation in cancer progression and metastasis in animal cancer models [67]. NETs cover cancer cells and protect them from the immune system[68]. In this scenario, IL-8 is important in attracting myeloid cells/MDSCs, resulting in enhanced NET formation in the tumor microenvironment [59].

In this study, we treated patients with VA two weeks before initiating Avelumab. We aimed to determine the immunomodulatory effects of this HDAC inhibitor [69]. HDAC inhibitors target different classes of HDAC enzymes, and their effect is selective and depends on the enzymatic activity of HDACs in different cells. For example, Entinostat selectively targets PMN-MDSCs and modulates their functions [70]. Due

to this selective property of HDAC inhibitors, the immunomodulatory role of VA in cancer requires further investigation. In our study, VA combined with anti-PD-L1 to enhance the response against cancer. Signaling dynamics two weeks after VA initiation disclosed a substantial difference between NRs and Rs. The downregulated signaling pathways such as HIF-1α and glycolysis, JAK/STAT, and STAT3 favor immune system deactivation. HIF-1α induces genes related to the glycolytic pathway, and enhanced glycolysis supports MDSCs' expansion and function [71]. Similarly, STAT3 signaling plays a complex role in immune cell growth, differentiation, and apoptosis and is involved in the negative regulation of immune responses, mainly regulated by IL-6 through JAK/STAT signaling [40]. FLT3 (FMS-like tyrosine kinase 3) and ID-1signaling are expressed mainly in progenitors and stem cells, and downregulated pathways related to FLT3 and ID1 in NRs following VA therapy might be in the direction of MDSC attenuation [72,73]. In general, VA modulates innate and adaptive immune cells [29]. It has been shown to induce T cell apoptosis [74], modulate NK cells, DCs, and macrophages, and regulate the inhibitory effects of MDSCs [75]. In particular, VA inhibits HDAC class I (HDAC 1, 2, 3) and impairs the immunosuppressive function of PMN-MDSC[43]. In support of this possibility, it has been reported that VA enhances anti-PD-L1 immunotherapy by attenuating MDSCs [76]. HDAC activity has been reported to be selective and higher in MDSCs [75]. Likewise, in our cohort, the effect of VA was prominent in NRs, which might be due to the possible enrichment of NRs' PBMCs with MDSCs. The repressing effect of VA on immune signature in our cohort agrees with the attenuating impact of VA on MDSCs and myeloid cells [43].

Despite a similar pattern for the frequency of myeloid cells in NR and R groups at the baseline, differential VA effects might result in observed differences in their clinical outcomes. In contrast, we observed an activated innate immune response in the R group based on the upregulation of TREM-1 and TLR signaling pathways. These canonical pathways are mainly related to myeloid cell functions [77] and stress response [78]. It has been shown that anti-PD-L1 therapy initiates a perceptible inflammatory signature in $CD14^+$ monocytes with elevated levels of myeloid-derived cytokine [44]. Moreover, an activated inflammatory myeloid cell signature with the upregulation of Heparin-binding EGF-like factor (HBEGF), THBS (thrombospondin-1), IL-1β, CXCL1, CXCL2, NLRP3 and increase in inflammasome-associated cytokines(IL-1β, IL-18) have been demonstrated upon anti-PD-L1 therapy [44]. Also, MDSCs upregulate PD-L1 under hypoxia through HIF-1α by binding to HRE (hypoxia-response element) in the proximal PD-L1 promotor, which provides a negative signal by attenuating their suppressive function [79]. PD-L1 is mainly expressed in M-MDSCs and, to a lesser extent, in PMN-MDSCs [80]. In NRs, we did not observe substantial changes in signaling pathways in C2. The enriched presence of immunosuppressive myeloid cells may be a reason for this. On the contrary, a more inflammatory response was associated with anti-tumor immunity in the R group.

We are aware of multiple study limitations. The small cohort size is our major limitation. Also, due to the poor quality of RNA, we had to exclude some samples/time points. Due to the small sample size, we could not group complete responders from partial responders and stable NRs from progressive disease cases. These examples emphasize a larger cohort size to compare immune responses in various subpopulations. Also, longitudinal follow-up beyond the endpoint is required to

differentiate long-lasting responders from those that may acquire resistance to immunotherapy. Moreover, we were limited with the sample size to perform additional functional assays to better characterize cellular immune components in Rs vs. NRs.

We found elevated baseline plasma IL-8/IL-18 levels and activated IL-8/IL-18 signaling pathways in NRs. Even at the endpoint, the NR group exhibited a similar pattern of enhanced IL-8/IL-18 signaling accompanied by the NET formation pathway. The chemotactic effects of the IL-8/IL-18 signaling pathway on MDSCs, neutrophils, and other myeloid cells [58,59] may contribute to the acquired resistance against ICIs.

Our findings highlight the importance of peripheral blood immune signature as a non-invasive biomarker source to characterize Rs and NRs in a clinical setting. Such studies could be applied to larger cohorts to identify a specific immune signature for precision medicine.

4.5 Acknowledgements

We thank all cancer patients who enrolled in the LATENT clinical trial study and donated their blood, dedicating their time to this study. We thank the clinical trial study staff and, coordinators, medical laboratory staff at the Cross-Cancer Institute (CCI) for sample collection. We thank the Applied Genomic Centre of the University of Alberta for sample handling and preparation and Dr. Juan Jovel for guiding the RNA sequencing analysis.

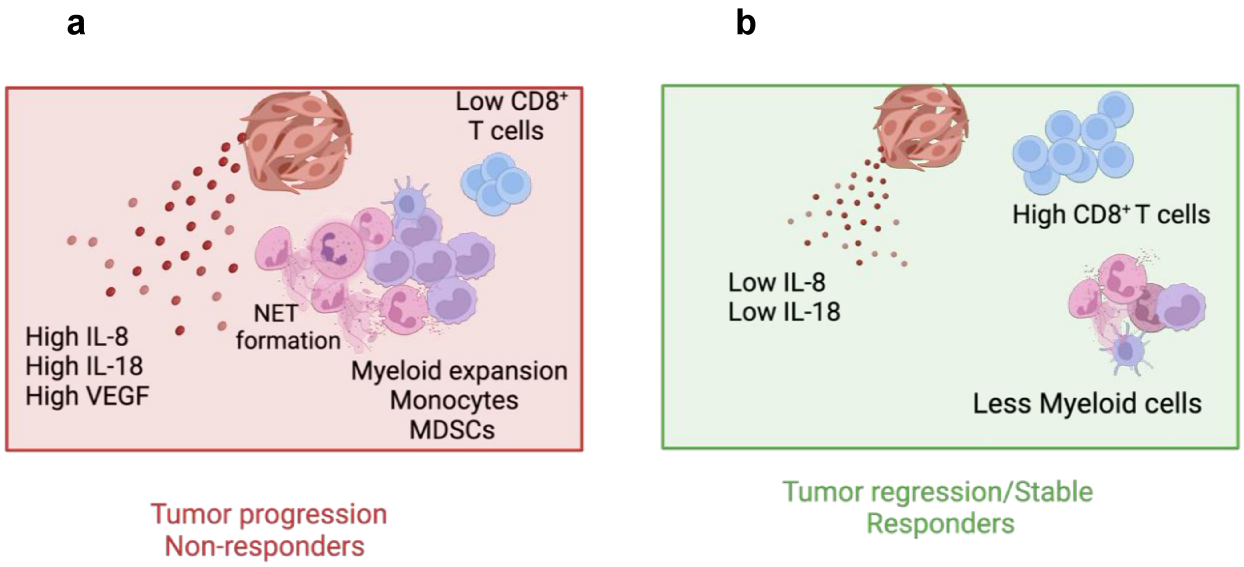


Fig. 7. Graphical summary of findings in non-responders and responders. (a) NRs to immunotherapy exhibited a higher baseline plasma IL-8, IL-18 concentrations, increased VEGF levels at the EP accompanied by a myeloid subset expansion, decreased $CD8^+$ T cells frequency, activated IL-8 signaling, and NET formation. **(b)** Rs had a lower baseline level of IL-8/IL-18, higher $CD8^+$ T cells, and lesser myeloid cells at the EP.

4.6 Supplementary Table 1

Response Category	Sex	Primary Tumor Site	Stage	Best Response
R1	F	Anal	IV	iCCR
R2	F	Unknown	IV	iCPR
R3	M	Tonsil	II	iSD
R4	F	Cervix	IV	iSD
NR1	F	Cervix	III	iCPD
NR2	M	Oropharynx	IVB	iCPD
NR3	M	Tonsil	III	NE/Clinically Progressive
NR4	F	Vulva	III	iUPD
NR5	F	Anal	IV	iUPD
NR6	F	Anal	IV	iUPD
NR7	M	Anal	IV	iUPD

Supplementary Table 1. Patients' characteristics. M: Male, F: Female, iUPD: Unconfirmed progressive disease, iCCR: Confirmed complete response, iCPD: Confirmed progressive disease, NE: Response was not evaluated, iUPR: Unconfirmed partial response, iCPR: Confirmed partial response, iSD: Stable disease, R: Responders (iCCR+ iCPR+iUPR+ iSD), NR: Non-responders (iCPD+ iUPD+ Clinically progressive).

# Clinical Trial NO	# Sample NO	# Response Category	Cycle of Treatment	RIN
CCI-19	NB1	R2	C0	7.5
CCI-19	NB2	R2	C2	9.2
CCI-19	NB3	R2	EP	8.6
CCI-20	NB5	NR4	C0	7.4
CCI-20	NB6	NR4	C1	8.4
CCI-20	NB7	NR4	C2	8.8
CCI-20	NB8	NR4	EP	9.5
CCI-27	NB18	R4	C0	6.8
CCI-27	NB19	R4	C1	9.4
CCI-27	NB20	R4	C2	9.5
CCI-27	NB21	R4	EP	6.5
CCI-29	NB23	NR2	C0	8.6
CCI-29	NB24	NR2	C1	9.8
CCI-29	NB25	NR2	C2	9.2
CCI-29	NB26	NR2	EP	8.6
CCI-30	NB27	NR3	C0	9.3
CCI-30	NB28	NR3	C1	8.5
CCI-30	NB29	NR3	C2	9.3
CCI-31	NB30	R3	C0	7.5
CCI-31	NB31	R3	C1	9.5

CCI-31	NB32	R3	C2	7.7
CCI-31	NB33	R3	EP	7.6
CCI-33	NB35	R1	C0	7.1
CCI-33	NB36	R1	C1	8.9
CCI-33	NB37	R1	C2	8.9
CCI-33	NB38	R1	EP	8.9
CCI-38	NB40	NR5	C0	8.8
CCI-38	NB41	NR5	C1	9.2
CCI-38	NB42	NR5	C2	7.1
CCI-38	NB43	NR5	EP	8.5
CCI-40	NB44	NR7	C0	8.4
CCI-40	NB45	NR7	C1	9.5
CCI-40	NB46	NR7	C2	9.6
CCI-40	NB47	NR7	EP	9.6
CCI-43	NB52	NR6	C0	7.3
CCI-43	NB54	NR6	EP	6.6
CCI-17	NB55	NR1	C0	7.8
CCI-17	NB57	NR1	C2	7
CCI-17	NB58	NR1	EP	8.4

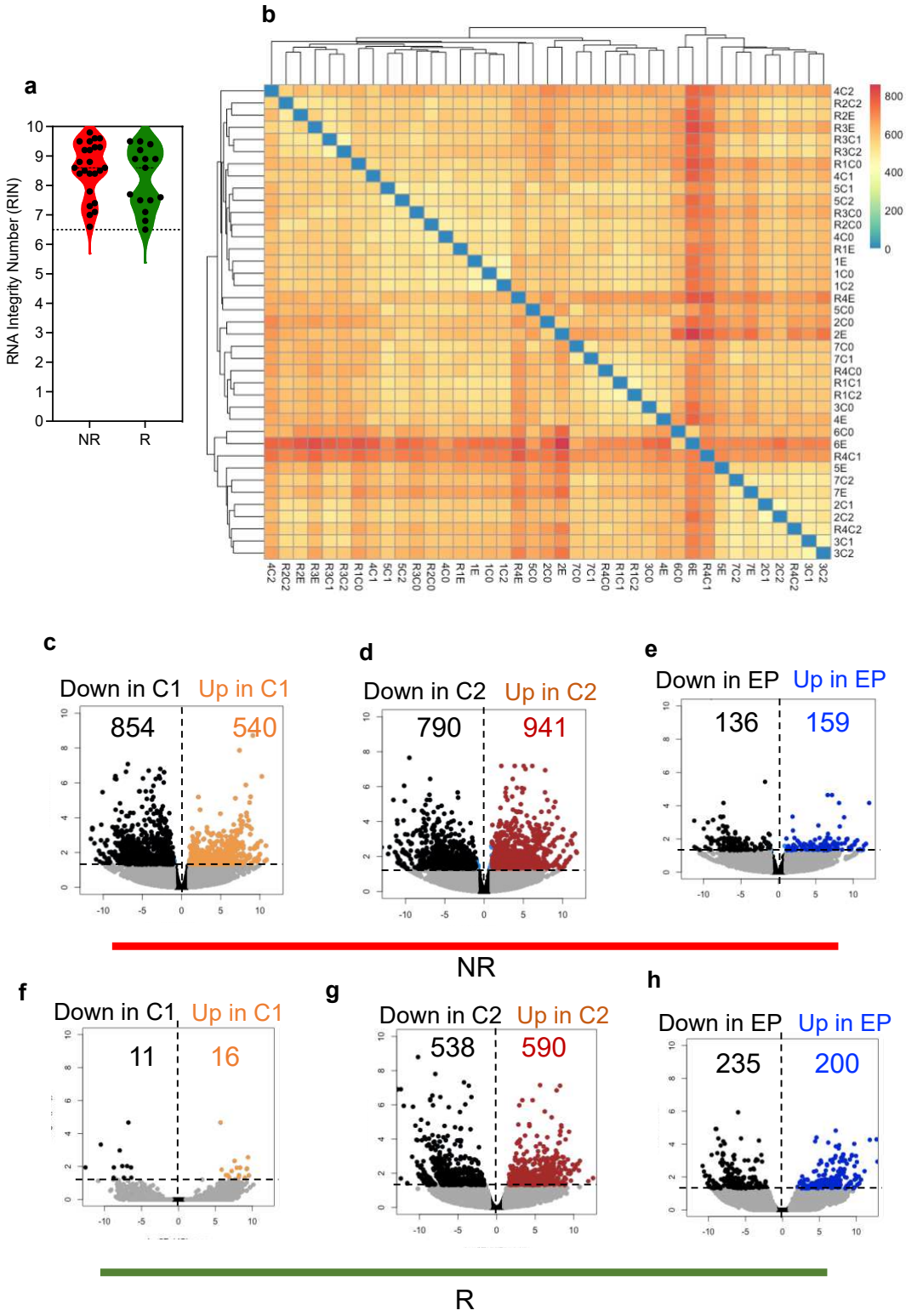
Supplementary Table 2. Samples submitted for RNA sequencing. R: Responders, NR: Non-responders, C0: Cycle 0, C1: Cycle 1, C2: Cycle 2, EP: Endpoint, RIN: RNA Integrity Number.

Response Category	Tumor Site	Sex	Stage	Best Response
NR	Penis	M	IV	iUPD
R	Larynx	M	IVC	iCCR
R	Penis	M	IV	iCPR
R	Penis	M	IV	iUPR
R	Cervix	F	IVB	iSD
NR	Tonsil	M	IVC	iUPD
R	Nasopharynx	M	III	iUPR
NR	Tonsil	M	IV	iUPD
NR	Cervix	F	IVB	iCPD
NR	Anal	F	IV	iUPD

Supplementary Table 3. HPV-associated metastatic carcinoma patients' characteristics (Ancillary cohort for plasma analytes validation). R: Responders (iCCR+ iCPR+iUPR+ iSD), NR: Non-responders (iCPD+ iUPD+ Clinically progressive), M: Male, F: Female, iUPD: Unconfirmed progressive disease, iCCR: Confirmed complete response, iCPD: Confirmed progressive disease, NE: Response was not evaluated, iUPR: Unconfirmed partial response, iCPR: Confirmed partial response, iSD: Stable disease.

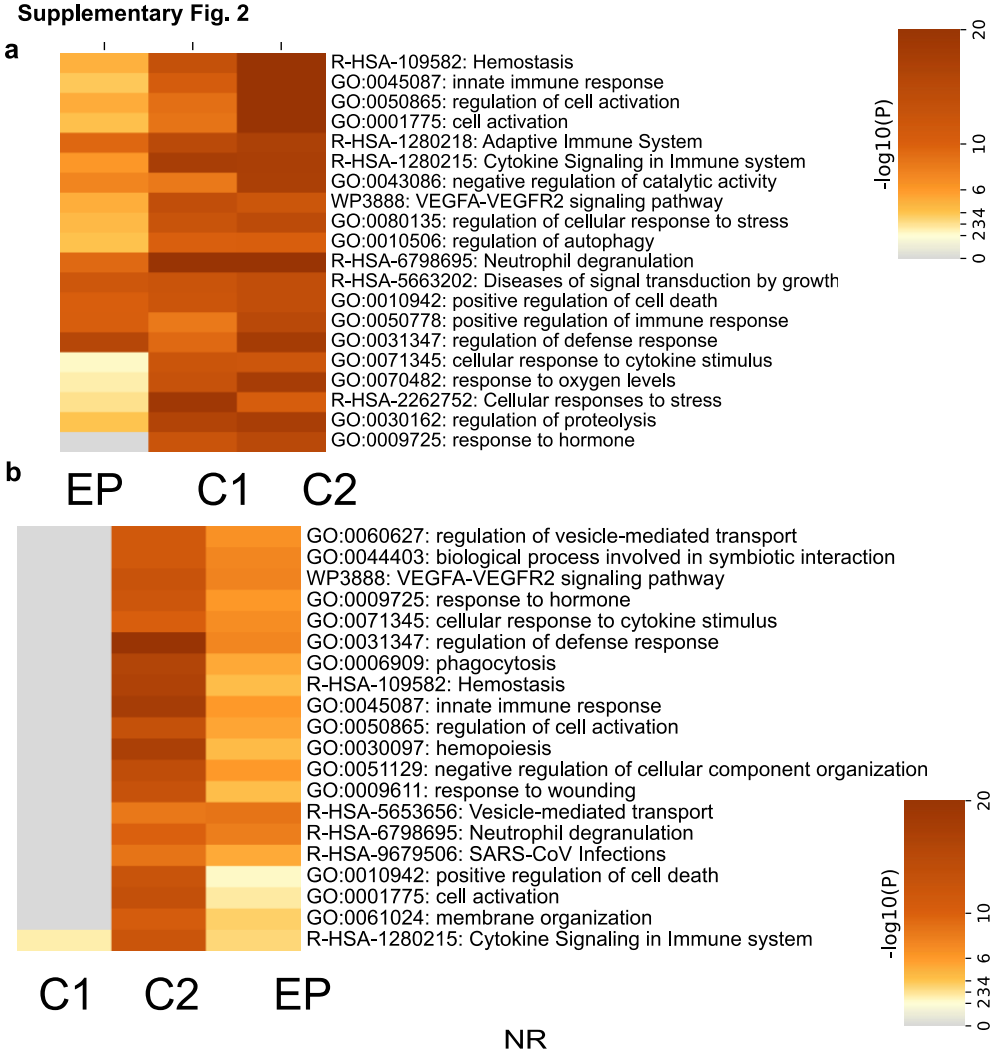
4.7 Supplementary Figures

Supplementary Fig. 1



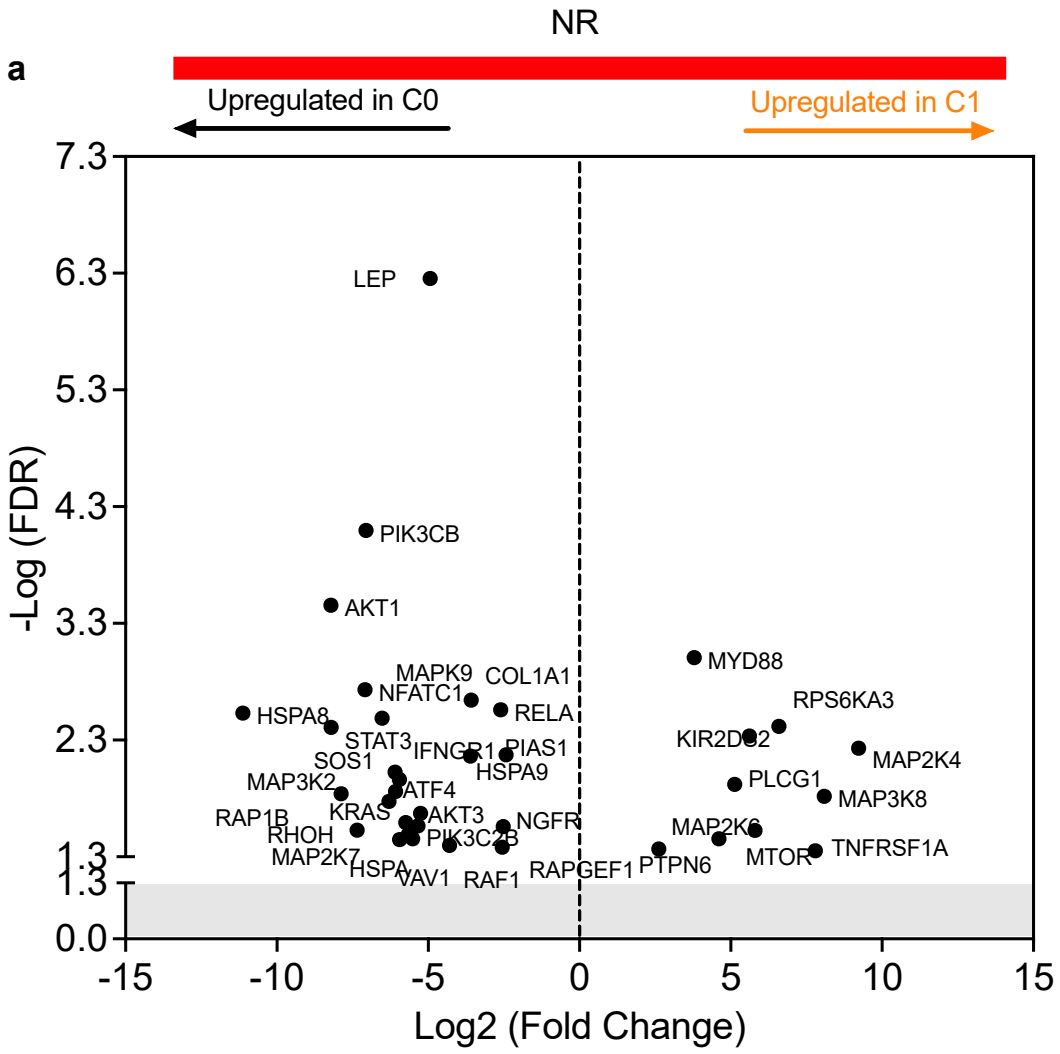
Supplementary Fig. 1. (a) Cumulative data showing RNA Integrity Number (RIN) of NRs' and Rs' samples. **(b)** Heatmap showing the Euclidean distance between samples as calculated from the regularized log transformation. **(c-e)** Volcano plots illustrating $\log_2$ (fold change) and -log (p_{adj}) of transcripts in NRs comparing different time points. Each dot represents one transcript. The threshold was set as absolute $\log_2$ (fold change) >1 and -log (p_{adj}) > 1.3. **(f-h)** Volcano plots illustrating $\log_2$ (fold change) and -log (p_{adj}) of transcripts in Rs comparing different time points. Each dot represents one transcript. The threshold was set as absolute $\log_2$ (fold change) >1 and -log (p_{adj}) > 1.3.

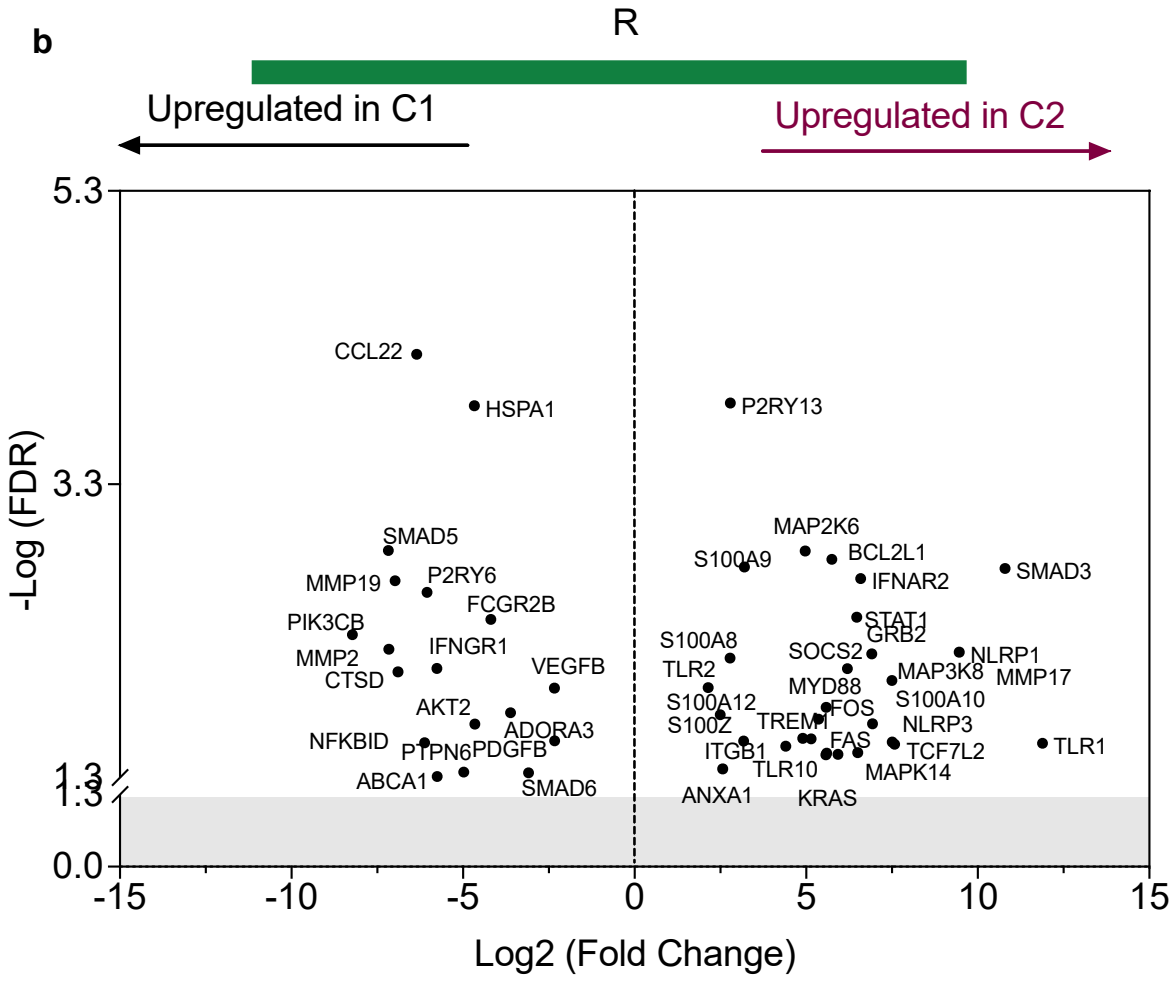
Supplementary Fig. 2



Supplementary Fig. 2. (a) Functional pathway enrichment in NRs at C1, C2, and EP compared to C0, analysed in Metascape for (KEGG, Reactome, GO, and Wikipathways). The heatmap cells are colored based on the value of enriched terms, and white cells represent the lack of enrichment for that specific term. **(b)** Functional pathway enrichment in Rs in the Cycle1, Cycle2, and Endpoint compared to the baseline, analysed in Metascape for Reactome, GO, and WikiPathways. The heatmap cells are colored based on the value of enriched terms, and white cells represent the lack of enrichment for that specific term. **(c)** . Volcano plot showing differentially expressed transcripts in NRs (C1 compared to C0) related to B cell/T cell receptor signaling obtained from IPA. Threshold was set as absolute $\log_2$ (fold change) > 1, and -log (FDR) > 1.3.

Supplementary Fig. 3




b
R
Upregulated in C1
Upregulated in C2
5.3
3.3
1.3
1.3
0.0
-Log (FDR)
-15
-10
-5
0
5
10
15
Log2 (Fold Change)
CCL22
HSPA1
SMAD5
MMP19
P2RY6
FCGR2B
PIK3CB
IFNGR1
MMP2
CTSD
VEGFB
AKT2
ADORA3
NFKBID
PTPN6
PDGFB
ABCA1
SMAD6
P2RY13
MAP2K6
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STAT1
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MMP17
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S100A10
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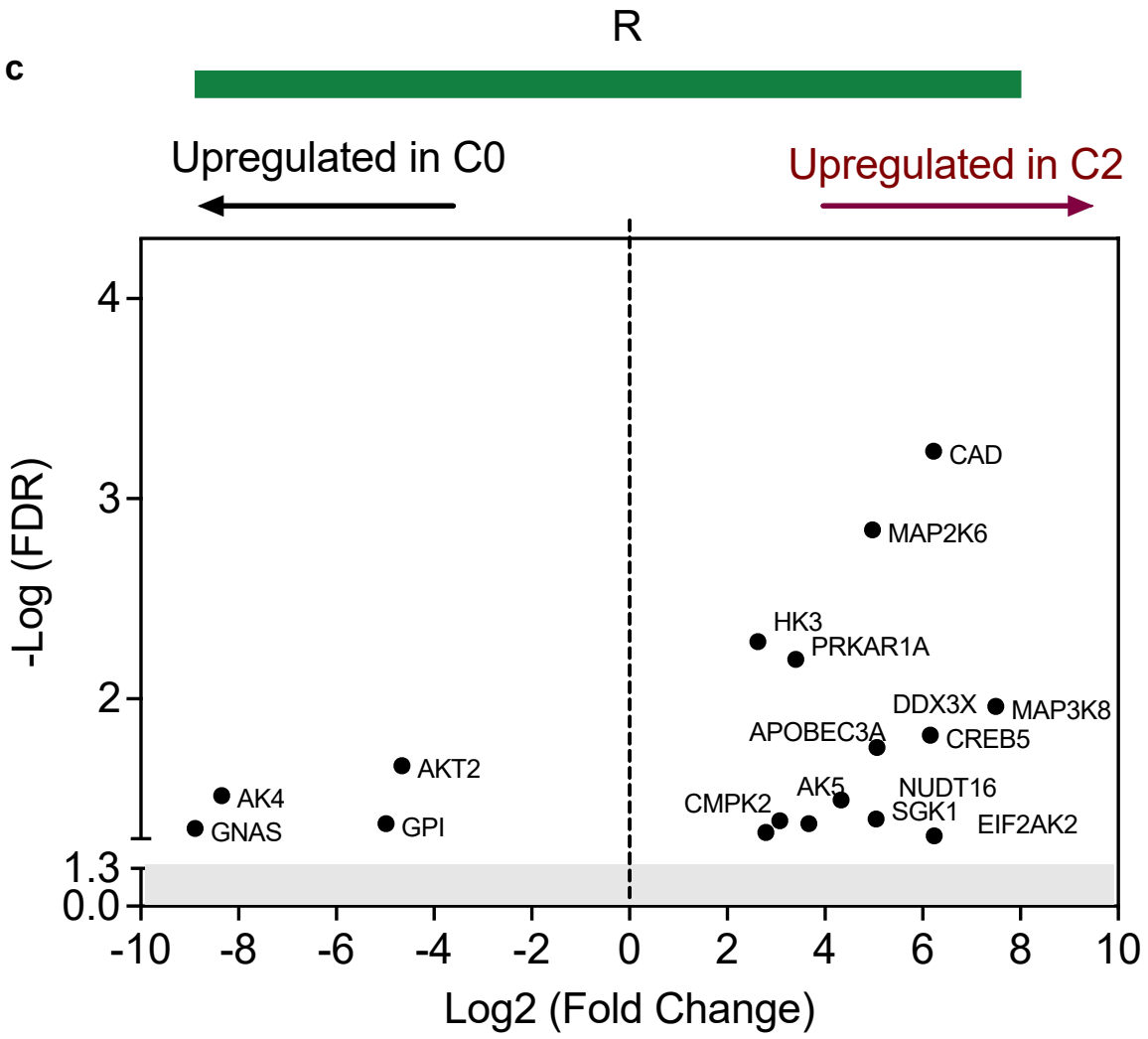
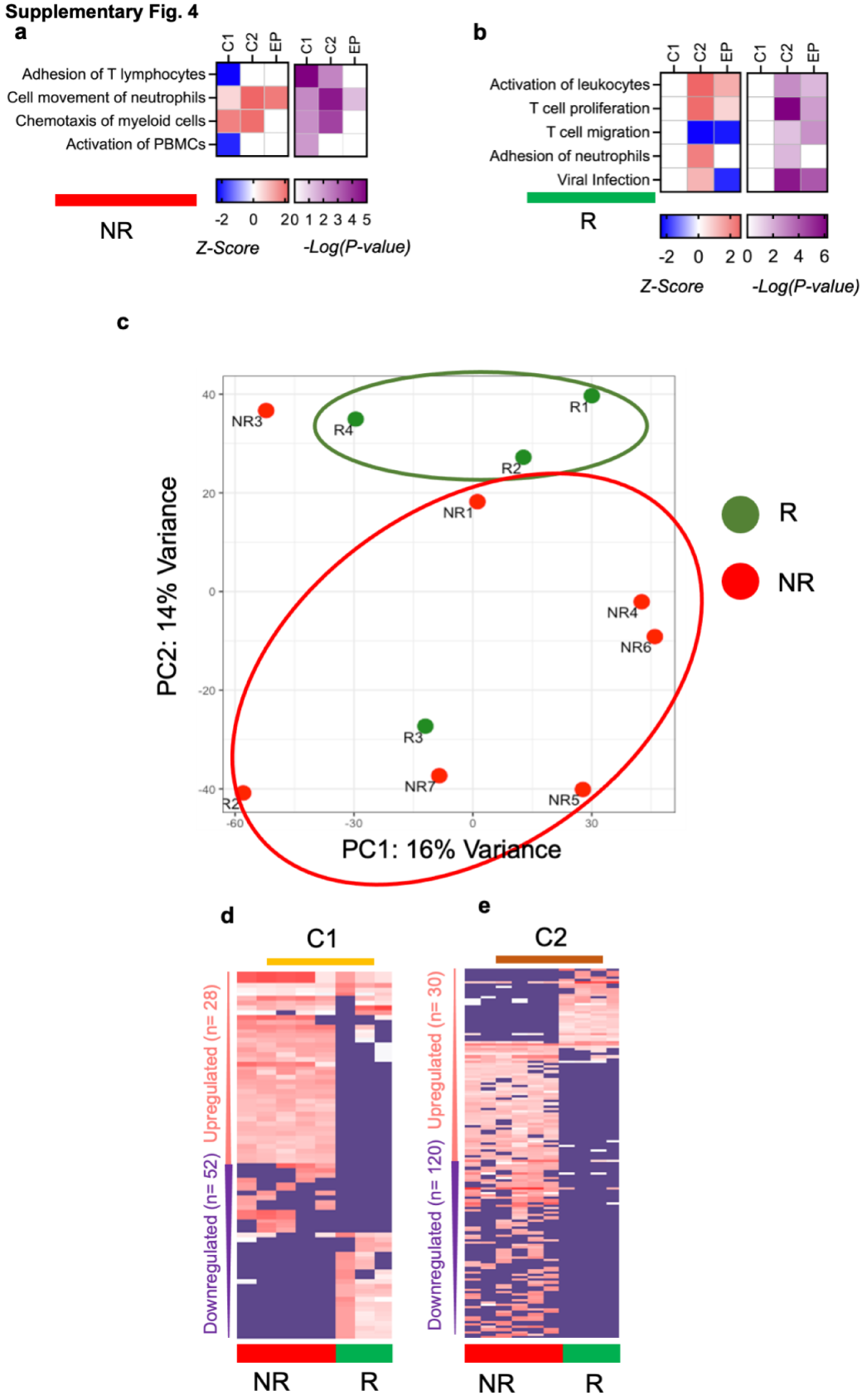
KRAS

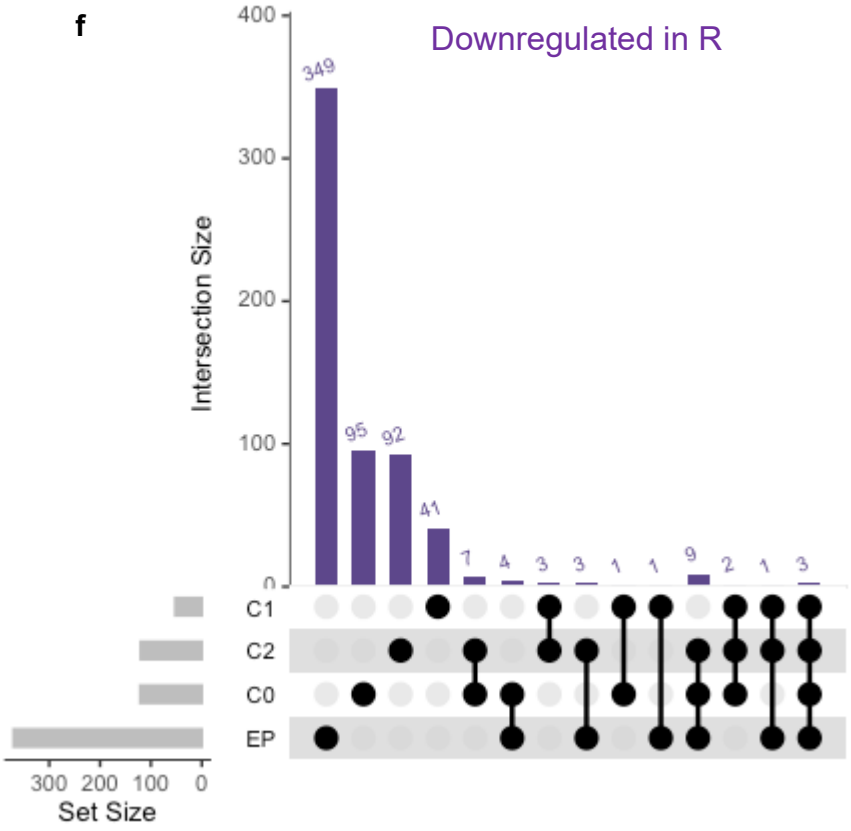


Supplementary Fig.3. (a) Volcano plot showing differentially expressed transcripts in NRs (C1 compared to C0) related to HMGB1, GM-CSF, IL-23, FLT3, IL-3, and IL-8 signaling obtained from IPA. Threshold was set as absolute log_2 (fold change) > 1, and -log (FDR) > 1.3. **(b)** Volcano plot showing differentially expressed transcripts in Rs (C2 compared to C0) related to innate signaling pathways (S100 family, PKR in interferon induction and antiviral response, Toll-like Receptor, TREM1) obtained from IPA. Threshold was set as absolute log_2 (fold change) > 1, and -log (FDR) > 1.3. **(c)** Volcano plot showing differentially expressed transcripts in Rs (C2 compared to C0) related to metabolic pathways (Pyrimidine Ribonucleotides De Novo Biosyntheis, Salvage Pathways of Pyrimidine Ribonucleotides, and PFKFB4 signaling) obtained from IPA. Threshold was set as absolute log_2 (fold change) > 1, and -log (FDR) > 1.3.

Supplementary Fig. 4



f


Downregulated in R
Intersection Size
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200
100
0
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95
92
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7
4
3
3
1
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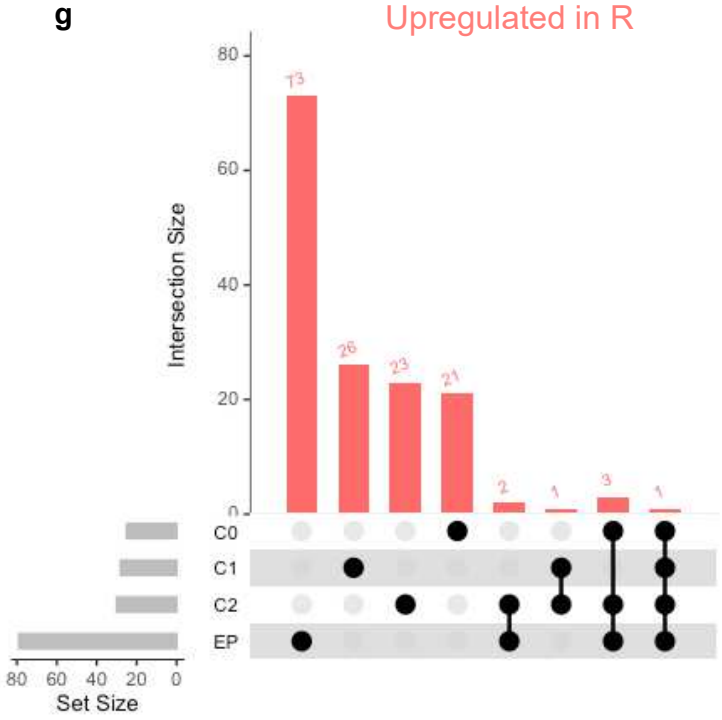
9
2
1
3
C1
C2
C0
EP
300 200 100 0
Set Size

g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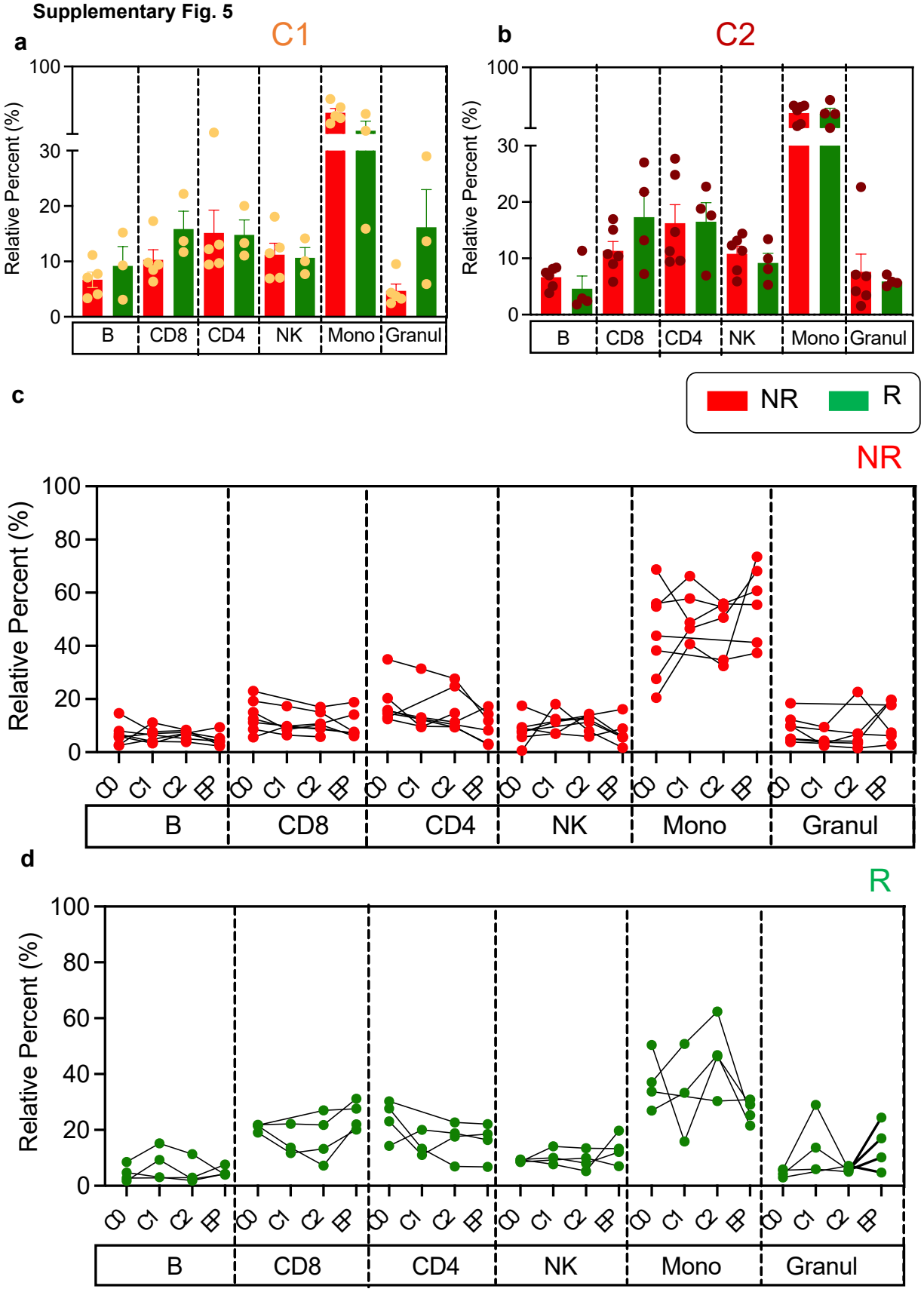
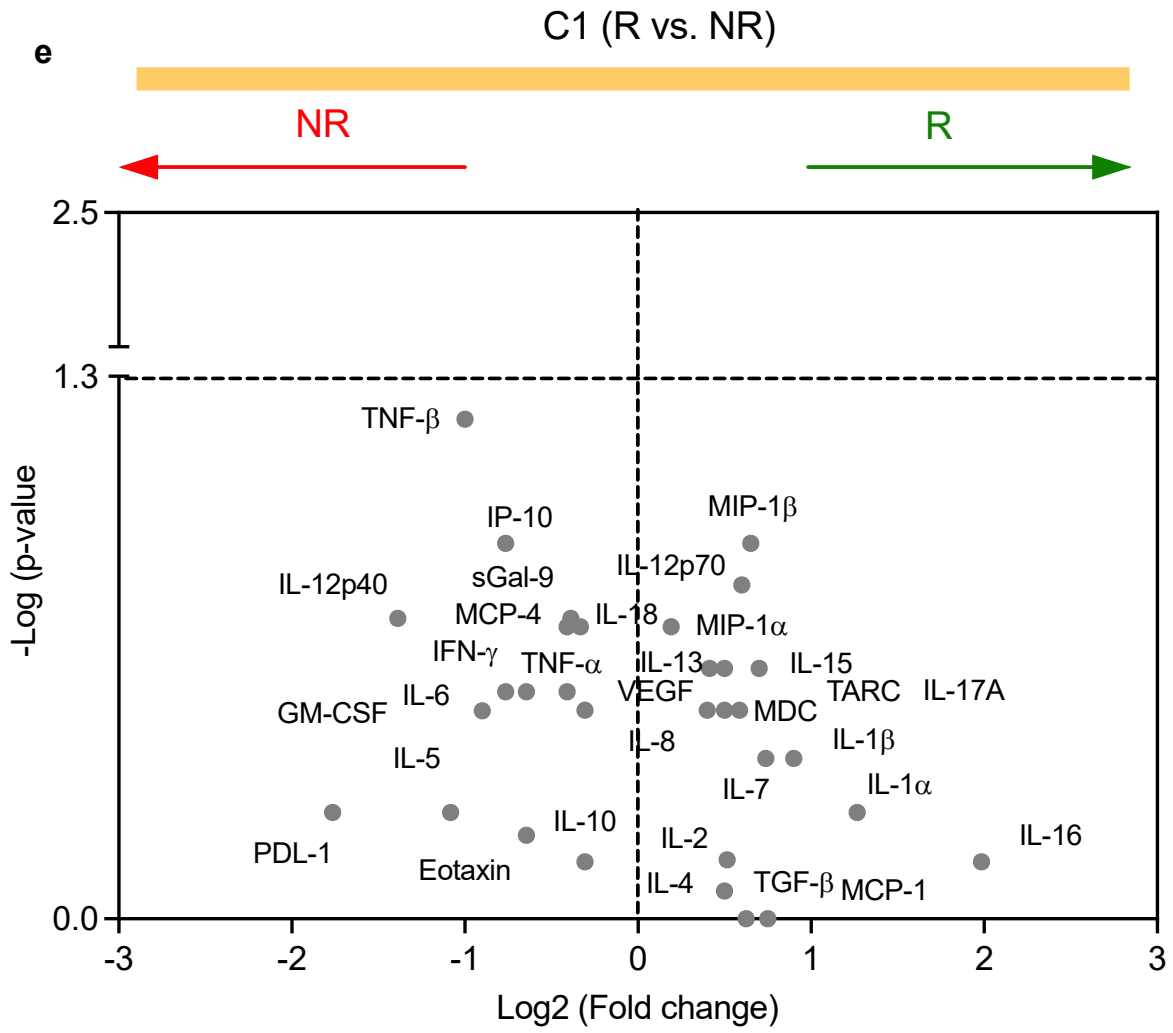
26
23
21
2
1
3
1
C0
C1
C2
EP
80 60 40 20 0
Set Size

Supplementary Fig. 4. (a) Heatmaps showing enriched functional pathways, comparing C1, C2, and EP to C0 in NRs, and **(b)** Rs. **(c)** Principal component analysis (PCA) plot on the Euclidian distances between NRs and Rs at C0, red dot represents (NR = non-responder), green dot represents (R = responder) **(d)** Heatmap showing differentially expressed transcripts in Rs vs NRs at C1, and **(e)** C2. For heat map plotting regularized logarithmic transformation (rlog) was priorly applied to the raw counts. Only transcripts with a False Discovery rate (FDR or p_{adj}) > 0.05, and an absolute $\log_2$ (fold change) > 1 are included. The number of downregulated or upregulated transcripts in each comparison is indicated with purple or Indian red vertical arrows on the right of each heatmap. The magnitude of the $\log_2$ (fold change) is depicted in color key bar beneath each heatmap. Purple represents downregulated and Indian red represents upregulated transcripts in Rs. **(f, g)** UpSet plots describing downregulated differentially expressed transcripts (Purple bars), and Upregulated (Indian red bars) in Rs vs NRs in one or more timepoints. Vertical bars represent intersection size which is the number of differentially expressed transcripts in one or more comparisons. Gray sidebars show the number of transcripts that were found differentially expressed in each timepoint. Black dots represent one timepoint comparisons, and black dots connected with a line indicate differentially expressed transcripts in more than one timepoint comparisons.

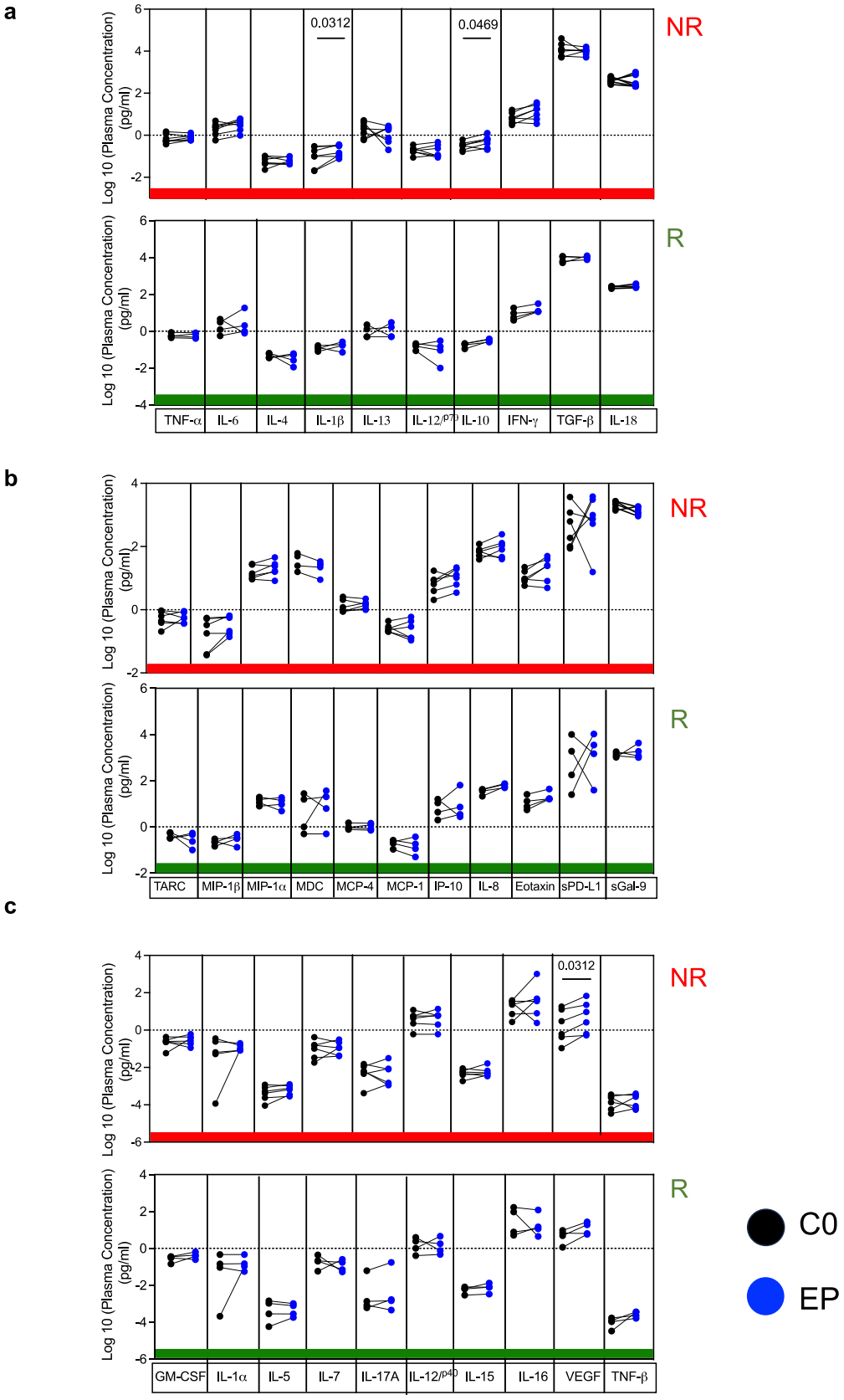
Supplementary Fig. 5



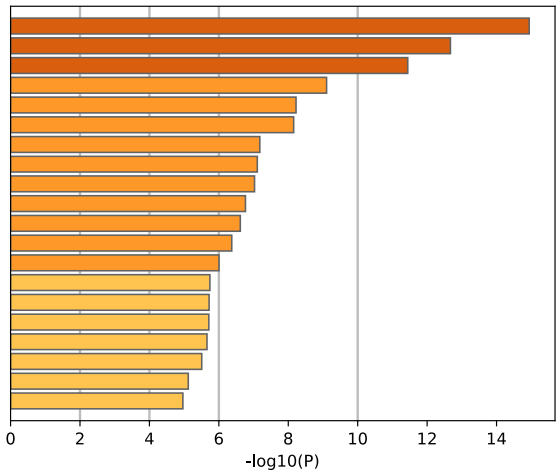


Supplementary Fig. 5. (a) Cumulative data comparing the relative percentages of 6 types of immune cells in Rs (green) and NRs (red) at C1, and **(b)** C2. The CIBERSORTx result of 22 cell types was merged into 6 subsets as the sum of the counts of B cells (B cells naïve + memory +Plasma cells), CD8 T cells, CD4 T cells (CD4 naïve + memory resting + memory activated + T follicular helper + T cells gamma delta), NK cells (NK resting + activated), Monocytes (Monocytes + M0 + M1 + M2 macrophages + DC resting + Dc activated), Granulocytes (Mast cells resting + Mast cells activated + Eosinophils + Neutrophils). **(c)** Longitudinal changes in cell fractions of 6 subsets inferred from CIBERSORTx at C0, C1, C2, and EP in NRs, and **(d)** Rs. **(e)** Volcano plot illustrating the significance and magnitude of the differences in soluble mediators in Rs vs NRs at C1. For volcano plot, absolute $\log_2$ (fold change) > 1 and -log (p-value) > 1.3 were considered as threshold for up or downregulation. P-value < 0.05 was considered as significant.

Supplementary Fig. 6

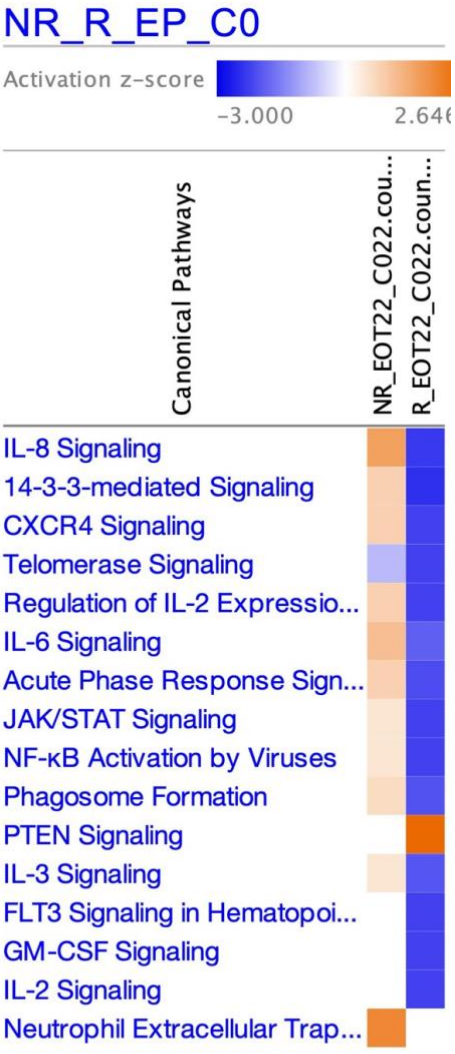


d C0 (R vs NR)



hsa05332: Graft-versus-host disease
GO:0002821: positive regulation of adaptive immune response
GO:0002696: positive regulation of leukocyte activation
GO:0002764: immune response-regulating signaling pathway
GO:0002700: regulation of production of molecular mediator of immune response
GO:0080135: regulation of cellular response to stress
GO:0045087: innate immune response
R-HSA-354192: Integrin signaling
R-HSA-6798695: Neutrophil degranulation
GO:0019218: regulation of steroid metabolic process
hsa04064: NF-kappa B signaling pathway
GO:0070665: positive regulation of leukocyte proliferation
GO:0043408: regulation of MAPK cascade
WP3645: NAD+ biosynthetic pathways
WP4754: IL-18 signaling pathway
GO:0006974: cellular response to DNA damage stimulus
M37: PID NFKAPPAB CANONICAL PATHWAY
M118: PID INTEGRIN A9B1 PATHWAY
GO:0045596: negative regulation of cell differentiation
GO:0031532: actin cytoskeleton reorganization

e



Supplementary Fig. 6. (a-c) Comparison of normalized plasma levels of soluble mediators from C0 to EP in NRs (tagged with red bar) and Rs (Tagged with green bar). *P-value* < 0.05 was considered as significant. **(d)** Enriched functional pathways analysed in Metascape for (KEGG, Reactome, GO, WikiPathways), comparing Rs vs NRs at C0. The colored bars show the -log (p-value) of the enriched term. **(e)** Comparison of the enriched signaling pathways in NRs (first column) and Rs (Second column). Each column represents the enriched terms at the EP vs C0. The right panel shows the activation Z-Score (Orange represents activation, blue represents inhibition), and the left panel represents the p-values.

4.8 References

1. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumors. Nat Rev Immunol [Internet]. 2012 [cited 2023 Feb 2];12:253–68. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3587148/

2. Zhao L, He R, Long H, Guo B, Jia Q, Qin D, et al. Late-stage tumors induce anemia and immunosuppressive extramedullary erythroid progenitor cells. Nat Med [Internet]. 2018 [cited 2023 Mar 11];24:1536–44. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6211844/

3. Pathria P, Louis TL, Varner JA. Targeting Tumor-Associated Macrophages in Cancer. Trends Immunol. 2019;40:310–27.

4. Wu W-C, Sun H-W, Chen H-T, Liang J, Yu X-J, Wu C, et al. Circulating hematopoietic stem and progenitor cells are myeloid-biased in cancer patients. Proc Natl Acad Sci U S A. 2014;111:4221–6.

5. Elahi S. Neglected Cells: Immunomodulatory Roles of CD71+ Erythroid Cells. Trends Immunol. 2019;40:181–5.

6. Anderson NM, Simon MC. The tumor microenvironment. Curr Biol [Internet]. 2020 [cited 2023 Feb 9];30:R921–5. Available from: https://www.sciencedirect.com/science/article/pii/S0960982220309337

7. Okoye IS, Houghton M, Tyrrell L, Barakat K, Elahi S. Coinhibitory Receptor Expression and Immune Checkpoint Blockade: Maintaining a Balance in CD8+ T Cell Responses to Chronic Viral Infections and Cancer. Front Immunol [Internet]. 2017 [cited 2023 Feb 9];8. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2017.01215

8. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. Nat Rev Immunol [Internet]. Nature Publishing Group; 2020 [cited 2023 Feb 9];20:651–68. Available from: https://www.nature.com/articles/s41577-020-0306-5

9. Ribas A, Wolchok JD. Cancer Immunotherapy Using Checkpoint Blockade. Science [Internet]. 2018 [cited 2023 Feb 9];359:1350–5. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7391259/

10. Groth C, Hu X, Weber R, Fleming V, Altevogt P, Utikal J, et al. Immunosuppression mediated by myeloid-derived suppressor cells (MDSCs) during tumor progression. Br J Cancer [Internet]. Nature Publishing Group; 2019 [cited 2023 Feb 2];120:16–25. Available from: https://www.nature.com/articles/s41416-018-0333-1

11. Bronte V, Serafini P, Apolloni E, Zanovello P. Tumor-induced immune dysfunctions caused by myeloid suppressor cells. J Immunother Hagerstown Md 1997. 2001;24:431–46.

12. Bronte V, Brandau S, Chen S-H, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. Nat Commun [Internet]. 2016 [cited 2023 Feb 13];7:12150. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4935811/

13. Hou A, Hou K, Huang Q, Lei Y, Chen W. Targeting Myeloid-Derived Suppressor Cell, a Promising Strategy to Overcome Resistance to Immune Checkpoint Inhibitors. Front Immunol. 2020;11:783.

14. Li X, Zhong J, Deng X, Guo X, Lu Y, Lin J, et al. Targeting Myeloid-Derived Suppressor Cells to Enhance the Anti-tumor Efficacy of Immune Checkpoint Blockade Therapy. Front Immunol [Internet]. 2021 [cited 2023 Feb 27];12. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2021.754196

15. Otter S, Whitaker S, Chatterjee J, Stewart A. The Human Papillomavirus as a Common Pathogen in Oropharyngeal, Anal and Cervical Cancers. Clin Oncol [Internet]. 2019 [cited 2023 Feb 9];31:81–90. Available from: https://www.sciencedirect.com/science/article/pii/S0936655518304655

16. Lechner M, Liu J, Masterson L, Fenton TR. HPV-associated oropharyngeal cancer: epidemiology, molecular biology, and clinical management. Nat Rev Clin Oncol [Internet]. Nature Publishing Group; 2022 [cited 2023 Mar 5];19:306–27. Available from: https://www.nature.com/articles/s41571-022-00603-7

17. Gao P, Lazare C, Cao C, Meng Y, Wu P, Zhi W, et al. Immune checkpoint inhibitors in the treatment of virus-associated cancers. J Hematol OncolJ Hematol Oncol [Internet]. 2019 [cited 2023 Feb 9];12:58. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6558794/

18. Liu C, Lu J, Tian H, Du W, Zhao L, Feng J, et al. Increased expression of PD-L1 by the human papillomavirus 16 E7 oncoprotein inhibits anticancer immunity. Mol Med Rep. 2017;15:1063–70.

19. Kuss-Duerkop SK, Westrich JA, Pyeon D. DNA Tumor Virus Regulation of Host DNA Methylation and Its Implications for Immune Evasion and Oncogenesis. Viruses. 2018;10:82.

20. Qi L, Li N, Lin A, Wang X, Cong J. Efficacy and safety of pembrolizumab on cervical cancer: A systematic review and single-arm meta-analysis. Front Oncol [Internet]. 2022 [cited 2023 Feb 9];12:910486. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9399507/

21. Li X, Su X, Liu R, Pan Y, Fang J, Cao L, et al. HDAC inhibition potentiates the anti-tumor activity of macrophages and enhances anti-PD-L1-mediated tumor suppression. Oncogene [Internet]. Nature Publishing Group; 2021 [cited 2023 Feb

14];40:1836–50. Available from: https://www.nature.com/articles/s41388-020-01636-x

22. Duenas-Gonzalez A, Candelaria M, Perez-Plascencia C, Perez-Cardenas E, de la Cruz-Hernandez E, Herrera LA. Valproic acid as epigenetic cancer drug: preclinical, clinical and transcriptional effects on solid tumors. Cancer Treat Rev. 2008;34:206–22.

23. Suraweera A, O'Byrne KJ, Richard DJ. Combination Therapy With Histone Deacetylase Inhibitors (HDACi) for the Treatment of Cancer: Achieving the Full Therapeutic Potential of HDACi. Front Oncol [Internet]. 2018 [cited 2022 Sep 3];8. Available from: https://www.frontiersin.org/articles/10.3389/fonc.2018.00092

24. Smith KT, Workman JL. Histone deacetylase inhibitors: anticancer compounds. Int J Biochem Cell Biol. 2009;41:21–5.

25. Cinatl J, Kotchetkov R, Blaheta R, Driever PH, Vogel JU, Cinatl J. Induction of differentiation and suppression of malignant phenotype of human neuroblastoma BE(2)-C cells by valproic acid: enhancement by combination with interferon-alpha. Int J Oncol. 2002;20:97–106.

26. Kawagoe R, Kawagoe H, Sano K. Valproic acid induces apoptosis in human leukemia cells by stimulating both caspase-dependent and -independent apoptotic signaling pathways. Leuk Res. 2002;26:495–502.

27. Feng S, Yang Y, Lv J, Sun L, Liu M. Valproic acid exhibits different cell growth arrest effects in three HPV-positive/negative cervical cancer cells and possibly via inducing Notch1 cleavage and E6 downregulation. Int J Oncol. 2016;49:422–30.

28. Mora-García M de L, Duenas-González A, Hernández-Montes J, De la Cruz-Hernández E, Pérez-Cárdenas E, Weiss-Steider B, et al. Up-regulation of HLA class-I antigen expression and antigen-specific CTL response in cervical cancer cells by the demethylating agent hydralazine and the histone deacetylase inhibitor valproic acid. J Transl Med [Internet]. 2006 [cited 2023 Feb 9];4:55. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1781077/

29. Soria-Castro R, Schcolnik-Cabrera A, Rodríguez-López G, Campillo-Navarro M, Puebla-Osorio N, Estrada-Parra S, et al. Exploring the Drug Repurposing Versatility of Valproic Acid as a Multifunctional Regulator of Innate and Adaptive Immune Cells. J Immunol Res. 2019;2019:9678098.

30. Okoye I, Xu L, Motamedi M, Parashar P, Walker JW, Elahi S. Galectin-9 expression defines exhausted T cells and impaired cytotoxic NK cells in patients with virus-associated solid tumors. J Immunother Cancer. 2020;8:e001849.
31. Seymour L, Bogaerts J, Perrone A, Ford R, Schwartz LH, Mandrekar S, et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. Lancet Oncol [Internet]. 2017 [cited 2023 Jan 23];18:e143–52. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5648544/

32. Shahbaz S, Jovel J, Elahi S. Differential transcriptional and functional properties of regulatory T cells in HIV-infected individuals on antiretroviral therapy and long-term non-progressors. Clin Transl Immunol. 2021;10:e1289.

33. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun. 2019;10:1523.

34. Newman AM, Steen CB, Liu CL, Gentles AJ, Chaudhuri AA, Scherer F, et al. Determining cell-type abundance and expression from bulk tissues with digital cytometry. Nat Biotechnol [Internet]. 2019 [cited 2023 Jan 23];37:773–82. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6610714/

35. Nadel BB, Oliva M, Shou BL, Mitchell K, Ma F, Montoya DJ, et al. Systematic evaluation of transcriptomics-based deconvolution methods and references using thousands of clinical samples. Brief Bioinform. 2021;22:bbab265.

36. Bozorgmehr N, Okoye I, Oyegbami O, Xu L, Fontaine A, Cox-Kennett N, et al. Expanded antigen-experienced CD160+CD8+effector T cells exhibit impaired effector functions in chronic lymphocytic leukemia. J Immunother Cancer. 2021;9:e002189.

37. Bozorgmehr N, Hnatiuk M, Peters AC, Elahi S. Depletion of polyfunctional CD26highCD8+ T cells repertoire in chronic lymphocytic leukemia. Exp Hematol Oncol. 2023;12:13.

38. AHS Cancer Control Alberta. The LATENT Trial: Lytic Activation To Enhance Neoantigen-directed Therapy A Study to Evaluate the Feasibility and Efficacy of the Combined Use of Avelumab With Valproic Acid for the Treatment of Virus-associated Cancer [Internet]. clinicaltrials.gov; 2019 Jul. Report No.: NCT03357757. Available from: https://clinicaltrials.gov/ct2/show/NCT03357757

39. Grunstein M. Histone acetylation in chromatin structure and transcription. Nature. 1997;389:349–52.

40. O'Shea JJ, Schwartz DM, Villarino AV, Gadina M, McInnes IB, Laurence A. The JAK-STAT Pathway: Impact on Human Disease and Therapeutic Intervention. Annu Rev Med [Internet]. 2015 [cited 2023 Feb 6];66:311–28. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5634336/

41. Addison CL, Daniel TO, Burdick MD, Liu H, Ehlert JE, Xue YY, et al. The CXC chemokine receptor 2, CXCR2, is the putative receptor for ELR+ CXC chemokine-induced angiogenic activity. J Immunol Baltim Md 1950. 2000;165:5269–77.

42. Stothers C, Luan L, Fensterheim B, Bohannon J. Hypoxia-inducible factor-1α regulation of myeloid cells. J Mol Med Berl Ger [Internet]. 2018 [cited 2023 Mar 1];96:1293–306. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6292431/

43. Xie Z, Ago Y, Okada N, Tachibana M. Valproic acid attenuates immunosuppressive function of myeloid-derived suppressor cells. J Pharmacol Sci. 2018;137:359–65.

44. Bar N, Costa F, Das R, Duffy A, Samur M, McCachren S, et al. Differential effects of PD-L1 versus PD-1 blockade on myeloid inflammation in human cancer. JCI Insight [Internet]. American Society for Clinical Investigation; 2020 [cited 2023 Feb 21];5. Available from: https://insight.jci.org/articles/view/129353

45. Fairbanks LD, Bofill M, Ruckemann K, Simmonds HA. Importance of ribonucleotide availability to proliferating T-lymphocytes from healthy humans. Disproportionate expansion of pyrimidine pools and contrasting effects of de novo synthesis inhibitors. J Biol Chem. 1995;270:29682–9.

46. Bertheloot D, Latz E, Franklin BS. Necroptosis, pyroptosis and apoptosis: an intricate game of cell death. Cell Mol Immunol [Internet]. Nature Publishing Group; 2021 [cited 2023 Feb 2];18:1106–21. Available from: https://www.nature.com/articles/s41423-020-00630-3

47. Dumitru CA, Moses K, Trellakis S, Lang S, Brandau S. Neutrophils and granulocytic myeloid-derived suppressor cells: immunophenotyping, cell biology and clinical relevance in human oncology. Cancer Immunol Immunother CII. 2012;61:1155–67.

48. Li K, Shi H, Zhang B, Ou X, Ma Q, Chen Y, et al. Myeloid-derived suppressor cells as immunosuppressive regulators and therapeutic targets in cancer. Signal Transduct Target Ther [Internet]. Nature Publishing Group; 2021 [cited 2023 Mar 6];6:1–25. Available from: https://www.nature.com/articles/s41392-021-00670-9

49. Propper DJ, Balkwill FR. Harnessing cytokines and chemokines for cancer therapy. Nat Rev Clin Oncol [Internet]. Nature Publishing Group; 2022 [cited 2023 Feb 22];19:237–53. Available from: https://www.nature.com/articles/s41571-021-00588-9

50. Bozorgmehr N, Mashhouri S, Perez Rosero E, Xu L, Shahbaz S, Sligl W, et al. Galectin-9, a Player in Cytokine Release Syndrome and a Surrogate Diagnostic Biomarker in SARS-CoV-2 Infection. mBio. 2021;12:e00384-21.

51. Zhao S, Wu D, Wu P, Wang Z, Huang J. Serum IL-10 Predicts Worse Outcome in Cancer Patients: A Meta-Analysis. PLoS ONE [Internet]. 2015 [cited 2023 Mar 7];10:e0139598. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4595202/

52. Gelfo V, Romaniello D, Mazzeschi M, Sgarzi M, Grilli G, Morselli A, et al. Roles of IL-1 in Cancer: From Tumor Progression to Resistance to Targeted Therapies. Int J Mol Sci [Internet]. 2020 [cited 2023 Mar 7];21:6009. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7503335/

53. STATHOPOULOS J, ARMAKOLAS A, STATHOPOULOS GP, GOMATOS IP. Plasma VEGF levels in breast cancer patients with and without metastases. Oncol Lett

[Internet]. 2010 [cited 2023 Mar 7];1:739–41. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3436353/

54. Vetsika E-K, Koukos A, Kotsakis A. Myeloid-Derived Suppressor Cells: Major Figures that Shape the Immunosuppressive and Angiogenic Network in Cancer. Cells [Internet]. 2019 [cited 2023 Mar 7];8:1647. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6953061/

55. Yao Z, Zhao M, Gao G, Yang J, Wang Z, Liu Y. Prognostic Role of IL-18 in Various Human Cancers and Radiation Injuries: A Meta-Analysis. Dose-Response [Internet]. 2020 [cited 2023 Feb 22];18:1559325820931360. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7323287/

56. Xu Q, Ma H, Chang H, Feng Z, Zhang C, Yang X. The interaction of interleukin-8 and PTEN inactivation promotes the malignant progression of head and neck squamous cell carcinoma via the STAT3 pathway. Cell Death Dis [Internet]. Nature Publishing Group; 2020 [cited 2023 Feb 13];11:1–14. Available from: https://www.nature.com/articles/s41419-020-2627-5

57. Ben-Hamo R, Jacob Berger A, Gavert N, Miller M, Pines G, Oren R, et al. Predicting and affecting response to cancer therapy based on pathway-level biomarkers. Nat Commun [Internet]. Nature Publishing Group; 2020 [cited 2023 Feb 17];11:3296. Available from: https://www.nature.com/articles/s41467-020-17090-y

58. Nakamura K, Kassem S, Cleynen A, Chrétien M-L, Guillerey C, Putz EM, et al. Dysregulated IL-18 Is a Key Driver of Immunosuppression and a Possible Therapeutic Target in the Multiple Myeloma Microenvironment. Cancer Cell. 2018;33:634-648.e5.

59. Alfaro C, Teijeira A, Oñate C, Pérez G, Sanmamed MF, Andueza MP, et al. Tumor-Produced Interleukin-8 Attracts Human Myeloid-Derived Suppressor Cells and Elicits Extrusion of Neutrophil Extracellular Traps (NETs). Clin Cancer Res [Internet]. 2016 [cited 2023 Feb 24];22:3924–36. Available from: https://doi.org/10.1158/1078-0432.CCR-15-2463

60. Veglia F, Sanseviero E, Gabrilovich DI. Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity. Nat Rev Immunol [Internet]. Nature Publishing Group; 2021 [cited 2023 Feb 24];21:485–98. Available from: https://www.nature.com/articles/s41577-020-00490-y

61. Feng F, Zheng G, Wang Q, Liu S, Liu Z, Xu G, et al. Low lymphocyte count and high monocyte count predicts poor prognosis of gastric cancer. BMC Gastroenterol [Internet]. 2018 [cited 2023 Feb 26];18:148. Available from: https://doi.org/10.1186/s12876-018-0877-9

62. Bizzarri C, Beccari AR, Bertini R, Cavicchia MR, Giorgini S, Allegretti M. ELR+ CXC chemokines and their receptors (CXC chemokine receptor 1 and CXC chemokine receptor 2) as new therapeutic targets. Pharmacol Ther [Internet]. 2006 [cited 2023 Feb 13];112:139–49. Available from: https://www.sciencedirect.com/science/article/pii/S0163725806000556

63. Yuen KC, Liu L-F, Gupta V, Madireddi S, Keerthivasan S, Li C, et al. High systemic and tumor-associated IL-8 correlates with reduced clinical benefit of PD-L1 blockade. Nat Med. 2020;26:693–8.

64. Lechner MG, Liebertz DJ, Epstein AL. CHARACTERIZATION OF CYTOKINE-INDUCED MYELOID-DERIVED SUPPRESSOR CELLS FROM NORMAL HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS. J Immunol Baltim Md 1950 [Internet]. 2010 [cited 2023 Feb 2];185:2273–84. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2923483/

65. Tobin RP, Jordan KR, Kapoor P, Spongberg E, Davis D, Vorwald VM, et al. IL-6 and IL-8 Are Linked With Myeloid-Derived Suppressor Cell Accumulation and Correlate With Poor Clinical Outcomes in Melanoma Patients. Front Oncol [Internet]. 2019 [cited 2023 Feb 13];9:1223. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6857649/

66. Zhang Y, Huang H, Coleman M, Ziemys A, Gopal P, Kazmi SM, et al. VEGFR2 activity on myeloid cells mediates immune suppression in the tumor microenvironment. JCI Insight [Internet]. American Society for Clinical Investigation; 2021 [cited 2023 Mar 3];6. Available from: https://insight.jci.org/articles/view/150735

67. Yang L, Liu Q, Zhang X, Liu X, Zhou B, Chen J, et al. DNA of neutrophil extracellular traps promotes cancer metastasis via CCDC25. Nature [Internet]. Nature Publishing Group; 2020 [cited 2023 Feb 26];583:133–8. Available from: https://www.nature.com/articles/s41586-020-2394-6

68. He X-Y, Ng D, Egeblad M. Caught in a Web: Emerging Roles of Neutrophil Extracellular Traps in Cancer. Annu Rev Cancer Biol [Internet]. 2022 [cited 2023 Feb 27];6:223–43. Available from: https://doi.org/10.1146/annurev-cancerbio-080421-015537

69. Nencioni A, Beck J, Werth D, Grünebach F, Patrone F, Ballestrero A, et al. Histone deacetylase inhibitors affect dendritic cell differentiation and immunogenicity. Clin Cancer Res Off J Am Assoc Cancer Res. 2007;13:3933–41.
70. Hashimoto A, Fukumoto T, Zhang R, Gabrilovich D. Selective targeting of different populations of myeloid-derived suppressor cells by histone deacetylase inhibitors. Cancer Immunol Immunother CII. 2020;69:1929–36.

71. Li J, Bolyard C, Xin G, Li Z. Targeting Metabolic Pathways of Myeloid Cells Improves Cancer Immunotherapy. Front Cell Dev Biol [Internet]. 2021 [cited 2023 Feb 27];9. Available from: https://www.frontiersin.org/articles/10.3389/fcell.2021.747863

72. Papaspyridonos M, Matei I, Huang Y, do Rosario Andre M, Brazier-Mitouart H, Waite JC, et al. Id1 suppresses anti-tumour immune responses and promotes tumour progression by impairing myeloid cell maturation. Nat Commun [Internet]. Nature

Publishing Group; 2015 [cited 2023 Feb 2];6:6840. Available from: https://www.nature.com/articles/ncomms7840

73. Kazi JU, Rönnstrand L. FMS-like Tyrosine Kinase 3/FLT3: From Basic Science to Clinical Implications. Physiol Rev [Internet]. American Physiological Society; 2019 [cited 2023 Feb 14];99:1433–66. Available from: https://journals.physiology.org/doi/full/10.1152/physrev.00029.2018

74. Lv J, Du C, Wei W, Wu Z, Zhao G, Li Z, et al. The Antiepileptic Drug Valproic Acid Restores T Cell Homeostasis and Ameliorates Pathogenesis of Experimental Autoimmune Encephalomyelitis. J Biol Chem [Internet]. 2012 [cited 2023 Feb 2];287:28656–65. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3436564/

75. Cui Y, Cai J, Wang W, Wang S. Regulatory Effects of Histone Deacetylase Inhibitors on Myeloid-Derived Suppressor Cells. Front Immunol [Internet]. 2021 [cited 2023 Feb 14];12:690207. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8208029/

76. Adeshakin AO, Yan D, Zhang M, Wang L, Adeshakin FO, Liu W, et al. Blockade of myeloid-derived suppressor cell function by valproic acid enhanced anti-PD-L1 tumor immunotherapy. Biochem Biophys Res Commun [Internet]. 2020 [cited 2023 Feb 14];522:604–11. Available from: https://www.sciencedirect.com/science/article/pii/S0006291X19322831

77. Arts RJW, Joosten LAB, van der Meer JWM, Netea MG. TREM-1: intracellular signaling pathways and interaction with pattern recognition receptors. J Leukoc Biol. 2013;93:209–15.

78. Cesaro T, Michiels T. Inhibition of PKR by Viruses. Front Microbiol [Internet]. 2021 [cited 2023 Feb 6];12:757238. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8573351/

79. Noman MZ, Desantis G, Janji B, Hasmim M, Karray S, Dessen P, et al. PD-L1 is a novel direct target of HIF-1α, and its blockade under hypoxia enhanced MDSC-mediated T cell activation. J Exp Med [Internet]. 2014 [cited 2023 Feb 27];211:781–90. Available from: https://doi.org/10.1084/jem.20131916

80. Jachetti E, Sangaletti S, Chiodoni C, Ferrara R, Colombo MP. Modulation of PD-1/PD-L1 axis in myeloid-derived suppressor cells by anti-cancer treatments. Cell Immunol [Internet]. 2021 [cited 2023 Feb 27];362:104301. Available from: https://www.sciencedirect.com/science/article/pii/S0008874921000204

5 Chapter 5: General discussion and conclusion

5.1 Discussion

CTLs are the primary immune cells that can eliminate tumor cells. CTLs recognize the processed tumor antigen-specific peptide bound to HLA class I on antigen-presenting or tumor cells [1]. As a result, CTLs are primed via multiple signaling cascades, then kill tumor cells through diverse effector functions such as granule-mediated cytotoxicity (perforin and granzymes), death ligand/receptor killing (FASL: FAS), and the secretion of several cytokines and chemokines [2]. These effector pathways induce apoptotic cell death in tumor cells by prompting programmed intracellular signaling events [2,3]. The significance of CTL function in controlling tumor growth and elimination is evident from numerous cancer studies [4]. For instance, the predictive value of intra-tumoral lymphocytes in tumors has been demonstrated [5]. In addition, the inception of the tumor mutations and downregulation of tumor antigens as an evasion mechanism from CTL recognition signifies the role of CTLs in tumor elimination [6]. Another piece of evidence confirming the crucial role of CTLs in anti-tumor defense is the improved cancer prognosis following immunotherapy [7].

However, despite CTL's crucial role in fighting against cancer, they gradually get exhausted. T cell exhaustion is defined as hyporesponsive T cells to chronic antigenic stimulation in chronic viral infections and cancers [8]. Exhausted CTLs are characterized by epigenetic, transcriptional, and metabolic profile alterations, and decreased effector functions accompanied by a sustained co-expression of multiple co-inhibitory receptors [9]. The mechanism(s) underlying the upregulation of co-

inhibitory receptors and, subsequently, the induction of CTL exhaustion is diverse. Several associating factors in the tumor microenvironment (TME), such as tumor antigen load and potency, soluble mediators (cytokines, chemokines, exosomes, and metabolomics), immunoregulatory cells, as well as cell-intrinsic changes in transcriptional profiles have been recognized [10]. In general, tumors adopt strategies to escape T cell immunity by the induction of dysfunctional T cells in both types of hematological and solid cancers [11].

Co-inhibitory receptors are the hallmark of T cell exhaustion. They are various types of molecules that have evolved to prevent the overactivation of immune cells or assist in regulating immune responses. However, during antigen persistence in chronic conditions such as cancers, and chronic viral infections, the expression of co-inhibitory receptors is elevated and sustained [12]. Coinhibitory receptors are expressed on the surface of T cells and, upon interaction with their cognate ligands on antigen-presenting cells, induce an inhibitory signal, resulting in effector CTL function inhibition. This interaction site is called an immune checkpoint [13]. While the transient expression of immune checkpoint pathways is crucial to control physiologic inflammatory responses in cancers, their persistent upregulation facilitates tumor escape from T cell anti-tumor response. Ever since, various types of co-inhibitory receptors and their ligands have been identified (PD-1, CTLA-4, TIM-3, 2B4, TIGIT, CD160, BTLA, GAL-9, and LAG-3) [14] and the list is growing. However, monoclonal antibodies can block or modulate the receptor-ligand interaction of immune checkpoints [15]. So far, monoclonal antibodies against Cytotoxic T Lymphocyte Associated Antigen 4 (CTLA-4) (Ipilimumab), PD-1 (Nivolumab, Pembrolizumab, Cemiplimab), and PDL-1(Avelumab, Durvalumab, Atezolizumab)

have received US FDA approval with promising durable clinical responses in some cancers [16,17]. While immune checkpoint blockade therapies show progress in cancer treatment, most patients still fail to respond [18]. Therefore, a better understanding of the biological properties of these coinhibitory receptors in different types of cancers merits further investigations.

Similarities and differences in T cell responses in hematologic malignancies and solid tumors have been reflected in their outcome to various T cell-based immunotherapy methods [11]. As hematologic malignancies arise from immune cells, this suggests a robust immune dysfunction that impacts anti-tumor defense. T cells are mainly defective in sensing and priming immune responses [19]. This inadequate priming might explain why immune checkpoint blockade therapy is less efficient than solid tumors [11]. Some studies in multiple myeloma have shown that T cells are more anergic or senescent rather than exhausted [20].

In contrast, immune checkpoint blockade therapy in solid tumors has been promising, while CAR T cell therapies are falling behind [21]. It seems that T cell trafficking and penetration of T cells into tumors are restricted in solid tumors due to physical barriers in the tumor microenvironment that dampens the adoptive T cell transfer therapy [22]. The early mechanism of immune escape in solid tumors is the loss of immunogenicity of cancer [23]. Tumors such as melanoma with high tumor mutation burden possess higher neoantigens and increased immunogenicity and respond better to immune checkpoint blockade therapy [24]. Loss of immunogenicity in solid tumors also dampens CAR T cell therapy as a specific tumor antigen is not available to target tumor cells with CAR T cells [25]. Therefore, collective evidence necessitates the investigation of T-cell responses in various types of cancers.

The role of CD160 on $CD8^+$ T cells is a matter of controversy. Both co-inhibitory and co-stimulatory parts of CD160 on $CD8^+$ T cells have been reported in various diseases. These disparities seem related to the interaction with its cognate ligands (MHC-I, HVEM) that send inhibitory or stimulatory signals, respectively [26,27]. The exhausted phenotype of $CD160^+$ $CD8^+$ T cells has been reported in HIV [28], HTLV-1 [29], and pancreatic carcinoma [30], whereas co-stimulatory roles have been written in Listeria monocytogenes [27], and allograft skin rejections[31]. Soluble CD160 acts as an immune evasion mediator in melanoma [32], and also reports have shown that soluble CD160 binds to the MHC-I complex and interdicts binding of $CD8^+$ T cells to MHC-I to blunt cytotoxicity [33]. Upregulation of several co-inhibitory receptors in CLL that induce exhausted T cell phenotype has been reported [34,35].

In the first study, we showed the expansion of $CD160^+CD8^+$ T cells in CLL patients. Despite of Lower frequency of $CD160^+CD8^+$ T cells than $2B4^+CD8^+$ and $TIGIT^+CD8^+$ T cells in CLL patients, $CD160^+$ $CD8^+$ T cells were prominently dysfunctional. Further characterization revealed that $CD160^+$ $CD8^+$ T cells are effector T cells with impaired cytokine secretion and degranulation ability. However, $CD160^+$ $CD8^+$ T cells maintained their proliferative capacity, contrasting with the latest report on virus infections [28]. CD160 is co-expressed with other coinhibitory molecules such as 2B4 and TIGIT, and CD160 expression dictated the inhibitory role of CD160 on 2B4 expressing $CD8^+$ T cells in CLL. Few tumor-specific antigens have been identified in CLL due to heterogeneous clones of malignant B cells [36]; we used immunophenotyping markers and confirmed that $CD160^+$ $CD8^+$ T cells are highly

antigen-experienced. Less antigen-experienced T cells have shown increased anti-tumor properties in adoptive T cell transfer studies [37].

We used several approaches to find proposed mechanisms to expand CD160 expressing $CD8^+$ T cells in CLL. Finally, we devised three proposed mechanisms that need further in-depth investigations. First, we observed increased surface CD160 expression following prolonged T cell activation (6 days), agreeing that chronic stimulation upregulates co-inhibitory receptors [38]. Second, for the first time, we detected EVs as a source of CD160 molecule in the plasma of CLL patients that can be taken up by T cells. This observation is consistent with a recent study on the role of EVs in inducing T cell dysfunction in CLL [39]. Further studies to define the effect of EVs on the effector function of T cells are warranted. Third, a substantial rise in IL-16 levels in the plasma of CLL patients compared to HCs was observed, consistent with another study [40]. A significant correlation was detected between plasma IL-16 levels and the cancer stage. Likewise, in multiple myeloma, a profound expression of IL-16 in the BM has been reported that increases the survival of malignant cells [41]. Moreover, a positive correlation between the plasma IL-16 concentrations and percentages of $CD160^+CD8^+$ T cells suggests a complex mechanism, which demands future investigations. In summary, our results demonstrated the abundance of CD160-expressing $CD8^+$ T cells in CLL patients. Our observations revealed $CD160^+CD8^+$ T cells are intrinsically dysfunctional in CLL.

It has been proven that tumor shapes the differentiation and development of T cells [42]. Although short-lived effector cells are essential in the early stages of CTL activation to eliminate tumor cells, memory T cells remain for a long time in

circulation or secondary lymphoid organs, and due to their longevity and polyfunctionality, they play an essential role in cancer [42]. However, chronic antigenic stimulation in cancer causes the accumulation of terminally differentiated effector T cells, while memory T cells are more desirable in the context of cancers [43]. Identifying and infusing selected polyfunctional $CD8^+$ T cells with enhanced anti-tumor properties into cancer patients is applied in adoptive T cell transfer therapy [44].

The second study found a deficiency of CD26-expressing $CD8^+$ T cells in CLL patients. So far, to our knowledge, the role of $CD26^+CD8^+$ T cells in cancer models has not been well-defined. However, polyfunctional $CD4^+CD26^{high}$ T cells exhibit markers of stemness/migration and elicit anti-tumor activity in different malignancies [45]. Moreover, the enzymatic activity of CD26 in cancer models has not been thoroughly investigated. For instance, one group reported that inhibiting CD26 was linked to enhanced anti-tumor immunity [46]. Conversely, another group revealed that CD26 inhibition led to tumor progression and metastasis [47]. As such, further investigation in cancer models to thoroughly investigate the immunological role of CD26 on T cells and beyond is necessary. Such studies will assist in understanding if anti-tumor immunity could be improved by genetic or therapeutic manipulation of CD26 expression.

To perform in-depth characterization of $CD26^+CD8^+$ T cells, we separated $CD26^+CD8^+$ T cells into $CD26^{low}$/$CD26^{high}$ and observed that the frequencies of both populations declined in CLL patients. $CD26^{neg}$ $CD8^+$ T cells were mainly terminal effectors, whereas $CD26^{high}CD8^+$ T cells were predominantly transitional and effector memory

cells, as reported elsewhere [48]. $CD26^{low}$ subset was densely populated with a mixture of naïve stem cells and central memory $CD8^+$ T cells. Moreover, $CD26^{high}CD8^+$ T cells demonstrated abundant CXCR3/CCR6/CCR4 expressing cells, consistent with a Tc1/Tc17 phenotype [49].

$CD26^{high}$ expression has been suggested as a surrogate marker for MAIT cells [50]. Characterization of $CD26^+CD8^+$ T cells based on the defined surrogate markers for MAIT cells ($CD161^{high}$ TVα7.2$^+$) [50] showed a heterogeneous population enriched with MAIT-like cells. However, MR-1 tetramers have been suggested to confirm the presence of MAIT cells within $CD26^{high}CD8^+$ T cells [50]. $CD26^+$ $CD8^+$ T cells demonstrated higher migratory propensity that may support their trafficking to lymph nodes and inflamed organs or the TME. Whether this mobile capacity interprets their paucity in blood circulation needs to be elucidated.

Furthermore, we found that $CD26^{high}$ $CD8^+$ T cells are polyfunctional, producing multiple cytokines, and have the plasticity to further increase the production of GzmB, perforin, and IFN-γ in response to cytokines or TCR stimulation. High GzmK contents and greater IL-2 expression capacity in $CD26^{high}CD8^+$ T cells are consistent with this notion [51,52]. Therefore, $CD26^{high}CD8^+$T cells exert polyfunctionality, stemness, and migration that can survive and eliminate cancer cells. These properties may suggest that these cells are a potential candidate for adoptive T cell transfer therapies and define a potential mechanism for the susceptibility to recurrent bacterial infections and tumor progression in CLL patients.

Moreover, we found high levels of Gal-9 in the plasma of CLL patients. Gal-9 strongly correlates with increased pro-inflammatory cytokines/chemokines [53]. The apoptotic impact of Gal-9 on $CD8^+$ T cells has already been reported [54]. In

agreement, we discovered that $CD26^{high}CD8^{+}$ T cells were highly susceptible to Gal-9-induced apoptosis following treatment with Gal-9 and IL-18+IL-12+IL-15 *in vitro*. Therefore, the inflammatory environment of CLL with the higher levels of IL-18, IL-12, IL-15, and Gal-9 released from CLL cells might explain the reduction of $CD26^{high}CD8^{+}$ T cells polyfunctional reservoir in CLL. Whether $CD26^{+}$ T cells depletion in CLL could be inhabited by targeting Gal-9 needs further investigation.

Despite employing a coinhibitory receptor/ligand mechanism, tumor cells use diverse strategies to suppress CTL functions and escape the immune responses [55]. Tumor cells generate and secret soluble mediators, including growth factors, cytokines, and chemokines, to shape the surrounding environment and immune cells in favor of their growth [56]. These soluble mediators are involved in angiogenesis, stromal cell changes, and recruiting immune suppressor cells in TME. Moreover, suppressor cells such as MDSCs, tumor-associated macrophages (TAMs), neutrophils, Tregs, and $CD71^{+}$ erythroid cells (CECs) play an important role in cancer progression [57–60]. It has become evident that tumor recruits and exploits myeloid cells to convert them into immunosuppressive cells as an anti-tumor immune response evasion mechanism [61,62]. The TME of solid tumors is a prolific environment for trafficking and manipulating MDSCs [63]. Given their immunosuppressive properties, there is growing evidence that these myeloid cells suppress T cell effector functions and hinder the efficacy of immune checkpoint inhibitors [64]. Identifying primary or acquired resistance to such therapies at the baseline or during treatment should be defined [65,66].

Since HPV-associated carcinomas modulate the expression of co-inhibitory receptors, it is becoming evident that they are potential candidates for immune checkpoint

inhibitor therapies [67,68]. The FDA also approved pembrolizumab (anti-PD-1) therapy for metastatic cervical cancer in 2018 [69]. However, a requirement to employ combined treatment options has emerged as some patients fail to respond to monotherapy. For instance, combinations of immune checkpoint inhibitors with histone deacetylases (HDACs) inhibitors have shown promising results by enhancing anti-tumor immunity [70].

In the third study, we evaluated the immune transcriptome and plasma cytokine levels of HPV-associated squamous cell carcinoma patients by analyzing their PBMCs in responders (Rs) versus non-responders (NRs) at the baseline and throughout the treatment. Basal plasma IL-8/IL-18 levels were higher in the NR group, along with activated IL-8/IL-18 signaling pathways in circulating immune cells. Following treatment, the NR group demonstrated further upregulation of IL-8 signaling at the endpoint escorted by the NET formation pathway activation and upraised plasma IL-18. In line with our observations, several reports support the role of IL-8 in both angiogenesis, tumor progression and poor immune response to anti-PD-L1 [71]. Likewise, IL-18 is associated with poor prognosis as an immunosuppressor cytokine in cancer [72]. Our data support the sensitivity and specificity of plasma IL-8 and IL-18 as predictive biomarkers in NRs. Although implications of IL-8 as a prognostic biomarker in cancers have been demonstrated [71], the importance of plasma IL-18 as a prognostic biomarker in cancer has been controversial [73] and merits further investigations. Deconvoluting transcriptome data with digital cytometry (CIBERSORTx) [74] revealed a higher myeloid-to-lymphoid ratio in NRs compared to Rs in peripheral blood at the endpoint, confirmed by flow cytometry. We found an

enriched myeloid transcriptome in NRs with prominent neutrophil-related transcripts. Notably, CSF1, IL1R1, MMP7, and PTGES2, which are related to suppressive myeloid functions, were higher in NRs [75]. These observations suggest that myeloid compartment enrichment following avelumab therapy might be a resistance strategy mechanism. In the agreement, MDSCs' role in mediating resistance and poor prognosis in gastric cancer has been reported [76]. Analyzing transcriptome after valproic acid (VA) treatment showed a substantial difference between NRs and Rs.
In summary, downregulated signaling pathways in HIF-1α, glycolysis, JAK/STAT, and STAT3 favor immune system deactivation. MDSCs upregulate glycolysis to support their expansion and function [77]. Therefore, Inhibited glycolytic pathways accompanied by enriched myeloid signature might suggest the attenuating effect of VA on MDSCs as reported elsewhere [78,79]. Also, we found evidence of neutrophil NET formation in NRs at the endpoint, which implies a mechanism of immune evasion as reported elsewhere [80, 81].In this scenario, IL-8 is essential in attracting myeloid cells/MDSCs, and enhancing NET formation in the tumor microenvironment [82]. Thus, our results support the value of bulk RNAseq of the peripheral blood as a non-invasive approach to predict response to immune checkpoint inhibitor therapy.

5.2 Limitations and recommendations

5.2.1 Study 1

Expanded antigen experienced CD160^{+}CD8^{+} effector T Cells exhibit impaired effector functions in chronic lymphocytic leukemia.

One of this study's limitations is the size and composition of the cohort. In our cohort, most patients were naïve and in the early stages of the disease. Due to the small size

of the cohort, stratification of patients to naïve and treated was not appropriate; if treated patients were receiving various types of therapies, that makes the comparison vague. We could not correlate all the findings with the prognostic markers, such as IgHV mutation and FISH cytogenetic results, as they were unavailable for some patients. Unfortunately, we could not obtain the whole blood cell count from HCs for comparisons. Therefore, there is a possibility that the dominancy of malignant B cells in CLL patients skews the T cell count reported in PBMCs of CLL patients.

Moreover, the differential proportion of T cell subsets in CLL versus HC, such as decreased naïve and increased effector populations in CLL, might influence the EOMES, TCF1, and T-bet expression as these markers are limited to specific subsets. It is recommended these markers should be measured and compared in each subgroup. Comparing immunological changes in CLL-BM versus HC-BM may aid in further understanding the role of CD160 in T cells. We did not have access to HC-BM for such comparisons. Furthermore, studying the CD160 expressing $CD8^+$ T cells in the lymph node of CLL patients is valuable as the lymph node serves as the tumor microenvironment in CLL [83]. Due to an invasive procedure and limited application of lymph node biopsy in some CLL patients, samples were unavailable for such considerations. In addition, we couldn't analyze the $CD160^+$ EVs for their impact on T cells in CLL as we did not have access to larger blood volumes. In addition, the IL-16 effect on T cell function in CLL merits further investigation.

5.2.2 Study 2

Depletion of polyfunctional $CD26^{high}CD8^{+}$ T cells repertoire in chronic lymphocytic leukemia.

A small/single-centered cohort was a limitation of this study. Conducting similar studies in larger cohorts from multiple centers is recommended. Moreover, extending these studies to other hematologic or solid cancers assists in understanding the role of $CD26^{+}CD8^{+}$ T cells in cancer. As reported, age factor might impact $CD26^{+}$ T cells frequency [84] however, this was not proved in our cohort. We know that circulating T cells might have different properties from lymph-node-derived T cells. As mentioned in the first study, obtaining lymph node samples was impossible. Performing more in-depth analyses, such as RNA sequencing or single cell sequencing on different subsets of $CD26^{+}$ T cells in CLL patients, will be very informative. We could not collect larger blood volumes from CLL patients to conduct these studies.

5.2.3 Study 3

Transcriptomic profiling of peripheral blood Cells in HPV-associated carcinoma patients receiving the combined valproic acid and avelumab therapy.

Once again, the small cohort size was the main limitation of this study. Samples were obtained from the clinical trial study; each sample represented a single time point and was not replaceable. We had to exclude some samples/time points due to the poor quality of RNA. Due to the small cohort size, we were unable to stratify partial/complete responders and progressive/stable non-responders into separate

groups. These cases make a point of testing in a larger cohort to compare immune responses in various subgroups. Moreover, longitudinal follow-up exceeding the endpoint is necessary to identify long-lasting responders who establish acquired resistance during immunotherapy. Limited blood samples prohibited conducting further functional assays to characterize cellular immune components in responding groups. Single-cell RNA sequencing might be better approach for finding differences at certain cell level. Also, access to tumor tissues for analyzing tumor infiltrative cells was impossible. Hence, comparing circulating with tumor-infiltrating immune cells might shed light on understanding the immune evasion mechanism in the tumor microenvironment.

5.3 Conclusions

In conclusion, despite the limitations of the studies, we highlighted three different mechanisms that might be responsible for inducing T-cell dysfunction in CLL and HPV-associated carcinoma. First, we found expanded dysfunctional $CD160^{+}$ $CD8^{+}$ T cells in CLL. In this regard, we found three proposed mechanisms for developing these cells, prolonged TCR stimulation, up-taking of CD160 containing EVs, and plasma IL-16 elevation. Second, we discovered the depletion of $CD26^{+}CD8^{+}$ T cells in CLL, affecting both $CD26^{low}$ and $CD26^{high}$ subsets. We proved that these cells have different properties. In particular, $CD26^{high}CD8^{+}$ T cells are highly polyfunctional in generating multiple cytokines, proliferation abilities, plasticity in response to stimulations, migratory capacities, and are enriched with MAIT cells. Besides, we found Gal-9 and cytokine cocktail (IL-18+IL-15+IL-12) selectively induced apoptosis of $CD26^{high}$ populations. These findings suggest that the inflammatory milieu of CLL is a

predisposing factor for the depletion of $CD26^{high}CD8^{+}$ T cells. Third, analyzing the transcriptome of HPV-associated carcinoma patients receiving combined Avelumab (anti-PD-L1) and valproic acid revealed that MDSCs were accumulated in non-responders. Their ability to NET formation might be a potential resistance mechanism to therapy. Also, elevated plasma IL-8/IL-18 in non-responders might be linked to the accumulation of MDSCs. In addition, the dampening effect of Valproic acid on circulating immune cells, which was mainly related to myeloid cells, proposes valproic acid as an excellent candidate to inhibit MDSCs activity. These mechanisms have clinical implications, so further investigations in larger cohorts are warranted. A summary of our findings is depicted in Figure 1.

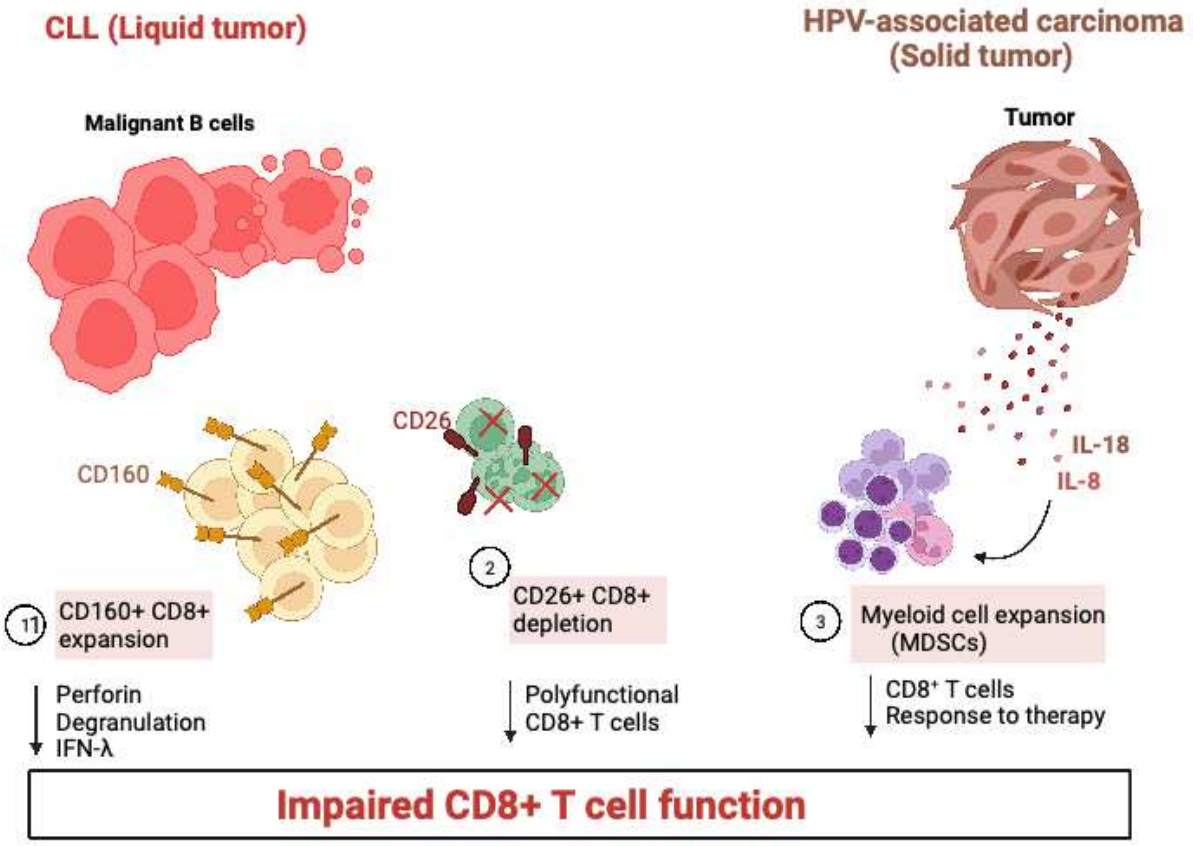


Fig. 1. The summary figure shows the different mechanisms leading to the impaired CD8^{+} T cell function in CLL and HPV-associated carcinoma

(Created in Biorenders).

5.4 References

1. Zamora AE, Crawford JC, Thomas PG. Hitting the target: how T cells detect and eliminate tumors. J Immunol Baltim Md 1950 [Internet]. 2018 [cited 2023 Mar 31];200:392–9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6116355/

2. Martínez-Lostao L, Anel A, Pardo J. How Do Cytotoxic Lymphocytes Kill Cancer Cells? Clin Cancer Res [Internet]. 2015 [cited 2023 Mar 31];21:5047–56. Available from: https://doi.org/10.1158/1078-0432.CCR-15-0685

3. Bolitho P, Voskoboinik I, Trapani JA, Smyth MJ. Apoptosis induced by the lymphocyte effector molecule perforin. Curr Opin Immunol. 2007;19:339–47.

4. Hashimoto M, Kamphorst AO, Im SJ, Kissick HT, Pillai RN, Ramalingam SS, et al. CD8 T Cell Exhaustion in Chronic Infection and Cancer: Opportunities for Interventions. Annu Rev Med [Internet]. 2018 [cited 2023 Mar 31];69:301–18. Available from: https://doi.org/10.1146/annurev-med-012017-043208

5. Clemente CG, Mihm MC, Bufalino R, Zurrida S, Collini P, Cascinelli N. Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. Cancer. 1996;77:1303–10.

6. Beatty GL, Gladney WL. Immune escape mechanisms as a guide for cancer immunotherapy. Clin Cancer Res Off J Am Assoc Cancer Res [Internet]. 2015 [cited 2023 Mar 31];21:687–92. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4334715/

7. Farhood B, Najafi M, Mortezaee K. CD8+ cytotoxic T lymphocytes in cancer immunotherapy: A review. J Cell Physiol. 2019;234:8509–21.

8. Blank CU, Haining WN, Held W, Hogan PG, Kallies A, Lugli E, et al. Defining "T cell exhaustion." Nat Rev Immunol. 2019;19:665–74.

9. Wherry EJ. T cell exhaustion. Nat Immunol. 2011;12:492–9.

10. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. Nat Rev Immunol. 2015;15:486–99.

11. Montironi C, Muñoz-Pinedo C, Eldering E. Hematopoietic versus Solid Cancers and T Cell Dysfunction: Looking for Similarities and Distinctions. Cancers [Internet]. 2021 [cited 2023 Mar 31];13:284. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7828769/

12. Okoye IS, Houghton M, Tyrrell L, Barakat K, Elahi S. Coinhibitory Receptor Expression and Immune Checkpoint Blockade: Maintaining a Balance in CD8+ T Cell Responses to Chronic Viral Infections and Cancer. Front Immunol. 2017;8:1215.

13. McLane LM, Abdel-Hakeem MS, Wherry EJ. CD8 T Cell Exhaustion During Chronic Viral Infection and Cancer. Annu Rev Immunol [Internet]. 2019 [cited 2021 Jun 1];37:457–95. Available from: https://doi.org/10.1146/annurev-immunol-041015-055318

14. Andrews LP, Yano H, Vignali DAA. Inhibitory receptors and ligands beyond PD-1, PD-L1 and CTLA-4: breakthroughs or backups. Nat Immunol [Internet]. 2019 [cited 2021 Oct 27];20:1425–34. Available from: https://www.nature.com/articles/s41590-019-0512-0

15. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer [Internet]. Nature Publishing Group; 2012 [cited 2021 May 17];12:252–64. Available from: https://www.nature.com/articles/nrc3239

16. Wilson RAM, Evans TRJ, Fraser AR, Nibbs RJB. Immune checkpoint inhibitors: new strategies to checkmate cancer. Clin Exp Immunol [Internet]. 2018 [cited 2021 Jun 1];191:133–48. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5758374/

17. Vaddepally RK, Kharel P, Pandey R, Garje R, Chandra AB. Review of Indications of FDA-Approved Immune Checkpoint Inhibitors per NCCN Guidelines with the Level of Evidence. Cancers. 2020;12.

18. Koustas E, Sarantis P, Papavassiliou AG, Karamouzis MV. The Resistance Mechanisms of Checkpoint Inhibitors in Solid Tumors. Biomolecules [Internet]. 2020 [cited 2023 Apr 6];10:666. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7277892/

19. Curran EK, Godfrey J, Kline J. Mechanisms of Immune Tolerance in Leukemia and Lymphoma. Trends Immunol. 2017;38:513–25.

20. Suen H, Brown R, Yang S, Weatherburn C, Ho PJ, Woodland N, et al. Multiple myeloma causes clonal T-cell immunosenescence: identification of potential novel targets for promoting tumour immunity and implications for checkpoint blockade. Leukemia [Internet]. Nature Publishing Group; 2016 [cited 2023 Mar 31];30:1716–24. Available from: https://www.nature.com/articles/leu201684

21. Jafferji MS, Yang JC. Adoptive T-Cell Therapy for Solid Malignancies. Surg Oncol Clin N Am. 2019;28:465–79.

22. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell. 2012;21:309–22.

23. Balachandran VP, Łuksza M, Zhao JN, Makarov V, Moral JA, Remark R, et al. Identification of unique neoantigen qualities in long-term survivors of pancreatic cancer. Nature. 2017;551:512–6.

24. O'Donnell JS, Teng MWL, Smyth MJ. Cancer immunoediting and resistance to T cell-based immunotherapy. Nat Rev Clin Oncol. 2019;16:151–67.

25. Martinez M, Moon EK. CAR T Cells for Solid Tumors: New Strategies for Finding, Infiltrating, and Surviving in the Tumor Microenvironment. Front Immunol. 2019;10:128.

26. Cai G, Anumanthan A, Brown JA, Greenfield EA, Zhu B, Freeman GJ. CD160 inhibits activation of human CD4+ T cells through interaction with herpesvirus entry mediator. Nat Immunol. 2008;9:176–85.

27. Tan CL, Peluso MJ, Drijvers JM, Mera CM, Grande SM, Brown KE, et al. CD160 Stimulates CD8+ T Cell Responses and Is Required for Optimal Protective Immunity to Listeria monocytogenes. ImmunoHorizons. 2018;2:238–50.

28. Peretz Y, He Z, Shi Y, Yassine-Diab B, Goulet J-P, Bordi R, et al. CD160 and PD-1 co-expression on HIV-specific CD8 T cells defines a subset with advanced dysfunction. PLoS Pathog. 2012;8:e1002840.

29. Chibueze CE, Yoshimitsu M, Arima N. CD160 expression defines a uniquely exhausted subset of T lymphocytes in HTLV-1 infection. Biochem Biophys Res Commun. 2014;453:379–84.

30. Liu S, Zhang W, Liu K, Wang Y. CD160 expression on CD8+ T cells is associated with active effector responses but limited activation potential in pancreatic cancer. Cancer Immunol Immunother CII. 2020;69:789–97.

31. Del Rio M-L, Nguyen TH, Tesson L, Heslan J-M, Gutierrez-Adan A, Fernandez-Gonzalez R, et al. The impact of CD160 deficiency on alloreactive CD8 T cell responses and allograft rejection. Transl Res J Lab Clin Med. 2022;239:103–23.

32. Gauci M-L, Giustiniani J, Lepelletier C, Garbar C, Thonnart N, Dumaz N, et al. The soluble form of CD160 acts as a tumor mediator of immune escape in melanoma. Cancer Immunol Immunother CII. 2022;71:2731–42.

33. Giustiniani J, Marie-Cardine A, Bensussan A. A soluble form of the MHC class I-specific CD160 receptor is released from human activated NK lymphocytes and inhibits cell-mediated cytotoxicity. J Immunol Baltim Md 1950. 2007;178:1293–300.

34. Ramsay AG, Clear AJ, Fatah R, Gribben JG. Multiple inhibitory ligands induce impaired T-cell immunologic synapse function in chronic lymphocytic leukemia that can be blocked with lenalidomide: establishing a reversible immune evasion mechanism in human cancer. Blood. 2012;120:1412–21.

35. Riches JC, Davies JK, McClanahan F, Fatah R, Iqbal S, Agrawal S, et al. T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. Blood. 2013;121:1612–21.

36. Krackhardt AM, Witzens M, Harig S, Hodi FS, Zauls AJ, Chessia M, et al. Identification of tumor-associated antigens in chronic lymphocytic leukemia by SEREX. Blood [Internet]. American Society of Hematology; 2002 [cited 2020 Oct 14];100:2123–31. Available from: https://ashpublications.org/blood/article/100/6/2123/16598/Identification-of-tumor-associated-antigens-in

37. Ca K, L G, Np R. Sorting through subsets: which T-cell populations mediate highly effective adoptive immunotherapy? J Immunother Hagerstown Md 1997 [Internet]. J Immunother; 2012 [cited 2020 Oct 14];35:651–60. Available from: https://pubmed.ncbi.nlm.nih.gov/23090074/

38. A L, De S, H P, D Z, Sa FM. Inhibitory Receptor Expression Depends More Dominantly on Differentiation and Activation than "Exhaustion" of Human CD8 T Cells [Internet]. Front. Immunol. Front Immunol; 2013 [cited 2020 Oct 14]. Available from: https://pubmed.ncbi.nlm.nih.gov/24391639/

39. Böttcher M, Böttcher-Loschinski R, Kahlfuss S, Aigner M, Gießl A, Mackensen A, et al. CLL-Derived Extracellular Vesicles Impair T-Cell Activation and Foster T-Cell Exhaustion via Multiple Immunological Checkpoints. Cells. 2022;11:2176.

40. Bellomo G, Allegra A, Alonci A, Quartarone E, Pizzoleo MA, Cincotta M, et al. Serum levels of interleukin-16 in lymphoid malignancies. Leuk Lymphoma. 2007;48:1225–7.

41. Atanackovic D, Hildebrandt Y, Templin J, Cao Y, Keller C, Panse J, et al. Role of interleukin 16 in multiple myeloma. J Natl Cancer Inst. 2012;104:1005–20.

42. Barnaba V. T Cell Memory in Infection, Cancer, and Autoimmunity. Front Immunol [Internet]. 2022 [cited 2023 Apr 2];12. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2021.811968

43. Takahashi H, Sakakura K, Ida S, Kawabata-Iwakawa R, Matsuyama T, Tada H, et al. Circulating naïve and effector memory T cells correlate with prognosis in head and neck squamous cell carcinoma. Cancer Sci [Internet]. 2022 [cited 2023 Apr 2];113:53–64. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8748237/

44. Busch DH, Fräßle SP, Sommermeyer D, Buchholz VR, Riddell SR. Role of memory T cell subsets for adoptive immunotherapy. Semin Immunol [Internet]. 2016 [cited 2023 Apr 2];28:28–34. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5027130/

45. Bailey SR, Nelson MH, Majchrzak K, Bowers JS, Wyatt MM, Smith AS, et al. Human CD26high T cells elicit tumor immunity against multiple malignancies via enhanced

migration and persistence. Nat Commun [Internet]. Nature Publishing Group; 2017 [cited 2022 Jun 15];8:1961. Available from: https://www.nature.com/articles/s41467-017-01867-9

46. Barreira da Silva R, Laird ME, Yatim N, Fiette L, Ingersoll MA, Albert ML. Dipeptidylpeptidase 4 inhibition enhances lymphocyte trafficking, improving both naturally occurring tumor immunity and immunotherapy. Nat Immunol. 2015;16:850–8.

47. Wang H, Liu X, Long M, Huang Y, Zhang L, Zhang R, et al. NRF2 activation by antioxidant antidiabetic agents accelerates tumor metastasis. Sci Transl Med. 2016;8:334ra51.

48. Hatano R, Ohnuma K, Yamamoto J, Dang NH, Morimoto C. CD26-mediated co-stimulation in human CD8(+) T cells provokes effector function via pro-inflammatory cytokine production. Immunology. 2013;138:165–72.

49. Mousset CM, Hobo W, Woestenenk R, Preijers F, Dolstra H, van der Waart AB. Comprehensive Phenotyping of T Cells Using Flow Cytometry. Cytom Part J Int Soc Anal Cytol. 2019;95:647–54.

50. Gherardin NA, Souter MN, Koay H, Mangas KM, Seemann T, Stinear TP, et al. Human blood MAIT cell subsets defined using MR1 tetramers. Immunol Cell Biol [Internet]. 2018 [cited 2023 Apr 2];96:507–25. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6446826/

51. Kahan SM, Bakshi RK, Ingram JT, Hendrickson RC, Lefkowitz EJ, Crossman DK, et al. Intrinsic IL-2 production by effector CD8 T cells affects IL-2 signaling and promotes fate decisions, stemness, and protection. Sci Immunol [Internet]. American Association for the Advancement of Science; 2022 [cited 2022 Jun 14];7:eabl6322. Available from: https://www.science.org/doi/10.1126/sciimmunol.abl6322

52. Bouwman AC, van Daalen KR, Crnko S, ten Broeke T, Bovenschen N. Intracellular and Extracellular Roles of Granzyme K. Front Immunol [Internet]. 2021 [cited 2022 Jun 14];12. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2021.677707

53. Bozorgmehr N, Mashhouri S, Perez Rosero E, Xu L, Shahbaz S, Sligl W, et al. Galectin-9, a Player in Cytokine Release Syndrome and a Surrogate Diagnostic Biomarker in SARS-CoV-2 Infection. mBio. 2021;12:e00384-21.

54. Lhuillier C, Barjon C, Niki T, Gelin A, Praz F, Morales O, et al. Impact of Exogenous Galectin-9 on Human T Cells. J Biol Chem [Internet]. 2015 [cited 2023 Mar 23];290:16797–811. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4505427/

55. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol [Internet]. 2012 [cited 2023 Feb

2];12:253–68. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3587148/

56. Anderson NM, Simon MC. The tumor microenvironment. Curr Biol [Internet]. 2020 [cited 2023 Feb 9];30:R921–5. Available from: https://www.sciencedirect.com/science/article/pii/S0960982220309337

57. Zhao L, He R, Long H, Guo B, Jia Q, Qin D, et al. Late-stage tumors induce anemia and immunosuppressive extramedullary erythroid progenitor cells. Nat Med [Internet]. 2018 [cited 2023 Mar 11];24:1536–44. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6211844/

58. Pathria P, Louis TL, Varner JA. Targeting Tumor-Associated Macrophages in Cancer. Trends Immunol. 2019;40:310–27.

59. Wu W-C, Sun H-W, Chen H-T, Liang J, Yu X-J, Wu C, et al. Circulating hematopoietic stem and progenitor cells are myeloid-biased in cancer patients. Proc Natl Acad Sci U S A. 2014;111:4221–6.

60. Elahi S. Neglected Cells: Immunomodulatory Roles of CD71+ Erythroid Cells. Trends Immunol. 2019;40:181–5.

61. Groth C, Hu X, Weber R, Fleming V, Altevogt P, Utikal J, et al. Immunosuppression mediated by myeloid-derived suppressor cells (MDSCs) during tumour progression. Br J Cancer [Internet]. Nature Publishing Group; 2019 [cited 2023 Feb 2];120:16–25. Available from: https://www.nature.com/articles/s41416-018-0333-1

62. Bronte V, Serafini P, Apolloni E, Zanovello P. Tumor-induced immune dysfunctions caused by myeloid suppressor cells. J Immunother Hagerstown Md 1997. 2001;24:431–46.

63. Bronte V, Brandau S, Chen S-H, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. Nat Commun [Internet]. 2016 [cited 2023 Feb 13];7:12150. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4935811/

64. Hou A, Hou K, Huang Q, Lei Y, Chen W. Targeting Myeloid-Derived Suppressor Cell, a Promising Strategy to Overcome Resistance to Immune Checkpoint Inhibitors. Front Immunol. 2020;11:783.

65. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. Nat Rev Immunol [Internet]. Nature Publishing Group; 2020 [cited 2023 Feb 9];20:651–68. Available from: https://www.nature.com/articles/s41577-020-0306-5

66. Ribas A, Wolchok JD. Cancer Immunotherapy Using Checkpoint Blockade. Science [Internet]. 2018 [cited 2023 Feb 9];359:1350–5. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7391259/

67. Kuss-Duerkop SK, Westrich JA, Pyeon D. DNA Tumor Virus Regulation of Host DNA Methylation and Its Implications for Immune Evasion and Oncogenesis. Viruses. 2018;10:82.

68. Liu C, Lu J, Tian H, Du W, Zhao L, Feng J, et al. Increased expression of PD-L1 by the human papillomavirus 16 E7 oncoprotein inhibits anticancer immunity. Mol Med Rep. 2017;15:1063–70.

69. Qi L, Li N, Lin A, Wang X, Cong J. Efficacy and safety of pembrolizumab on cervical cancer: A systematic review and single-arm meta-analysis. Front Oncol [Internet]. 2022 [cited 2023 Feb 9];12:910486. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9399507/

70. Li X, Su X, Liu R, Pan Y, Fang J, Cao L, et al. HDAC inhibition potentiates anti-tumor activity of macrophages and enhances anti-PD-L1-mediated tumor suppression. Oncogene [Internet]. Nature Publishing Group; 2021 [cited 2023 Feb 14];40:1836–50. Available from: https://www.nature.com/articles/s41388-020-01636-x

71. Yuen KC, Liu L-F, Gupta V, Madireddi S, Keerthivasan S, Li C, et al. High systemic and tumor-associated IL-8 correlates with reduced clinical benefit of PD-L1 blockade. Nat Med. 2020;26:693–8.

72. Nakamura K, Kassem S, Cleynen A, Chrétien M-L, Guillerey C, Putz EM, et al. Dysregulated IL-18 Is a Key Driver of Immunosuppression and a Possible Therapeutic Target in the Multiple Myeloma Microenvironment. Cancer Cell. 2018;33:634-648.e5.

73. Yao Z, Zhao M, Gao G, Yang J, Wang Z, Liu Y. Prognostic Role of IL-18 in Various Human Cancers and Radiation Injuries: A Meta-Analysis. Dose-Response [Internet]. 2020 [cited 2023 Feb 22];18:1559325820931360. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7323287/

74. Newman AM, Steen CB, Liu CL, Gentles AJ, Chaudhuri AA, Scherer F, et al. Determining cell-type abundance and expression from bulk tissues with digital cytometry. Nat Biotechnol [Internet]. 2019 [cited 2023 Jan 23];37:773–82. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6610714/

75. Veglia F, Sanseviero E, Gabrilovich DI. Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity. Nat Rev Immunol [Internet]. Nature Publishing Group; 2021 [cited 2023 Feb 24];21:485–98. Available from: https://www.nature.com/articles/s41577-020-00490-y

76. Feng F, Zheng G, Wang Q, Liu S, Liu Z, Xu G, et al. Low lymphocyte count and high monocyte count predicts poor prognosis of gastric cancer. BMC Gastroenterol [Internet]. 2018 [cited 2023 Feb 26];18:148. Available from: https://doi.org/10.1186/s12876-018-0877-9

77. Li J, Bolyard C, Xin G, Li Z. Targeting Metabolic Pathways of Myeloid Cells Improves Cancer Immunotherapy. Front Cell Dev Biol [Internet]. 2021 [cited 2023

Feb 27];9. Available from: https://www.frontiersin.org/articles/10.3389/fcell.2021.747863

78. Soria-Castro R, Schcolnik-Cabrera A, Rodríguez-López G, Campillo-Navarro M, Puebla-Osorio N, Estrada-Parra S, et al. Exploring the Drug Repurposing Versatility of Valproic Acid as a Multifunctional Regulator of Innate and Adaptive Immune Cells. J Immunol Res. 2019;2019:9678098.

79. Adeshakin AO, Yan D, Zhang M, Wang L, Adeshakin FO, Liu W, et al. Blockade of myeloid-derived suppressor cell function by valproic acid enhanced anti-PD-L1 tumor immunotherapy. Biochem Biophys Res Commun [Internet]. 2020 [cited 2023 Feb 14];522:604–11. Available from: https://www.sciencedirect.com/science/article/pii/S0006291X19322831

80. Yang L, Liu Q, Zhang X, Liu X, Zhou B, Chen J, et al. DNA of neutrophil extracellular traps promotes cancer metastasis via CCDC25. Nature [Internet]. Nature Publishing Group; 2020 [cited 2023 Feb 26];583:133–8. Available from: https://www.nature.com/articles/s41586-020-2394-6

81. He X-Y, Ng D, Egeblad M. Caught in a Web: Emerging Roles of Neutrophil Extracellular Traps in Cancer. Annu Rev Cancer Biol [Internet]. 2022 [cited 2023 Feb 27];6:223–43. Available from: https://doi.org/10.1146/annurev-cancerbio-080421-015537

82. Alfaro C, Teijeira A, Oñate C, Pérez G, Sanmamed MF, Andueza MP, et al. Tumor-Produced Interleukin-8 Attracts Human Myeloid-Derived Suppressor Cells and Elicits Extrusion of Neutrophil Extracellular Traps (NETs). Clin Cancer Res [Internet]. 2016 [cited 2023 Feb 24];22:3924–36. Available from: https://doi.org/10.1158/1078-0432.CCR-15-2463

83. Svanberg R, Janum S, Patten PEM, Ramsay AG, Niemann CU. Targeting the tumor microenvironment in chronic lymphocytic leukemia. Haematologica. 2021;106:2312–24.

84. Detel D, Baticic L, Varljen J. The influence of age on intestinal dipeptidyl peptidase IV (DPP IV/CD26), disaccharidases, and alkaline phosphatase enzyme activity in C57BL/6 mice. Exp Aging Res. 2008;34:49–62.

Bibliography

Chapter 1

1. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. Nat Rev Immunol [Internet]. 2020 [cited 2021 Oct 26];20:651–68. Available from: https://www.nature.com/articles/s41577-020-0306-5

2. Bai R, Lv Z, Xu D, Cui J. Predictive biomarkers for cancer immunotherapy with immune checkpoint inhibitors. Biomark Res [Internet]. 2020 [cited 2021 Oct 27];8:34. Available from: https://doi.org/10.1186/s40364-020-00209-0

3. Chronic Lymphocytic Leukemia - Cancer Stat Facts [Internet]. SEER. [cited 2023 Mar 9]. Available from: https://seer.cancer.gov/statfacts/html/clyl.html

4. Yao Y, Lin X, Li F, Jin J, Wang H. The global burden and attributable risk factors of chronic lymphocytic leukemia in 204 countries and territories from 1990 to 2019: analysis based on the global burden of disease study 2019. Biomed Eng OnLine [Internet]. 2022 [cited 2023 Mar 11];21:4. Available from: https://doi.org/10.1186/s12938-021-00973-6

5. Key Statistics for Chronic Lymphocytic Leukemia [Internet]. [cited 2023 Mar 9]. Available from: https://www.cancer.org/cancer/chronic-lymphocytic-leukemia/about/key-statistics.html

6. Chiorazzi N, Rai KR, Ferrarini M. Chronic lymphocytic leukemia. N Engl J Med. 2005;352:804–15.

7. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood [Internet]. 2016 [cited 2021 Apr 13];127:2375–90. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4874220/

8. Seifert M, Sellmann L, Bloehdorn J, Wein F, Stilgenbauer S, Dürig J, et al. Cellular origin and pathophysiology of chronic lymphocytic leukemia. J Exp Med [Internet]. 2012 [cited 2021 Feb 18];209:2183–98. Available from: https://doi.org/10.1084/jem.20120833

9. Kipps TJ, Stevenson FK, Wu CJ, Croce CM, Packham G, Wierda WG, et al. Chronic lymphocytic leukaemia. Nat Rev Dis Primer [Internet]. 2017 [cited 2023 Mar 10];3:16096. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5336551/

10. Cerhan JR, Slager SL. Familial predisposition and genetic risk factors for lymphoma. Blood. 2015;126:2265–73.

11. Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, et al. Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med. 2000;343:78–85.

12. Baumann Kreuziger LM, Tarchand G, Morrison VA. The impact of Agent Orange exposure on presentation and prognosis of patients with chronic lymphocytic leukemia. Leuk Lymphoma. 2014;55:63–6.

13. Schinasi LH, De Roos AJ, Ray RM, Edlefsen KL, Parks CG, Howard BV, et al. Insecticide exposure and farm history in relation to risk of lymphomas and leukemias in the Women's Health Initiative observational study cohort. Ann Epidemiol. 2015;25:803–10.

14. Kikushige Y, Ishikawa F, Miyamoto T, Shima T, Urata S, Yoshimoto G, et al. Self-renewing hematopoietic stem cell is the primary target in pathogenesis of human chronic lymphocytic leukemia. Cancer Cell. 2011;20:246–59.

15. Landau DA, Tausch E, Taylor-Weiner AN, Stewart C, Reiter JG, Bahlo J, et al. Mutations driving CLL and their evolution in progression and relapse. Nature [Internet]. 2015 [cited 2023 Mar 10];526:525–30. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4815041/

16. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature [Internet]. Nature Publishing Group; 2009 [cited 2023 Mar 10];461:1071–8. Available from: https://www.nature.com/articles/nature08467

17. Landau DA, Clement K, Ziller MJ, Boyle P, Fan J, Gu H, et al. Locally Disordered Methylation Forms the Basis of Intratumor Methylome Variation in Chronic Lymphocytic Leukemia. Cancer Cell [Internet]. 2014 [cited 2023 Mar 10];26:813–25. Available from: https://www.sciencedirect.com/science/article/pii/S1535610814004164

18. Burger JA, Ghia P, Rosenwald A, Caligaris-Cappio F. The microenvironment in mature B-cell malignancies: a target for new treatment strategies. Blood [Internet]. 2009 [cited 2023 Mar 10];114:3367–75. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4969052/

19. Morrison VA. Infectious complications in patients with chronic lymphocytic leukemia: pathogenesis, spectrum of infection, and approaches to prophylaxis. Clin Lymphoma Myeloma. 2009;9:365–70.

20. Dearden C. Disease-specific complications of chronic lymphocytic leukemia. Hematol Am Soc Hematol Educ Program. 2008;450–6.

21. Hallek M, Al-Sawaf O. Chronic lymphocytic leukemia: 2022 update on diagnostic and therapeutic procedures. Am J Hematol [Internet]. 2021 [cited 2023 Mar 11];96:1679–705. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/ajh.26367

22. Brugiatelli M, Bandini G, Barosi G, Lauria F, Liso V, Marchetti M, et al. Management of chronic lymphocytic leukemia: practice guidelines from the Italian Society of Hematology, the Italian Society of Experimental Hematology and the Italian Group for Bone Marrow Transplantation. Haematologica. 2006;91:1662–73.

23. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Döhner H, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood. 2008;111:5446–56.

24. Zenz T, Mertens D, Küppers R, Döhner H, Stilgenbauer S. From pathogenesis to treatment of chronic lymphocytic leukaemia. Nat Rev Cancer. 2010;10:37–50.

25. Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS. Clinical staging of chronic lymphocytic leukemia. Blood. 1975;46:219–34.

26. Binet JL, Auquier A, Dighiero G, Chastang C, Piguet H, Goasguen J, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer. 1981;48:198–206.

27. Moreno C, Montserrat E. New prognostic markers in chronic lymphocytic leukemia. Blood Rev. 2008;22:211–9.

28. Zenz T, Eichhorst B, Busch R, Denzel T, Häbe S, Winkler D, et al. TP53 mutation and survival in chronic lymphocytic leukemia. J Clin Oncol Off J Am Soc Clin Oncol. 2010;28:4473–9.

29. Foà R, Del Giudice I, Cuneo A, Del Poeta G, Ciolli S, Di Raimondo F, et al. Chlorambucil plus rituximab with or without maintenance rituximab as first-line treatment for elderly chronic lymphocytic leukemia patients. Am J Hematol. 2014;89:480–6.

30. Freeman CL, Gribben JG. Immunotherapy in Chronic Lymphocytic Leukaemia (CLL). Curr Hematol Malig Rep [Internet]. 2016 [cited 2023 Mar 11];11:29–36. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4796351/

31. Fischer K, Bahlo J, Fink AM, Goede V, Herling CD, Cramer P, et al. Long-term remissions after FCR chemoimmunotherapy in previously untreated patients with CLL: updated results of the CLL8 trial. Blood. 2016;127:208–15.

32. Sher T, Miller KC, Lawrence D, Whitworth A, Hernandez-Ilizaliturri F, Czuczman MS, et al. Efficacy of lenalidomide in patients with chronic lymphocytic leukemia with high-risk cytogenetics. Leuk Lymphoma. 2010;51:85–8.

33. Ramsay AG, Clear AJ, Fatah R, Gribben JG. Multiple inhibitory ligands induce impaired T-cell immunologic synapse function in chronic lymphocytic leukemia that can be blocked with lenalidomide: establishing a reversible immune evasion mechanism in human cancer. Blood. 2012;120:1412–21.

34. Greaves P, Gribben JG. The role of B7 family molecules in hematologic malignancy. Blood. 2013;121:734–44.

35. Riches JC, Davies JK, McClanahan F, Fatah R, Iqbal S, Agrawal S, et al. T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. Blood. 2013;121:1612–21.

36. Rogers KA, Huang Y, Dotson E, Lundberg J, Andritsos LA, Awan FT, et al. Use of PD-1 (PDCD1) inhibitors for the treatment of Richter syndrome: experience at a single academic centre. Br J Haematol. 2019;185:363–6.

37. Sagiv-Barfi I, Kohrt HEK, Czerwinski DK, Ng PP, Chang BY, Levy R. Therapeutic antitumor immunity by checkpoint blockade is enhanced by ibrutinib, an inhibitor of both BTK and ITK. Proc Natl Acad Sci U S A. 2015;112:E966-972.

38. Ding W, LaPlant BR, Call TG, Parikh SA, Leis JF, He R, et al. Pembrolizumab in patients with CLL and Richter transformation or with relapsed CLL. Blood. 2017;129:3419–27.

39. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med. 2011;365:725–33.

40. Turtle CJ, Hay KA, Hanafi L-A, Li D, Cherian S, Chen X, et al. Durable Molecular Remissions in Chronic Lymphocytic Leukemia Treated With CD19-Specific Chimeric Antigen Receptor-Modified T Cells After Failure of Ibrutinib. J Clin Oncol Off J Am Soc Clin Oncol. 2017;35:3010–20.

41. Frey NV, Gill S, Hexner EO, Schuster S, Nasta S, Loren A, et al. Long-Term Outcomes From a Randomized Dose Optimization Study of Chimeric Antigen Receptor Modified T Cells in Relapsed Chronic Lymphocytic Leukemia. J Clin Oncol Off J Am Soc Clin Oncol. 2020;38:2862–71.

42. Siddiqi T. Updated Follow-up of Patients with Relapsed/Refractory Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma Treated with Lisocabtagene Maraleucel in the Phase 1 Monotherapy Cohort of Transcend CLL 004, Including High-Risk and Ibrutinib-Treated Patients. ASH; 2020 [cited 2023 Mar 11]. Available from: https://ash.confex.com/ash/2020/webprogram/Paper140491.html

43. Gribben JG. Stem cell transplantation in chronic lymphocytic leukemia. Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant. 2009;15:53–8.

44. Dreger P, Schetelig J, Andersen N, Corradini P, van Gelder M, Gribben J, et al. Managing high-risk CLL during transition to a new treatment era: stem cell transplantation or novel agents? Blood. 2014;124:3841–9.

45. Petlickovski A, Laurenti L, Li X, Marietti S, Chiusolo P, Sica S, et al. Sustained signaling through the B-cell receptor induces Mcl-1 and promotes survival of chronic lymphocytic leukemia B cells. Blood [Internet]. 2005 [cited 2023 Mar 11];105:4820–

7. Available from: https://www.sciencedirect.com/science/article/pii/S0006497120534198

46. Wiestner A. Emerging role of kinase-targeted strategies in chronic lymphocytic leukemia. Hematol Am Soc Hematol Educ Program. 2012;2012:88–96.

47. Roberts AW, Davids MS, Pagel JM, Kahl BS, Puvvada SD, Gerecitano JF, et al. Targeting BCL2 with Venetoclax in Relapsed Chronic Lymphocytic Leukemia. N Engl J Med [Internet]. Massachusetts Medical Society; 2016 [cited 2023 Mar 11];374:311–22. Available from: https://doi.org/10.1056/NEJMoa1513257

48. Sthoeger ZM, Sthoeger D, Shtalrid M, Sigler E, Geltner D, Berrebi A. Mechanism of autoimmune hemolytic anemia in chronic lymphocytic leukemia. Am J Hematol. 1993;43:259–64.

49. Cheson BD, Heitner Enschede S, Cerri E, Desai M, Potluri J, Lamanna N, et al. Tumor Lysis Syndrome in Chronic Lymphocytic Leukemia with Novel Targeted Agents. The Oncologist [Internet]. 2017 [cited 2023 Apr 3];22:1283–91. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5679833/

50. Beiggi S, Johnston JB, Seftel MD, Pitz MW, Kumar R, Banerji V, et al. Increased risk of second malignancies in chronic lymphocytic leukaemia patients as compared with follicular lymphoma patients: a Canadian population-based study. Br J Cancer [Internet]. 2013 [cited 2023 Apr 3];109:1287–90. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3778273/

51. Eversman A, Cao S, Boughan KM, Gallogly MM, Malek E, Metheny L III, et al. Risk of Secondary Malignancy in CLL Patients Treated with Novel Targeted Agents. Blood [Internet]. 2020 [cited 2023 Apr 3];136:46–7. Available from: https://doi.org/10.1182/blood-2020-140042

52. Solomon BM, Rabe KG, Slager SL, Brewer JD, Cerhan JR, Shanafelt TD. Overall and cancer-specific survival of patients with breast, colon, kidney, and lung cancers with and without chronic lymphocytic leukemia: a SEER population-based study. J Clin Oncol Off J Am Soc Clin Oncol. 2013;31:930–7.

53. Teh BW, Tam CS, Handunnetti S, Worth LJ, Slavin MA. Infections in patients with chronic lymphocytic leukaemia: Mitigating risk in the era of targeted therapies. Blood Rev. 2018;32:499–507.

54. Caligaris-Cappio F. Role of the microenvironment in chronic lymphocytic leukaemia. Br J Haematol [Internet]. 2003 [cited 2023 Mar 10];123:380–8. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1365-2141.2003.04679.x

55. Ghia P, Strola G, Granziero L, Geuna M, Guida G, Sallusto F, et al. Chronic lymphocytic leukemia B cells are endowed with the capacity to attract CD4+, CD40L+ T cells by producing CCL22. Eur J Immunol [Internet]. 2002 [cited 2023 Mar 10];32:1403–13. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/1521-

4141%28200205%2932%3A5%3C1403%3A%3AAID-IMMU1403%3E3.0.CO%3B2-Y

56. Bürkle A, Niedermeier M, Schmitt-Gräff A, Wierda WG, Keating MJ, Burger JA. Overexpression of the CXCR5 chemokine receptor, and its ligand, CXCL13 in B-cell chronic lymphocytic leukemia. Blood [Internet]. 2007 [cited 2023 Mar 10];110:3316–25. Available from: https://doi.org/10.1182/blood-2007-05-089409

57. Burger JA, Quiroga MP, Hartmann E, Bürkle A, Wierda WG, Keating MJ, et al. High-level expression of the T-cell chemokines CCL3 and CCL4 by chronic lymphocytic leukemia B cells in nurselike cell cocultures and after BCR stimulation. Blood [Internet]. 2009 [cited 2023 Mar 10];113:3050–8. Available from: https://doi.org/10.1182/blood-2008-07-170415

58. Kontoyiannis DP, Georgiadou SP, Wierda WG, Wright S, Albert ND, Ferrajoli A, et al. Impaired bactericidal but not fungicidal activity of polymorphonuclear neutrophils in patients with chronic lymphocytic leukemia. Leuk Lymphoma. 2013;54:1730–3.

59. Maffei R, Bulgarelli J, Fiorcari S, Bertoncelli L, Martinelli S, Guarnotta C, et al. The monocytic population in chronic lymphocytic leukemia shows altered composition and deregulation of genes involved in phagocytosis and inflammation. Haematologica [Internet]. 2013 [cited 2021 Feb 27];98:1115–23. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3696616/

60. Huergo-Zapico L, Acebes-Huerta A, Gonzalez-Rodriguez AP, Contesti J, Gonzalez-García E, Payer AR, et al. Expansion of NK cells and reduction of NKG2D expression in chronic lymphocytic leukemia. Correlation with progressive disease. PloS One. 2014;9:e108326.

61. Marshall JS, Warrington R, Watson W, Kim HL. An introduction to immunology and immunopathology. Allergy Asthma Clin Immunol [Internet]. 2018 [cited 2023 Mar 12];14:49. Available from: https://doi.org/10.1186/s13223-018-0278-1

62. Füst G, Miszlay Z, Czink E, Varga L, Pálóczi K, Szegedi G, et al. C1 and C4 abnormalities in chronic lymphocytic leukaemia and their significance. Immunol Lett. 1987;14:255–9.

63. Rozman C, Montserrat E, Viñolas N. Serum immunoglobulins in B-chronic lymphocytic leukemia. Natural history and prognostic significance. Cancer. 1988;61:279–83.

64. Visentin A, Compagno N, Cinetto F, Imbergamo S, Zambello R, Piazza F, et al. Clinical profile associated with infections in patients with chronic lymphocytic leukemia. Protective role of immunoglobulin replacement therapy. Haematologica [Internet]. 2015 [cited 2021 Mar 8];100:e515–8. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4666345/

65. Rizzo D, Chauzeix J, Trimoreau F, Woillard JB, Genevieve F, Bouvier A, et al. IgM peak independently predicts treatment-free survival in chronic lymphocytic leukemia

and correlates with accumulation of adverse oncogenetic events. Leukemia [Internet]. Nature Publishing Group; 2015 [cited 2021 Mar 8];29:337–45. Available from: https://www.nature.com/articles/leu2014198

66. Grywalska E, Zaborek M, Łyczba J, Hrynkiewicz R, Bębnowska D, Becht R, et al. Chronic Lymphocytic Leukemia-Induced Humoral Immunosuppression: A Systematic Review. Cells. 2020;9.

67. DiLillo DJ, Weinberg JB, Yoshizaki A, Horikawa M, Bryant JM, Iwata Y, et al. Chronic lymphocytic leukemia and regulatory B cells share IL-10 competence and immunosuppressive function. Leukemia [Internet]. Nature Publishing Group; 2013 [cited 2021 Mar 8];27:170–82. Available from: https://www.nature.com/articles/leu2012165

68. Apollonio B, Scielzo C, Bertilaccio MTS, Ten Hacken E, Scarfò L, Ranghetti P, et al. Targeting B-cell anergy in chronic lymphocytic leukemia. Blood. 2013;121:3879–88, S1-8.

69. Coelho V, Krysov S, Steele A, Sanchez Hidalgo M, Johnson PW, Chana PS, et al. Identification in CLL of circulating intraclonal subgroups with varying B-cell receptor expression and function. Blood [Internet]. 2013 [cited 2021 Mar 8];122:2664–72. Available from: https://doi.org/10.1182/blood-2013-02-485425

70. Christopoulos P, Pfeifer D, Bartholomé K, Follo M, Timmer J, Fisch P, et al. Definition and characterization of the systemic T-cell dysregulation in untreated indolent B-cell lymphoma and very early CLL. Blood. 2011;117:3836–46.

71. Pourgheysari B, Bruton R, Parry H, Billingham L, Fegan C, Murray J, et al. The number of cytomegalovirus-specific CD4+ T cells is markedly expanded in patients with B-cell chronic lymphocytic leukemia and determines the total CD4+ T-cell repertoire. Blood. 2010;116:2968–74.

72. Forconi F, Moss P. Perturbation of the normal immune system in patients with CLL. Blood. 2015;126:573–81.

73. Pizzolo G, Chilosi M, Ambrosetti A, Semenzato G, Fiore-Donati L, Perona G. Immunohistologic study of bone marrow involvement in B-chronic lymphocytic leukemia. Blood. 1983;62:1289–96.

74. Patten PEM, Buggins AGS, Richards J, Wotherspoon A, Salisbury J, Mufti GJ, et al. CD38 expression in chronic lymphocytic leukemia is regulated by the tumor microenvironment. Blood. 2008;111:5173–81.

75. Görgün G, Holderried TAW, Zahrieh D, Neuberg D, Gribben JG. Chronic lymphocytic leukemia cells induce changes in gene expression of CD4 and CD8 T cells. J Clin Invest. 2005;115:1797–805.

76. Ramsay AG, Johnson AJ, Lee AM, Gorgün G, Le Dieu R, Blum W, et al. Chronic lymphocytic leukemia T cells show impaired immunological synapse formation that can be reversed with an immunomodulating drug. J Clin Invest. 2008;118:2427–37.

77. Roessner PM, Seiffert M. T-cells in chronic lymphocytic leukemia: Guardians or drivers of disease? Leukemia [Internet]. Nature Publishing Group; 2020 [cited 2023 Mar 12];34:2012–24. Available from: https://www.nature.com/articles/s41375-020-0873-2

78. Palma M, Gentilcore G, Heimersson K, Mozaffari F, Näsman-Glaser B, Young E, et al. T cells in chronic lymphocytic leukemia display dysregulated expression of immune checkpoints and activation markers. Haematologica [Internet]. 2017 [cited 2023 Mar 12];102:562–72. Available from: https://haematologica.org/article/view/8007

79. Giannopoulos K, Schmitt M, Kowal M, Wlasiuk P, Bojarska-Junak A, Chen J, et al. Characterization of regulatory T cells in patients with B-cell chronic lymphocytic leukemia. Oncol Rep. 2008;20:677–82.

80. Le Saos-Patrinos C, Loizon S, Zouine A, Turpin D, Dilhuydy M-S, Blanco P, et al. Elevated levels of circulatory follicular T helper cells in chronic lymphocytic leukemia contribute to B cell expansion. J Leukoc Biol [Internet]. 2023 [cited 2023 Mar 12];113:305–14. Available from: https://doi.org/10.1093/jleuko/qiad006

81. Zhang N, Bevan MJ. CD8+ T Cells: Foot Soldiers of the Immune System. Immunity [Internet]. Elsevier; 2011 [cited 2023 Mar 12];35:161–8. Available from: https://www.cell.com/immunity/abstract/S1074-7613(11)00303-7

82. Krzewski K, Coligan J. Human NK cell lytic granules and regulation of their exocytosis. Front Immunol [Internet]. 2012 [cited 2023 Mar 12];3. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2012.00335

83. Darmon AJ, Ley TJ, Nicholson DW, Bleackley RC. Cleavage of CPP32 by granzyme B represents a critical role for granzyme B in the induction of target cell DNA fragmentation. J Biol Chem. 1996;271:21709–12.

84. Aguiló JI, Anel A, Catalán E, Sebastián A, Acín-Pérez R, Naval J, et al. Granzyme B of cytotoxic T cells induces extramitochondrial reactive oxygen species production via caspase-dependent NADPH oxidase activation. Immunol Cell Biol. 2010;88:545–54.

85. Lord SJ, Rajotte RV, Korbutt GS, Bleackley RC. Granzyme B: a natural born killer. Immunol Rev. 2003;193:31–8.

86. Atkinson EA, Barry M, Darmon AJ, Shostak I, Turner PC, Moyer RW, et al. Cytotoxic T lymphocyte-assisted suicide. Caspase 3 activation is primarily the result of the direct action of granzyme B. J Biol Chem. 1998;273:21261–6.

87. Barry M, Heibein JA, Pinkoski MJ, Lee SF, Moyer RW, Green DR, et al. Granzyme B short-circuits the need for caspase 8 activity during granule-mediated cytotoxic T-lymphocyte killing by directly cleaving Bid. Mol Cell Biol. 2000;20:3781–94.

88. Voskoboinik I, Whisstock JC, Trapani JA. Perforin and granzymes: function, dysfunction and human pathology. Nat Rev Immunol. 2015;15:388–400.

89. Thiery J, Keefe D, Boulant S, Boucrot E, Walch M, Martinvalet D, et al. Perforin pores in the endosomal membrane trigger release of endocytosed granzyme B to the cytosol of target cells. Nat Immunol [Internet]. 2011 [cited 2023 Mar 13];12:770–7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3140544/

90. Shi L, Keefe D, Durand E, Feng H, Zhang D, Lieberman J. Granzyme B binds to target cells mostly by charge and must be added at the same time as perforin to trigger apoptosis. J Immunol Baltim Md 1950. 2005;174:5456–61.

91. Wherry EJ. T cell exhaustion. Nat Immunol. 2011;12:492–9.

92. Blank CU, Haining WN, Held W, Hogan PG, Kallies A, Lugli E, et al. Defining "T cell exhaustion." Nat Rev Immunol. 2019;19:665–74.

93. Okoye IS, Houghton M, Tyrrell L, Barakat K, Elahi S. Coinhibitory Receptor Expression and Immune Checkpoint Blockade: Maintaining a Balance in CD8+ T Cell Responses to Chronic Viral Infections and Cancer. Front Immunol. 2017;8:1215.

94. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12:252–64.

95. Schnell A, Bod L, Madi A, Kuchroo VK. The yin and yang of co-inhibitory receptors: toward anti-tumor immunity without autoimmunity. Cell Res [Internet]. Nature Publishing Group; 2020 [cited 2023 Apr 3];30:285–99. Available from: https://www.nature.com/articles/s41422-020-0277-x

96. Andrews LP, Yano H, Vignali DAA. Inhibitory receptors and ligands beyond PD-1, PD-L1 and CTLA-4: breakthroughs or backups. Nat Immunol. 2019;20:1425–34.

97. McLane LM, Abdel-Hakeem MS, Wherry EJ. CD8 T Cell Exhaustion During Chronic Viral Infection and Cancer. Annu Rev Immunol. 2019;37:457–95.

98. Philip M, Schietinger A. Heterogeneity and fate choice: T cell exhaustion in cancer and chronic infections. Curr Opin Immunol. 2019;58:98–103.

99. Vaddepally RK, Kharel P, Pandey R, Garje R, Chandra AB. Review of Indications of FDA-Approved Immune Checkpoint Inhibitors per NCCN Guidelines with the Level of Evidence. Cancers. 2020;12:738.

100. McKinney EF, Lee JC, Jayne DRW, Lyons PA, Smith KGC. T cell exhaustion, costimulation and clinical outcome in autoimmunity and infection. Nature [Internet].

2015 [cited 2021 Mar 29];523:612–6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4623162/

101. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. Nat Rev Immunol. 2015;15:486–99.

102. Vlachonikola E, Stamatopoulos K, Chatzidimitriou A. T Cells in Chronic Lymphocytic Leukemia: A Two-Edged Sword. Front Immunol [Internet]. 2021 [cited 2023 Apr 3];11. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2020.612244

103. Curran EK, Godfrey J, Kline J. Mechanisms of Immune Tolerance in Leukemia and Lymphoma. Trends Immunol. 2017;38:513–25.

104. Brusa D, Serra S, Coscia M, Rossi D, D'Arena G, Laurenti L, et al. The PD-1/PD-L1 axis contributes to T-cell dysfunction in chronic lymphocytic leukemia. Haematologica [Internet]. 2013 [cited 2023 Apr 3];98:953–63. Available from: https://haematologica.org/article/view/6698

105. Sadik A, Somarribas Patterson LF, Öztürk S, Mohapatra SR, Panitz V, Secker PF, et al. IL4I1 Is a Metabolic Immune Checkpoint that Activates the AHR and Promotes Tumor Progression. Cell. 2020;182:1252-1270.e34.

106. Peters FS, Strefford JC, Eldering E, Kater AP. T-cell dysfunction in chronic lymphocytic leukemia from an epigenetic perspective. Haematologica [Internet]. 2021 [cited 2023 Apr 3];106:1234–43. Available from: https://haematologica.org/article/view/haematol.2020.267914

107. Maïza H, Leca G, Mansur IG, Schiavon V, Boumsell L, Bensussan A. A novel 80-kD cell surface structure identifies human circulating lymphocytes with natural killer activity. J Exp Med. 1993;178:1121–6.

108. El-Far M, Pellerin C, Pilote L, Fortin J-F, Lessard IAD, Peretz Y, et al. CD160 isoforms and regulation of CD4 and CD8 T-cell responses. J Transl Med. 2014;12:217.

109. Ortonne N, Ram-Wolff C, Giustiniani J, Marie-Cardine A, Bagot M, Mecheri S, et al. Human and mouse mast cells express and secrete the GPI-anchored isoform of CD160. J Invest Dermatol. 2011;131:916–24.

110. Henry A, Boulagnon-Rombi C, Menguy T, Giustiniani J, Garbar C, Mascaux C, et al. CD160 Expression in Retinal Vessels Is Associated With Retinal Neovascular Diseases. Invest Ophthalmol Vis Sci. 2018;59:2679–86.

111. Farren TW, Giustiniani J, Liu F-T, Tsitsikas DA, Macey MG, Cavenagh JD, et al. Differential and tumor-specific expression of CD160 in B-cell malignancies. Blood. 2011;118:2174–83.

112. Giustiniani J, Marie-Cardine A, Bensussan A. A soluble form of the MHC class I-specific CD160 receptor is released from human activated NK lymphocytes and inhibits cell-mediated cytotoxicity. J Immunol Baltim Md 1950. 2007;178:1293–300.

113. Tu TC, Brown NK, Kim T-J, Wroblewska J, Yang X, Guo X, et al. CD160 is essential for NK-mediated IFN-γ production. J Exp Med. 2015;212:415–29.

114. Maeda M, Carpenito C, Russell RC, Dasanjh J, Veinotte LL, Ohta H, et al. Murine CD160, Ig-like receptor on NK cells and NKT cells, recognizes classical and nonclassical MHC class I and regulates NK cell activation. J Immunol Baltim Md 1950. 2005;175:4426–32.

115. Rodriguez-Barbosa JI, Schneider P, Weigert A, Lee K-M, Kim T-J, Perez-Simon J-A, et al. HVEM, a cosignaling molecular switch, and its interactions with BTLA, CD160 and LIGHT. Cell Mol Immunol. 2019;16:679–82.

116. Cai G, Anumanthan A, Brown JA, Greenfield EA, Zhu B, Freeman GJ. CD160 inhibits activation of human CD4+ T cells through interaction with herpesvirus entry mediator. Nat Immunol. 2008;9:176–85.

117. Peretz Y, He Z, Shi Y, Yassine-Diab B, Goulet J-P, Bordi R, et al. CD160 and PD-1 co-expression on HIV-specific CD8 T cells defines a subset with advanced dysfunction. PLoS Pathog. 2012;8:e1002840.

118. Tan CL, Peluso MJ, Drijvers JM, Mera CM, Grande SM, Brown KE, et al. CD160 Stimulates CD8+ T Cell Responses and Is Required for Optimal Protective Immunity to Listeria monocytogenes. ImmunoHorizons. 2018;2:238–50.

119. Del Rio M-L, Nguyen TH, Tesson L, Heslan J-M, Gutierrez-Adan A, Fernandez-Gonzalez R, et al. The impact of CD160 deficiency on alloreactive CD8 T cell responses and allograft rejection. Transl Res J Lab Clin Med. 2022;239:103–23.

120. Liu F-T, Giustiniani J, Farren T, Jia L, Bensussan A, Gribben JG, et al. CD160 signaling mediates PI3K-dependent survival and growth signals in chronic lymphocytic leukemia. Blood [Internet]. 2010 [cited 2023 Mar 23];115:3079–88. Available from: https://doi.org/10.1182/blood-2009-08-239483

121. Chibueze CE, Yoshimitsu M, Arima N. CD160 expression defines a uniquely exhausted subset of T lymphocytes in HTLV-1 infection. Biochem Biophys Res Commun. 2014;453:379–84.

122. Liu S, Zhang W, Liu K, Wang Y. CD160 expression on CD8+ T cells is associated with active effector responses but limited activation potential in pancreatic cancer. Cancer Immunol Immunother CII. 2020;69:789–97.

123. Gauci M-L, Giustiniani J, Lepelletier C, Garbar C, Thonnart N, Dumaz N, et al. The soluble form of CD160 acts as a tumor mediator of immune escape in melanoma. Cancer Immunol Immunother CII. 2022;71:2731–42.

124. Cruikshank WW, Kornfeld H, Center DM. Interleukin-16. J Leukoc Biol. 2000;67:757–66.

125. Zhang Y, Center DM, Wu DM, Cruikshank WW, Yuan J, Andrews DW, et al. Processing and activation of pro-interleukin-16 by caspase-3. J Biol Chem. 1998;273:1144–9.

126. Cheng G, Ueda T, Eda F, Arima M, Yoshida N, Fukuda T. A549 cells can express interleukin-16 and stimulate eosinophil chemotaxis. Am J Respir Cell Mol Biol. 2001;25:212–8.

127. Chupp GL, Wright EA, Wu D, Vallen-Mashikian M, Cruikshank WW, Center DM, et al. Tissue and T cell distribution of precursor and mature IL-16. J Immunol Baltim Md 1950. 1998;161:3114–9.

128. Rumsaeng V, Cruikshank WW, Foster B, Prussin C, Kirshenbaum AS, Davis TA, et al. Human mast cells produce the CD4+ T lymphocyte chemoattractant factor, IL-16. J Immunol Baltim Md 1950. 1997;159:2904–10.

129. Lim KG, Wan HC, Bozza PT, Resnick MB, Wong DT, Cruikshank WW, et al. Human eosinophils elaborate the lymphocyte chemoattractants. IL-16 (lymphocyte chemoattractant factor) and RANTES. J Immunol Baltim Md 1950. 1996;156:2566–70.

130. Bellini A, Yoshimura H, Vittori E, Marini M, Mattoli S. Bronchial epithelial cells of patients with asthma release chemoattractant factors for T lymphocytes. J Allergy Clin Immunol. 1993;92:412–24.

131. Parada NA, Center DM, Kornfeld H, Rodriguez WL, Cook J, Vallen M, et al. Synergistic activation of CD4+ T cells by IL-16 and IL-2. J Immunol Baltim Md 1950. 1998;160:2115–20.

132. Mathy NL, Scheuer W, Lanzendörfer M, Honold K, Ambrosius D, Norley S, et al. Interleukin-16 stimulates the expression and production of pro-inflammatory cytokines by human monocytes. Immunology [Internet]. 2000 [cited 2023 Mar 24];100:63–9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2326980/

133. Baier M, Werner A, Bannert N, Metzner K, Kurth R. HIV suppression by interleukin-16. Nature. 1995;378:563.

134. Bellomo G, Allegra A, Alonci A, Quartarone E, Pizzoleo MA, Cincotta M, et al. Serum levels of interleukin-16 in lymphoid malignancies. Leuk Lymphoma. 2007;48:1225–7.

135. Mahindra A, Anderson KC. Role of Interleukin 16 in Multiple Myeloma Pathogenesis: A Potential Novel Therapeutic Target? JNCI J Natl Cancer Inst [Internet]. 2012 [cited 2023 Mar 24];104:964–5. Available from: https://doi.org/10.1093/jnci/djs274

136. Templin J, Luetkens T, Atanackovic D. Cytokine Interleukin-16 Supports Progression Of Multiple Myeloma Through Different Molecular Mechanisms. Blood [Internet]. 2013 [cited 2023 Mar 24];122:5367. Available from: https://www.sciencedirect.com/science/article/pii/S0006497119686258

137. Atanackovic D, Hildebrandt Y, Luetkens T, Zander AR, Bokemeyer C, Kroeger N. Interleukin-16 Is An Important Growth-Promoting Factor and a Novel Diagnostic and Therapeutic Target for Multiple Myeloma. Blood [Internet]. 2010 [cited 2023 Mar 24];116:4056. Available from: https://doi.org/10.1182/blood.V116.21.4056.4056

138. Yang H, Han Y, Wu L, Wu C. Diagnostic and prognostic value of serum interleukin-16 in patients with gastric cancer. Mol Med Rep [Internet]. Spandidos Publications; 2017 [cited 2023 Mar 24];16:9143–8. Available from: https://www.spandidos-publications.com/10.3892/mmr.2017.7688

139. Engel M, Hoffmann T, Wagner L, Wermann M, Heiser U, Kiefersauer R, et al. The crystal structure of dipeptidyl peptidase IV (CD26) reveals its functional regulation and enzymatic mechanism. Proc Natl Acad Sci U S A. 2003;100:5063–8.

140. Yaron A, Naider F, Scharpe S. Proline-Dependent Structural and Biological Properties of Peptides and Proteins. Crit Rev Biochem Mol Biol [Internet]. Taylor & Francis; 1993 [cited 2022 Jun 6];28:31–81. Available from: https://doi.org/10.3109/10409239309082572

141. Ahrén B, Hughes TE. Inhibition of dipeptidyl peptidase-4 augments insulin secretion in response to exogenously administered glucagon-like peptide-1, glucose-dependent insulinotropic polypeptide, pituitary adenylate cyclase-activating polypeptide, and gastrin-releasing peptide in mice. Endocrinology. 2005;146:2055–9.

142. Mortier A, Gouwy M, Van Damme J, Proost P, Struyf S. CD26/dipeptidylpeptidase IV—chemokine interactions: double-edged regulation of inflammation and tumor biology. J Leukoc Biol [Internet]. 2016 [cited 2022 Jun 29];99:955–69. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7166560/

143. Brezinschek RI, Lipsky PE, Galea P, Vita R, Oppenheimer-Marks N. Phenotypic characterization of CD4+ T cells that exhibit a transendothelial migratory capacity. J Immunol Baltim Md 1950. 1995;154:3062–77.

144. Shahbaz S, Xu L, Osman M, Sligl W, Shields J, Joyce M, et al. Erythroid precursors and progenitors suppress adaptive immunity and get invaded by SARS-CoV-2. Stem Cell Rep. 2021;16:1165–81.

145. Morimoto C, Torimoto Y, Levinson G, Rudd CE, Schrieber M, Dang NH, et al. 1F7, a novel cell surface molecule, involved in helper function of CD4 cells. J Immunol Baltim Md 1950. 1989;143:3430–9.

146. Bühling F, Junker U, Reinhold D, Neubert K, Jäger L, Ansorge S. Functional role of CD26 on human B lymphocytes. Immunol Lett. 1995;45:47–51.

147. Bühling F, Kunz D, Reinhold D, Ulmer AJ, Ernst M, Flad HD, et al. Expression and functional role of dipeptidyl peptidase IV (CD26) on human natural killer cells. Nat Immun. 1994;13:270–9.

148. Gliddon DR, Howard CJ. CD26 is expressed on a restricted subpopulation of dendritic cells in vivo. Eur J Immunol. 2002;32:1472–81.

149. Dang NH, Torimoto Y, Shimamura K, Tanaka T, Daley JF, Schlossman SF, et al. 1F7 (CD26): a marker of thymic maturation involved in the differential regulation of the CD3 and CD2 pathways of human thymocyte activation. J Immunol Baltim Md 1950. 1991;147:2825–32.

150. Yan S, Marguet D, Dobers J, Reutter W, Fan H. Deficiency of CD26 results in a change of cytokine and immunoglobulin secretion after stimulation by pokeweed mitogen. Eur J Immunol. 2003;33:1519–27.

151. Pinheiro MM, Stoppa CL, Valduga CJ, Okuyama CE, Gorjão R, Pereira RMS, et al. Sitagliptin inhibit human lymphocytes proliferation and Th1/Th17 differentiation in vitro. Eur J Pharm Sci Off J Eur Fed Pharm Sci. 2017;100:17–24.

152. Dong RP, Kameoka J, Hegen M, Tanaka T, Xu Y, Schlossman SF, et al. Characterization of adenosine deaminase binding to human CD26 on T cells and its biologic role in immune response. J Immunol [Internet]. American Association of Immunologists; 1996 [cited 2022 Jun 6];156:1349–55. Available from: https://www.jimmunol.org/content/156/4/1349

153. Martín M, Huguet J, Centelles JJ, Franco R. Expression of ecto-adenosine deaminase and CD26 in human T cells triggered by the TCR-CD3 complex. Possible role of adenosine deaminase as costimulatory molecule. J Immunol Baltim Md 1950. 1995;155:4630–43.

154. Ohnuma K, Uchiyama M, Yamochi T, Nishibashi K, Hosono O, Takahashi N, et al. Caveolin-1 triggers T-cell activation via CD26 in association with CARMA1. J Biol Chem. 2007;282:10117–31.

155. Bailey SR, Nelson MH, Majchrzak K, Bowers JS, Wyatt MM, Smith AS, et al. Human CD26high T cells elicit tumor immunity against multiple malignancies via enhanced migration and persistence. Nat Commun [Internet]. Nature Publishing Group; 2017 [cited 2023 Apr 7];8:1961. Available from: https://www.nature.com/articles/s41467-017-01867-9

156. Hatano R, Ohnuma K, Yamamoto J, Dang NH, Morimoto C. CD26-mediated co-stimulation in human CD8(+) T cells provokes effector function via pro-inflammatory cytokine production. Immunology. 2013;138:165–72.

157. Sharma PK, Wong EB, Napier RJ, Bishai WR, Ndung'u T, Kasprowicz VO, et al. High expression of CD26 accurately identifies human bacteria-reactive MR1-restricted MAIT cells. Immunology. 2015;145:443–53.

158. Treiner E, Duban L, Bahram S, Radosavljevic M, Wanner V, Tilloy F, et al. Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. Nature. 2003;422:164–9.

159. Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, Liu L, et al. MR1 presents microbial vitamin B metabolites to MAIT cells. Nature. 2012;491:717–23.

160. John S, Mishra R. Galectin-9: From cell biology to complex disease dynamics. J Biosci. 2016;41:507–34.

161. O'Brien MJ, Shu Q, Stinson WA, Tsou P-S, Ruth JH, Isozaki T, et al. A unique role for galectin-9 in angiogenesis and inflammatory arthritis. Arthritis Res Ther [Internet]. 2018 [cited 2023 Mar 22];20:31. Available from: https://doi.org/10.1186/s13075-018-1519-x

162. Oomizu S, Arikawa T, Niki T, Kadowaki T, Ueno M, Nishi N, et al. Cell surface galectin-9 expressing Th cells regulate Th17 and Foxp3+ Treg development by galectin-9 secretion. PloS One. 2012;7:e48574.

163. Merani S, Chen W, Elahi S. The bitter side of sweet: the role of Galectin-9 in immunopathogenesis of viral infections. Rev Med Virol. 2015;25:175–86.

164. Keryer-Bibens C, Pioche-Durieu C, Villemant C, Souquère S, Nishi N, Hirashima M, et al. Exosomes released by EBV-infected nasopharyngeal carcinoma cells convey the viral latent membrane protein 1 and the immunomodulatory protein galectin 9. BMC Cancer. 2006;6:283.

165. Reddy PBJ, Sehrawat S, Suryawanshi A, Rajasagi NK, Mulik S, Hirashima M, et al. Influence of galectin-9/Tim-3 interaction on herpes simplex virus-1 latency. J Immunol Baltim Md 1950. 2011;187:5745–55.

166. Moritoki M, Kadowaki T, Niki T, Nakano D, Soma G, Mori H, et al. Galectin-9 ameliorates clinical severity of MRL/lpr lupus-prone mice by inducing plasma cell apoptosis independently of Tim-3. PloS One. 2013;8:e60807.

167. Madireddi S, Eun S-Y, Lee S-W, Nemčovičová I, Mehta AK, Zajonc DM, et al. Galectin-9 controls the therapeutic activity of 4-1BB–targeting antibodies. J Exp Med [Internet]. 2014 [cited 2023 Mar 22];211:1433–48. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4076583/

168. Bi S, Hong PW, Lee B, Baum LG. Galectin-9 binding to cell surface protein disulfide isomerase regulates the redox environment to enhance T-cell migration and HIV entry. Proc Natl Acad Sci [Internet]. Proceedings of the National Academy of Sciences; 2011 [cited 2023 Mar 22];108:10650–5. Available from: https://www.pnas.org/doi/abs/10.1073/pnas.1017954108

169. Wu C, Thalhamer T, Franca RF, Xiao S, Wang C, Hotta C, et al. Galectin-9-CD44 interaction enhances stability and function of adaptive regulatory T cells. Immunity. 2014;41:270–82.

170. Anderson AC, Anderson DE, Bregoli L, Hastings WD, Kassam N, Lei C, et al. Promotion of tissue inflammation by the immune receptor Tim-3 expressed on innate immune cells. Science. 2007;318:1141–3.

171. Yang R, Sun L, Li C-F, Wang Y-H, Yao J, Li H, et al. Galectin-9 interacts with PD-1 and TIM-3 to regulate T cell death and is a target for cancer immunotherapy. Nat Commun [Internet]. Nature Publishing Group; 2021 [cited 2023 Mar 23];12:832. Available from: https://www.nature.com/articles/s41467-021-21099-2

172. Elahi S, Dinges WL, Lejarcegui N, Laing KJ, Collier AC, Koelle DM, et al. Protective HIV-specific CD8+ T cells evade Treg cell suppression. Nat Med. 2011;17:989–95.

173. Su EW, Bi S, Kane LP. Galectin-9 regulates T helper cell function independently of Tim-3. Glycobiology. 2011;21:1258–65.

174. Shahbaz S, Dunsmore G, Koleva P, Xu L, Houston S, Elahi S. Galectin-9 and VISTA Expression Define Terminally Exhausted T Cells in HIV-1 Infection. J Immunol Baltim Md 1950. 2020;204:2474–91.

175. Motamedi M, Shahbaz S, Fu L, Dunsmore G, Xu L, Harrington R, et al. Galectin-9 Expression Defines a Subpopulation of NK Cells with Impaired Cytotoxic Effector Molecules but Enhanced IFN-γ Production, Dichotomous to TIGIT, in HIV-1 Infection. ImmunoHorizons. 2019;3:531–46.

176. Dunsmore G, Rosero EP, Shahbaz S, Santer DM, Jovel J, Lacy P, et al. Neutrophils promote T-cell activation through the regulated release of CD44-bound Galectin-9 from the cell surface during HIV infection. PLoS Biol. 2021;19:e3001387.

177. Bozorgmehr N, Mashhouri S, Perez Rosero E, Xu L, Shahbaz S, Sligl W, et al. Galectin-9, a Player in Cytokine Release Syndrome and a Surrogate Diagnostic Biomarker in SARS-CoV-2 Infection. mBio. 2021;12:e00384-21.

178. Gonçalves Silva I, Yasinska IM, Sakhnevych SS, Fiedler W, Wellbrock J, Bardelli M, et al. The Tim-3-galectin-9 Secretory Pathway is Involved in the Immune Escape of Human Acute Myeloid Leukemia Cells. EBioMedicine. 2017;22:44–57.

179. Okoye I, Xu L, Motamedi M, Parashar P, Walker JW, Elahi S. Galectin-9 expression defines exhausted T cells and impaired cytotoxic NK cells in patients with virus-associated solid tumors. J Immunother Cancer [Internet]. 2020 [cited 2023 Mar 23];8:e001849. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7735134/

180. Lhuillier C, Barjon C, Niki T, Gelin A, Praz F, Morales O, et al. Impact of Exogenous Galectin-9 on Human T Cells. J Biol Chem [Internet]. 2015 [cited 2023 Mar 23];290:16797–811. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4505427/

181. Yang R, Sun L, Li C-F, Wang Y-H, Xia W, Liu B, et al. Development and characterization of anti-galectin-9 antibodies that protect T cells from galectin-9-induced cell death. J Biol Chem [Internet]. 2022 [cited 2023 Mar 23];298:101821. Available from: https://www.sciencedirect.com/science/article/pii/S0021925822002617

182. White MK, Pagano JS, Khalili K. Viruses and Human Cancers: a Long Road of Discovery of Molecular Paradigms. Clin Microbiol Rev [Internet]. American Society for Microbiology; 2014 [cited 2023 Mar 26];27:463–81. Available from: https://journals.asm.org/doi/10.1128/cmr.00124-13

183. Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, et al. The biology and life-cycle of human papillomaviruses. Vaccine. 2012;30 Suppl 5:F55-70.

184. Schiffman M, Doorbar J, Wentzensen N, de Sanjosé S, Fakhry C, Monk BJ, et al. Carcinogenic human papillomavirus infection. Nat Rev Dis Primer [Internet]. Nature Publishing Group; 2016 [cited 2023 Mar 26];2:1–20. Available from: https://www.nature.com/articles/nrdp201686

185. Liao C-I, Francoeur AA, Kapp DS, Caesar MAP, Huh WK, Chan JK. Trends in Human Papillomavirus–Associated Cancers, Demographic Characteristics, and Vaccinations in the US, 2001-2017. JAMA Netw Open [Internet]. 2022 [cited 2023 Mar 26];5:e222530. Available from: https://doi.org/10.1001/jamanetworkopen.2022.2530

186. de Martel C, Georges D, Bray F, Ferlay J, Clifford GM. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. Lancet Glob Health. 2020;8:e180–90.

187. Saraiya M, Unger ER, Thompson TD, Lynch CF, Hernandez BY, Lyu CW, et al. US Assessment of HPV Types in Cancers: Implications for Current and 9-Valent HPV Vaccines. JNCI J Natl Cancer Inst [Internet]. 2015 [cited 2023 Mar 26];107:djv086. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4838063/

188. Balasubramaniam SD, Balakrishnan V, Oon CE, Kaur G. Key Molecular Events in Cervical Cancer Development. Med Kaunas Lith. 2019;55:384.

189. Perkins RB, Guido RL, Saraiya M, Sawaya GF, Wentzensen N, Schiffman M, et al. Summary of Current Guidelines for Cervical Cancer Screening and Management of Abnormal Test Results: 2016–2020. J Womens Health [Internet]. 2021 [cited 2023 Mar 26];30:5–13. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8020523/

190. Peitsaro P, Johansson B, Syrjänen S. Integrated human papillomavirus type 16 is frequently found in cervical cancer precursors as demonstrated by a novel quantitative real-time PCR technique. J Clin Microbiol. 2002;40:886–91.

191. Jeon S, Allen-Hoffmann BL, Lambert PF. Integration of human papillomavirus type 16 into the human genome correlates with a selective growth advantage of cells. J Virol. 1995;69:2989–97.

192. Graham SV. The human papillomavirus replication cycle, and its links to cancer progression: a comprehensive review. Clin Sci Lond Engl 1979. 2017;131:2201–21.

193. Scheffner M, Münger K, Byrne JC, Howley PM. The state of the p53 and retinoblastoma genes in human cervical carcinoma cell lines. Proc Natl Acad Sci U S A. 1991;88:5523–7.

194. Pal A, Kundu R. Human Papillomavirus E6 and E7: The Cervical Cancer Hallmarks and Targets for Therapy. Front Microbiol [Internet]. 2020 [cited 2023 Mar 26];10. Available from: https://www.frontiersin.org/articles/10.3389/fmicb.2019.03116

195. Mietz JA, Unger T, Huibregtse JM, Howley PM. The transcriptional transactivation function of wild-type p53 is inhibited by SV40 large T-antigen and by HPV-16 E6 oncoprotein. EMBO J. 1992;11:5013–20.

196. El-Deiry WS. p21(WAF1) Mediates Cell-Cycle Inhibition, Relevant to Cancer Suppression and Therapy. Cancer Res. 2016;76:5189–91.

197. Wang J, Sampath A, Raychaudhuri P, Bagchi S. Both Rb and E7 are regulated by the ubiquitin proteasome pathway in HPV-containing cervical tumor cells. Oncogene [Internet]. Nature Publishing Group; 2001 [cited 2023 Mar 26];20:4740–9. Available from: https://www.nature.com/articles/1204655

198. Marth C, Landoni F, Mahner S, McCormack M, Gonzalez-Martin A, Colombo N. Cervical cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Ann Oncol [Internet]. Elsevier; 2017 [cited 2023 Apr 4];28:iv72–83. Available from: https://www.annalsofoncology.org/article/S0923-7534(19)42148-0/fulltext

199. Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. Nat Rev Dis Primer [Internet]. Nature Publishing Group; 2020 [cited 2023 Apr 4];6:1–22. Available from: https://www.nature.com/articles/s41572-020-00224-3

200. Mirghani H, Amen F, Blanchard P, Moreau F, Guigay J, Hartl D m., et al. Treatment de-escalation in HPV-positive oropharyngeal carcinoma: Ongoing trials, critical issues and perspectives. Int J Cancer [Internet]. 2015 [cited 2023 Mar 26];136:1494–503. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/ijc.28847

201. Rodríguez-Carunchio L, Soveral I, Steenbergen R, Torné A, Martinez S, Fusté P, et al. HPV-negative carcinoma of the uterine cervix: a distinct type of cervical cancer with poor prognosis. BJOG Int J Obstet Gynaecol [Internet]. 2015 [cited 2023 Mar 26];122:119–27. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/1471-0528.13071

202. Onuki M, Matsumoto K, Tenjimbayashi Y, Tasaka N, Akiyama A, Sakurai M, et al. Human papillomavirus genotype and prognosis of cervical cancer: Favorable survival of patients with HPV16-positive tumors. Papillomavirus Res [Internet]. 2018 [cited 2023 Mar 26];6:41–5. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6218653/

203. Rader JS, Tsaih S-W, Fullin D, Murray MW, Iden M, Zimmermann MT, et al. Genetic variations in human papillomavirus and cervical cancer outcomes. Int J Cancer [Internet]. 2019 [cited 2023 Mar 26];144:2206–14. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6450540/

204. Małusecka E, Chmielik E, Suwiński R, Giglok M, Lange D, Rutkowski T, et al. Significance of HPV16 Viral Load Testing in Anal Cancer. Pathol Oncol Res [Internet]. 2020 [cited 2023 Mar 26];26:2191–9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7471158/

205. Liu Y, Xu C, Pan J, Sun C, Zhou H, Meng Y. Significance of the viral load of high-risk HPV in the diagnosis and prediction of cervical lesions: a retrospective study. BMC Womens Health [Internet]. 2021 [cited 2023 Mar 26];21:353. Available from: https://doi.org/10.1186/s12905-021-01493-0

206. Frisch M, Biggar RJ, Engels EA, Goedert JJ, AIDS-Cancer Match Registry Study Group. Association of cancer with AIDS-related immunosuppression in adults. JAMA. 2001;285:1736–45.

207. Vajdic CM, McDonald SP, McCredie MRE, van Leeuwen MT, Stewart JH, Law M, et al. Cancer incidence before and after kidney transplantation. JAMA. 2006;296:2823–31.

208. Burmeister CA, Khan SF, Schäfer G, Mbatani N, Adams T, Moodley J, et al. Cervical cancer therapies: Current challenges and future perspectives. Tumour Virus Res [Internet]. 2022 [cited 2023 Mar 26];13:200238. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9062473/

209. Enwere EK, Kornaga EN, Dean M, Koulis TA, Phan T, Kalantarian M, et al. Expression of PD-L1 and presence of CD8-positive T cells in pre-treatment specimens of locally advanced cervical cancer. Mod Pathol Off J U S Can Acad Pathol Inc. 2017;30:577–86.

210. Quigley M, Pereyra F, Nilsson B, Porichis F, Fonseca C, Eichbaum Q, et al. Transcriptional analysis of HIV-specific CD8+ T cells shows that PD-1 inhibits T cell function by upregulating BATF. Nat Med [Internet]. 2010 [cited 2023 Apr 4];16:1147–51. Available from: https://europepmc.org/articles/PMC3326577

211. Hui E, Cheung J, Zhu J, Su X, Taylor MJ, Wallweber HA, et al. T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. Science. 2017;355:1428–33.

212. Cohen AC, Roane BM, Leath CA. Novel Therapeutics for Recurrent Cervical Cancer: Moving Towards Personalized Therapy. Drugs. 2020;80:217–27.

213. Duenas-Gonzalez A, Candelaria M, Perez-Plascencia C, Perez-Cardenas E, de la Cruz-Hernandez E, Herrera LA. Valproic acid as epigenetic cancer drug: preclinical, clinical and transcriptional effects on solid tumors. Cancer Treat Rev. 2008;34:206–22.

214. Mora-García M de L, Duenas-González A, Hernández-Montes J, De la Cruz-Hernández E, Pérez-Cárdenas E, Weiss-Steider B, et al. Up-regulation of HLA class-I antigen expression and antigen-specific CTL response in cervical cancer cells by the demethylating agent hydralazine and the histone deacetylase inhibitor valproic acid. J Transl Med [Internet]. 2006 [cited 2021 Jun 8];4:55. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1781077/

215. Grunstein M. Histone acetylation in chromatin structure and transcription. Nature. 1997;389:349–52.

216. Suraweera A, O'Byrne KJ, Richard DJ. Combination Therapy With Histone Deacetylase Inhibitors (HDACi) for the Treatment of Cancer: Achieving the Full Therapeutic Potential of HDACi. Front Oncol [Internet]. 2018 [cited 2022 Sep 3];8. Available from: https://www.frontiersin.org/articles/10.3389/fonc.2018.00092

217. Smith KT, Workman JL. Histone deacetylase inhibitors: anticancer compounds. Int J Biochem Cell Biol. 2009;41:21–5.

218. Marks P, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK. Histone deacetylases and cancer: causes and therapies. Nat Rev Cancer. 2001;1:194–202.

219. Soria-Castro R, Schcolnik-Cabrera A, Rodríguez-López G, Campillo-Navarro M, Puebla-Osorio N, Estrada-Parra S, et al. Exploring the Drug Repurposing Versatility of Valproic Acid as a Multifunctional Regulator of Innate and Adaptive Immune Cells. J Immunol Res. 2019;2019:9678098.

220. Reeves E, James E. Antigen processing and immune regulation in the response to tumours. Immunology. 2017;150:16–24.

221. Hemmat N, Baghi HB. Human papillomavirus E5 protein, the undercover culprit of tumorigenesis. Infect Agent Cancer. 2018;13:31.

222. Zitvogel L, Galluzzi L, Kepp O, Smyth MJ, Kroemer G. Type I interferons in anticancer immunity. Nat Rev Immunol. 2015;15:405–14.

223. Lau L, Gray EE, Brunette RL, Stetson DB. DNA tumor virus oncogenes antagonize the cGAS-STING DNA-sensing pathway. Science. 2015;350:568–71.

224. Canning M, Guo G, Yu M, Myint C, Groves MW, Byrd JK, et al. Heterogeneity of the Head and Neck Squamous Cell Carcinoma Immune Landscape and Its Impact on Immunotherapy. Front Cell Dev Biol. 2019;7:52.

225. Peltanova B, Raudenska M, Masarik M. Effect of tumor microenvironment on pathogenesis of the head and neck squamous cell carcinoma: a systematic review. Mol Cancer [Internet]. 2019 [cited 2023 Apr 4];18:63. Available from: https://doi.org/10.1186/s12943-019-0983-5

226. Ward MJ, Thirdborough SM, Mellows T, Riley C, Harris S, Suchak K, et al. Tumour-infiltrating lymphocytes predict for outcome in HPV-positive oropharyngeal cancer. Br J Cancer. 2014;110:489–500.

227. Ferris RL, Blumenschein G, Fayette J, Guigay J, Colevas AD, Licitra L, et al. Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. N Engl J Med. 2016;375:1856–67.

228. Wang J, Sun H, Zeng Q, Guo X-J, Wang H, Liu H-H, et al. HPV-positive status associated with inflamed immune microenvironment and improved response to anti-PD-1 therapy in head and neck squamous cell carcinoma. Sci Rep. 2019;9:13404.

229. Grabowska AK, Riemer AB. The invisible enemy - how human papillomaviruses avoid recognition and clearance by the host immune system. Open Virol J. 2012;6:249–56.

230. Gu Z, Shi W, Zhang L, Hu Z, Xu C. USP19 suppresses cellular type I interferon signaling by targeting TRAF3 for deubiquitination. Future Microbiol. 2017;12:767–79.

231. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol. 2012;12:253–68.

232. Groth C, Hu X, Weber R, Fleming V, Altevogt P, Utikal J, et al. Immunosuppression mediated by myeloid-derived suppressor cells (MDSCs) during tumour progression. Br J Cancer. 2019;120:16–25.

233. Bronte V, Serafini P, Apolloni E, Zanovello P. Tumor-Induced Immune Dysfunctions Caused by Myeloid Suppressor Cells. J Immunother [Internet]. 2001 [cited 2023 Mar 29];24:431. Available from: https://journals.lww.com/immunotherapy-journal/Abstract/2001/11000/Tumor_Induced_Immune_Dysfunctions_Caused_by.1.aspx

234. Bronte V, Brandau S, Chen S-H, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. Nat Commun [Internet]. Nature Publishing Group; 2016 [cited 2023 Mar 29];7:12150. Available from: https://www.nature.com/articles/ncomms12150

235. Condamine T, Dominguez GA, Youn J-I, Kossenkov AV, Mony S, Alicea-Torres K, et al. Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. Sci Immunol. 2016;1:aaf8943.

236. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol [Internet]. Nature Publishing Group; 2009 [cited 2023 Mar 29];9:162–74. Available from: https://www.nature.com/articles/nri2506

237. Li K, Shi H, Zhang B, Ou X, Ma Q, Chen Y, et al. Myeloid-derived suppressor cells as immunosuppressive regulators and therapeutic targets in cancer. Signal Transduct Target Ther [Internet]. Nature Publishing Group; 2021 [cited 2023 Mar 6];6:1–25. Available from: https://www.nature.com/articles/s41392-021-00670-9

238. Tartour E, Pere H, Maillere B, Terme M, Merillon N, Taieb J, et al. Angiogenesis and immunity: a bidirectional link potentially relevant for the monitoring of antiangiogenic therapy and the development of novel therapeutic combination with immunotherapy. Cancer Metastasis Rev. 2011;30:83–95.

239. Hou A, Hou K, Huang Q, Lei Y, Chen W. Targeting Myeloid-Derived Suppressor Cell, a Promising Strategy to Overcome Resistance to Immune Checkpoint Inhibitors. Front Immunol. 2020;11:783.

240. Bizzarri C, Beccari AR, Bertini R, Cavicchia MR, Giorgini S, Allegretti M. ELR+ CXC chemokines and their receptors (CXC chemokine receptor 1 and CXC chemokine receptor 2) as new therapeutic targets. Pharmacol Ther [Internet]. 2006 [cited 2023 Feb 13];112:139–49. Available from: https://www.sciencedirect.com/science/article/pii/S0163725806000556

241. Addison CL, Daniel TO, Burdick MD, Liu H, Ehlert JE, Xue YY, et al. The CXC chemokine receptor 2, CXCR2, is the putative receptor for ELR+ CXC chemokine-induced angiogenic activity. J Immunol Baltim Md 1950. 2000;165:5269–77.

242. Scheibenbogen C, Möhler T, Haefele J, Hunstein W, Keilholz U. Serum interleukin-8 (IL-8) is elevated in patients with metastatic melanoma and correlates with tumour load. Melanoma Res. 1995;5:179–81.

243. Xu Q, Ma H, Chang H, Feng Z, Zhang C, Yang X. The interaction of interleukin-8 and PTEN inactivation promotes the malignant progression of head and neck squamous cell carcinoma via the STAT3 pathway. Cell Death Dis [Internet]. Nature Publishing Group; 2020 [cited 2023 Feb 13];11:1–14. Available from: https://www.nature.com/articles/s41419-020-2627-5

244. Tobin RP, Jordan KR, Kapoor P, Spongberg E, Davis D, Vorwald VM, et al. IL-6 and IL-8 Are Linked With Myeloid-Derived Suppressor Cell Accumulation and Correlate With Poor Clinical Outcomes in Melanoma Patients. Front Oncol [Internet]. 2019 [cited 2023 Feb 13];9:1223. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6857649/

245. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science. 2004;303:1532–5.

246. Teijeira A, Garasa S, Ochoa MC, Villalba M, Olivera I, Cirella A, et al. IL8, Neutrophils, and NETs in a Collusion against Cancer Immunity and Immunotherapy.

Clin Cancer Res [Internet]. 2021 [cited 2023 Apr 4];27:2383–93. Available from: https://doi.org/10.1158/1078-0432.CCR-20-1319

247. Zhang Y, Guo L, Dai Q, Shang B, Xiao T, Di X, et al. A signature for pan-cancer prognosis based on neutrophil extracellular traps. J Immunother Cancer [Internet]. BMJ Specialist Journals; 2022 [cited 2023 Apr 4];10:e004210. Available from: https://jitc.bmj.com/content/10/6/e004210

248. Nie M, Yang L, Bi X, Wang Y, Sun P, Yang H, et al. Neutrophil Extracellular Traps Induced by IL8 Promote Diffuse Large B-cell Lymphoma Progression via the TLR9 Signaling. Clin Cancer Res Off J Am Assoc Cancer Res. 2019;25:1867–79.

249. Zhang Y, Chandra V, Riquelme Sanchez E, Dutta P, Quesada PR, Rakoski A, et al. Interleukin-17-induced neutrophil extracellular traps mediate resistance to checkpoint blockade in pancreatic cancer. J Exp Med. 2020;217:e20190354.

250. Shamseddine AA, Burman B, Lee NY, Zamarin D, Riaz N. Tumor Immunity and Immunotherapy for HPV-Related Cancers. Cancer Discov [Internet]. 2021 [cited 2023 Mar 26];11:1896–912. Available from: https://doi.org/10.1158/2159-8290.CD-20-1760

251. He X, Xu C. Immune checkpoint signaling and cancer immunotherapy. Cell Res [Internet]. 2020 [cited 2021 Oct 27];30:660–9. Available from: https://www.nature.com/articles/s41422-020-0343-4

252. Vaddepally RK, Kharel P, Pandey R, Garje R, Chandra AB. Review of Indications of FDA-Approved Immune Checkpoint Inhibitors per NCCN Guidelines with the Level of Evidence. Cancers. 2020;12.

253. Matić IZ, Đorđević M, Đorđić M, Grozdanić N, Damjanović A, Kolundžija B, et al. Dipeptidyl peptidase IV: serum activity and expression on lymphocytes in different hematological malignancies. Leuk Lymphoma. 2013;54:2701–6.

254. Tan EY, Mujoomdar M, Blay J. Adenosine Down-Regulates the Surface Expression of Dipeptidyl Peptidase IV on HT-29 Human Colorectal Carcinoma Cells. Am J Pathol [Internet]. 2004 [cited 2021 Oct 18];165:319–30. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1618535/

255. Vijayan D, Young A, Teng MWL, Smyth MJ. Targeting immunosuppressive adenosine in cancer. Nat Rev Cancer [Internet]. Nature Publishing Group; 2017 [cited 2021 Jun 3];17:709–24. Available from: https://www.nature.com/articles/nrc.2017.86

256. Metzemaekers M, Van Damme J, Mortier A, Proost P. Regulation of Chemokine Activity – A Focus on the Role of Dipeptidyl Peptidase IV/CD26. Front Immunol [Internet]. 2016 [cited 2023 Apr 4];7. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2016.00483

257. Frenel J-S, Le Tourneau C, O'Neil B, Ott PA, Piha-Paul SA, Gomez-Roca C, et al. Safety and Efficacy of Pembrolizumab in Advanced, Programmed Death Ligand 1-Positive Cervical Cancer: Results From the Phase Ib KEYNOTE-028 Trial. J Clin Oncol Off J Am Soc Clin Oncol. 2017;35:4035–41.

258. Khan O, La Thangue NB. HDAC inhibitors in cancer biology: emerging mechanisms and clinical applications. Immunol Cell Biol. 2012;90:85–94.

Chapter 2

1. Seifert M, Sellmann L, Bloehdorn J, Wein F, Stilgenbauer S, Dürig J, Küppers R. Cellular origin, and pathophysiology of chronic lymphocytic leukemia. J Exp Med. 2012 Nov 19;209(12):2183–98.

2. Scarfò L, Ferreri AJM, Ghia P. Chronic lymphocytic leukaemia. Crit Rev Oncol Hematol. 2016 Aug; 104:169–82.

3. Arruga F, Gyau BB, Iannello A, Vitale N, Vaisitti T, Deaglio S. Immune Response Dysfunction in Chronic Lymphocytic Leukemia: Dissecting Molecular Mechanisms and Microenvironmental Conditions. Int J Mol Sci [Internet]. 2020 Mar 6 [cited 2020 Oct 13];21(5). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7084946/

4. Christopoulos P, Pfeifer D, Bartholomé K, Follo M, Timmer J, Fisch P, Veelken H. Definition, and characterization of the systemic T-cell dysregulation in untreated indolent B-cell lymphoma and very early CLL. Blood. 2011 Apr 7;117(14):3836–46.

5. Porakishvili N, Roschupkina T, Kalber T, Jewell AP, Patterson K, Yong K, Lydyard PM. Expansion of CD4+ T cells with a cytotoxic phenotype in patients with B-chronic lymphocytic leukaemia (B-CLL). Clin Exp Immunol. 2001 Oct;126(1):29–36.

6. Ramsay AG, Clear AJ, Fatah R, Gribben JG. Multiple inhibitory ligands induce impaired T-cell immunologic synapse function in chronic lymphocytic leukemia that can be blocked with lenalidomide: establishing a reversible immune evasion mechanism in human cancer. Blood. 2012 Aug 16;120(7):1412–21.

7. Ramsay AG, Johnson AJ, Lee AM, Gorgün G, Le Dieu R, Blum W, Byrd JC, Gribben JG. Chronic lymphocytic leukemia T cells show impaired immunological synapse formation that can be reversed with an immunomodulating drug. J Clin Invest. 2008 Jul;118(7):2427–37.

8. Okoye IS, Houghton M, Tyrrell L, Barakat K, Elahi S. Coinhibitory Receptor Expression, and Immune Checkpoint Blockade: Maintaining a Balance in CD8+ T Cell Responses to Chronic Viral Infections and Cancer. Front Immunol. 2017; 8:1215.

9. Wherry EJ. T cell exhaustion. Nat Immunol [Internet]. 2011 Jun [cited 2020 Oct 13];12(6):492–9. Available from: https://www.nature.com/articles/ni.2035

10. McLane LM, Abdel-Hakeem MS, Wherry EJ. CD8 T Cell Exhaustion During Chronic Viral Infection and Cancer. Annu Rev Immunol. 2019;37(1):457–95.

11. Palma M, Gentilcore G, Heimersson K, Mozaffari F, Näsman-Glaser B, Young E, Rosenquist R, Hansson L, Österborg A, Mellstedt H. T cells in chronic lymphocytic leukemia display dysregulated expression of immune checkpoints and activation markers. Haematologica. 2017;102(3):562–72.

12. Riches JC, Davies JK, McClanahan F, Fatah R, Iqbal S, Agrawal S, Ramsay AG, Gribben JG. T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. Blood [Internet]. 2013 Feb 28 [cited 2020 Oct 13];121(9):1612–21. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3587324/

13. Hanna BS, Roessner PM, Yazdanparast H, Colomer D, Campo E, Kugler S, Yosifov D, Stilgenbauer S, Schmidt M, Gabriel R, Lichter P, Seiffert M. Control of chronic lymphocytic leukemia development by clonally-expanded CD8+ T-cells that undergo functional exhaustion in secondary lymphoid tissues. Leukemia. 2019;33(3):625–37.

14. Gassner FJ, Zaborsky N, Catakovic K, Rebhandl S, Huemer M, Egle A, Hartmann TN, Greil R, Geisberger R. Chronic lymphocytic leukaemia induces an exhausted T cell phenotype in the TCL1 transgenic mouse model. Br J Haematol. 2015 Aug;170(4):515–22.

15. Maïza H, Leca G, Leca G, Schiavon V, Boumsell L, Bensussan, A. A novel 80-kD cell surface structure identifies human circulating lymphocytes with natural killer activity. J Exp Med [Internet]. 1993 Sep 1 [cited 2020 Oct 13];178(3):1121–6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2191151/

16. Tsujimura K, Obata Y, Matsudaira Y, Nishida K, Akatsuka Y, Ito Y, Demachi-Okamura A, Kuzushima K, Takahashi T. Characterization of murine CD160+ CD8+ T lymphocytes. Immunol Lett. 2006 Jul 15;106(1):48–56.

17. Ortonne N, Ram-Wolff C, Giustiniani J, Marie-Cardine A, Bagot M, Mecheri S, Bensussan A. Human and mouse mast cells express and secrete the GPI-anchored isoform of CD160. J Invest Dermatol. 2011 Apr;131(4):916–24.

18. Farren TW, Giustiniani J, Liu F-T, Tsitsikas DA, Macey MG, Cavenagh JD, Oakervee HE, Taussig D, Newland AC, Calaminici M, Bensussan A, Jenner M, Gribben JG, Agrawal SG. Differential and tumor-specific expression of CD160 in B-cell malignancies. Blood. 2011 Aug 25;118(8):2174–83.

19. El-Far M, Pellerin C, Pilote L, Fortin J-F, Lessard IAD, Peretz Y, Wardrop E, Salois P, Bethell RC, Cordingley MG, Kukolj G. CD160 isoforms and regulation of CD4 and CD8 T-cell responses. J Transl Med. 2014 Sep 2; 12:217.

20. Giustiniani J, Marie-Cardine A, Bensussan A. A Soluble Form of the MHC Class I-Specific CD160 Receptor Is Released from Human Activated NK Lymphocytes and Inhibits Cell-Mediated Cytotoxicity. J Immunol [Internet]. 2007 Feb 1 [cited 2020 Oct

13];178(3):1293–300. Available from: https://www.jimmunol.org/content/178/3/1293

21. Maeda M, Carpenito C, Russell RC, Dasanjh J, Veinotte LL, Ohta H, Yamamura T, Tan R, Takei F. Murine CD160, Ig-like receptor on NK cells and NKT cells, recognizes classical and nonclassical MHC class I and regulates NK cell activation. J Immunol Baltim Md 1950. 2005 Oct 1;175(7):4426–32.

22. Tu TC, Brown NK, Kim T-J, Wroblewska J, Yang X, Guo X, Lee SH, Kumar V, Lee K-M, Fu Y-X. CD160 is essential for NK-mediated IFN-γ production. J Exp Med. 2015 Mar 9;212(3):415–29.

23. Rodriguez-Barbosa JI, Schneider P, Weigert A, Lee K-M, Kim T-J, Perez-Simon J-A, del Rio M-L. HVEM, a cosignaling molecular switch, and its interactions with BTLA, CD160 and LIGHT. Cell Mol Immunol [Internet]. 2019 Jul [cited 2020 Oct 13];16(7):679–82. Available from: https://www.nature.com/articles/s41423-019-0241-1

24. Kojima R, Kajikawa M, Shiroishi M, Kuroki K, Maenaka K. Molecular basis for herpesvirus entry mediator recognition by the human immune inhibitory receptor CD160 and its relationship to the cosignaling molecules BTLA and LIGHT. J Mol Biol. 2011 Nov 4;413(4):762–72.

25. Šedý JR, Bjordahl RL, Bekiaris V, Macauley MG, Ware BC, Norris PS, Lurain NS, Benedict CA, Ware CF. CD160 activation by herpesvirus entry mediator augments inflammatory cytokine production and cytolytic function by NK cells. J Immunol Baltim Md 1950. 2013 Jul 15;191(2):828–36.

26. Cai G, Anumanthan A, Brown JA, Greenfield EA, Zhu B, Freeman GJ. CD160 inhibits activation of human CD4 + T cells through interaction with herpesvirus entry mediator. Nat Immunol [Internet]. 2008 Feb [cited 2020 Oct 13];9(2):176–85. Available from: https://www.nature.com/articles/ni1554

27. Peretz Y, He Z, Shi Y, Yassine-Diab B, Goulet J-P, Bordi R, Filali-Mouhim A, Loubert J-B, El-Far M, Dupuy FP, Boulassel MR, Tremblay C, Routy J-P, Bernard N, Balderas R, Haddad EK, Sékaly R-P. CD160 and PD-1 co-expression on HIV-specific CD8 T cells defines a subset with advanced dysfunction. PLoS Pathog. 2012;8(8):e1002840.

28. Tan CL, Peluso MJ, Drijvers JM, Mera CM, Grande SM, Brown KE, Godec J, Freeman GJ, Sharpe AH. CD160 Stimulates CD8+ T Cell Responses and Is Required for Optimal Protective Immunity to Listeria monocytogenes. ImmunoHorizons [Internet]. 2018 Aug 1 [cited 2020 Oct 13];2(7):238–50. Available from: https://www.immunohorizons.org/content/2/7/238

29. del Rio M-L, Bravo Moral AM, Fernandez-Renedo C, Buhler L, Perez-Simon J-A, Chaloin O, Alvarez Nogal R, Fernandez-Caso M, Rodriguez-Barbosa J-I. Modulation of cytotoxic responses by targeting CD160 prolongs skin graft survival across major histocompatibility class I barrier. Transl Res [Internet]. 2017 Mar 1 [cited 2020 Oct

13];181:83-95.e3. Available from: http://www.sciencedirect.com/science/article/pii/S1931524416302018

30. Zhang Y, Liu Y, Liu H, Tang WH. Exosomes: biogenesis, biologic function and clinical potential. Cell Biosci [Internet]. 2019 Feb 15 [cited 2020 Oct 13];9(1):19. Available from: https://doi.org/10.1186/s13578-019-0282-2

31. Whiteside TL. Exosomes carrying immunoinhibitory proteins and their role in cancer. Clin Exp Immunol [Internet]. 2017 [cited 2020 Oct 13];189(3):259–67. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/cei.12974

32. Stages of chronic lymphocytic leukemia (CLL) - Canadian Cancer Society [Internet]. www.cancer.ca. [cited 2021 Feb 18]. Available from: https://www.cancer.ca:443/en/cancer-information/cancer-type/leukemia-chronic-lymphocytic-cll/staging/?region=on

33. Elahi S, Ertelt JM, Kinder JM, Jiang TT, Zhang X, Xin L, Chaturvedi V, Strong BS, Qualls JE, Steinbrecher KA, Kalfa TA, Shaaban AF, Way SS. Immunosuppressive CD71 + erythroid cells compromise neonatal host defence against infection. Nature [Internet]. 2013 Dec [cited 2020 Oct 23];504(7478):158–62. Available from: https://www.nature.com/articles/nature12675

34. Shahbaz S, Dunsmore G, Koleva P, Xu L, Houston S, Elahi S. Galectin-9 and VISTA Expression Define Terminally Exhausted T Cells in HIV-1 Infection. J Immunol Baltim Md 1950. 2020 May 1;204(9):2474–91.

35. Dunsmore G, Bozorgmehr N, Delyea C, Koleva P, Namdar A, Elahi S. Erythroid Suppressor Cells Compromise Neonatal Immune Response against Bordetella pertussis. J Immunol [Internet]. 2017 Aug 4 [cited 2020 Oct 23]; Available from: https://www.jimmunol.org/content/early/2017/08/04/jimmunol.1700742

36. Namdar A, Dunsmore G, Shahbaz S, Koleva P, Xu L, Jovel J, Houston S, Elahi S. CD71+ Erythroid Cells Exacerbate HIV-1 Susceptibility, Mediate trans-Infection, and Harbor Infective Viral Particles. mBio [Internet]. 2019 Dec 24 [cited 2020 Oct 23];10(6). Available from: https://mbio.asm.org/content/10/6/e02767-19

37. Morales-Kastresana A, Telford B, Musich TA, McKinnon K, Clayborne C, Braig Z, Rosner A, Demberg T, Watson DC, Karpova TS, Freeman GJ, DeKruyff RH, Pavlakis GN, Terabe M, Robert-Guroff M, Berzofsky JA, Jones JC. Labeling Extracellular Vesicles for Nanoscale Flow Cytometry. Sci Rep [Internet]. 2017 May 12 [cited 2021 Mar 3];7(1):1878. Available from: https://www.nature.com/articles/s41598-017-01731-2

38. Roederer M, Nozzi JL, Nason MC. SPICE: exploration and analysis of post-cytometric complex multivariate datasets. Cytom Part J Int Soc Anal Cytol. 2011 Feb;79(2):167–74.

39. Yun X, Zhang Y, Wang X. Recent progress of prognostic biomarkers and risk scoring systems in chronic lymphocytic leukemia. Biomark Res [Internet]. 2020 Sep

7 [cited 2021 Mar 3];8(1):40. Available from: https://doi.org/10.1186/s40364-020-00222-3

40. Fuertes Marraco SA, Neubert NJ, Verdeil G, Speiser DE. Inhibitory Receptors Beyond T Cell Exhaustion. Front Immunol. 2015; 6:310.

41. Podack ER, Young JD, Cohn ZA. Isolation and biochemical and functional characterization of perforin 1 from cytolytic T-cell granules. Proc Natl Acad Sci U S A. 1985 Dec;82(24):8629–33.

42. Lieberman J. The ABCs of granule-mediated cytotoxicity: new weapons in the arsenal. Nat Rev Immunol. 2003 May;3(5):361–70.

43. Zhao Y, Shao Q, Peng G. Exhaustion and senescence: two crucial dysfunctional states of T cells in the tumor microenvironment. Cell Mol Immunol. 2020 Jan;17(1):27–35.

44. Viganò S, Banga R, Bellanger F, Pellaton C, Farina A, Comte D, Harari A, Perreau M. CD160-Associated CD8 T-Cell Functional Impairment Is Independent of PD-1 Expression. PLOS Pathog. 2014 Sep 25;10(9):e1004380.

45. Romero P, Zippelius A, Kurth I, Pittet MJ, Touvrey C, Iancu EM, Corthesy P, Devevre E, Speiser DE, Rufer N. Four functionally distinct populations of human effector-memory CD8+ T lymphocytes. J Immunol Baltim Md 1950. 2007 Apr 1;178(7):4112–9.

46. Walker EB, Haley D, Petrausch U, Floyd K, Miller W, Sanjuan N, Alvord G, Fox BA, Urba WJ. Phenotype and functional characterization of long-term gp100-specific memory CD8+ T cells in disease-free melanoma patients before and after boosting immunization. Clin Cancer Res Off J Am Assoc Cancer Res. 2008 Aug 15;14(16):5270–83.

47. Elahi S, Dinges WL, Lejarcegui N, Laing KJ, Collier AC, Koelle DM, McElrath MJ, Horton H. Protective HIV-specific CD8+ T cells evade Treg cell suppression. Nat Med. 2011 Jul 17;17(8):989–95.

48. Lazarevic V, Glimcher LH, Lord GM. T-bet: a bridge between innate and adaptive immunity. Nat Rev Immunol [Internet]. 2013 Nov [cited 2020 Oct 13];13(11):777–89. Available from: https://www.nature.com/articles/nri3536

49. Chen Z, Ji Z, Ngiow SF, Manne S, Cai Z, Huang AC, Johnson J, Staupe RP, Bengsch B, Xu C, Yu S, Kurachi M, Herati RS, Vella LA, Baxter AE, Wu JE, Khan O, Beltra J-C, Giles JR, Stelekati E, McLane LM, Lau CW, Yang X, Berger SL, Vahedi G, Ji H, Wherry EJ. TCF-1-Centered Transcriptional Network Drives an Effector versus Exhausted CD8 T Cell-Fate Decision. Immunity [Internet]. 2019 Nov 19 [cited 2020 Oct 13];51(5):840-855.e5. Available from: http://www.sciencedirect.com/science/article/pii/S1074761319304091

50. Yeh Y-Y, Ozer HG, Lehman AM, Maddocks K, Yu L, Johnson AJ, Byrd JC. Characterization of CLL exosomes reveals a distinct microRNA signature and enhanced secretion by activation of BCR signaling. Blood. 2015 May 21;125(21):3297–305.

51. Yang Z-Z, Grote DM, Ziesmer SC, Niki T, Hirashima M, Novak AJ, Witzig TE, Ansell SM. IL-12 upregulates TIM-3 expression and induces T cell exhaustion in patients with follicular B cell non-Hodgkin lymphoma. J Clin Invest. 2012 Apr;122(4):1271–82.

52. Yan X-J, Dozmorov I, Li W, Yancopoulos S, Sison C, Centola M, Jain P, Allen SL, Kolitz JE, Rai KR, Chiorazzi N, Sherry B. Identification of outcome-correlated cytokine clusters in chronic lymphocytic leukemia. Blood. 2011 Nov 10;118(19):5201–10.

53. Bengsch B, Seigel B, Ruhl M, Timm J, Kuntz M, Blum HE, Pircher H, Thimme R. Coexpression of PD-1, 2B4, CD160 and KLRG1 on exhausted HCV-specific CD8+ T cells is linked to antigen recognition and T cell differentiation. PLoS Pathog. 2010 Jun 10;6(6):e1000947.

54. Liu S, Zhang W, Liu K, Wang Y. CD160 expression on CD8+ T cells is associated with active effector responses but limited activation potential in pancreatic cancer. Cancer Immunol Immunother [Internet]. 2020 May 1 [cited 2020 Oct 13];69(5):789–97. Available from: https://doi.org/10.1007/s00262-020-02500-3

55. He W, Wang B, Li Q, Yao Q, Jia X, Song R, Li S, Zhang J. Aberrant Expressions of Co-stimulatory and Co-inhibitory Molecules in Autoimmune Diseases. Front Immunol [Internet]. 2019 Feb 20 [cited 2020 Oct 13];10. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6391512/

56. Reiser J, Banerjee A. Effector, Memory, and Dysfunctional CD8+ T Cell Fates in the Antitumor Immune Response [Internet]. Vol. 2016, Journal of Immunology Research. Hindawi; 2016 [cited 2020 Oct 24]. p. e8941260. Available from: https://www.hindawi.com/journals/jir/2016/8941260/

57. Jia B, Zhao C, Rakszawski KL, Claxton DF, Ehmann WC, Rybka WB, Mineishi S, Wang M, Shike H, Bayerl MG, Sivik JM, Schell TD, Drabick JJ, Hohl RJ, Zheng H. Eomes $^{+}$ T-bet ^{low} CD8 $^{+}$ T Cells Are Functionally Impaired and Are Associated with Poor Clinical Outcome in Patients with Acute Myeloid Leukemia. Cancer Res [Internet]. 2019 Apr 1 [cited 2020 Oct 24];79(7):1635–45. Available from: http://cancerres.aacrjournals.org/lookup/doi/10.1158/0008-5472.CAN-18-3107

58. Josefsson SE, Huse K, Kolstad A, Beiske K, Pende D, Steen CB, Inderberg EM, Lingjærde OC, Østenstad B, Smeland EB, Levy R, Irish JM, Myklebust JH. T Cells Expressing Checkpoint Receptor TIGIT Are Enriched in Follicular Lymphoma Tumors and Characterized by Reversible Suppression of T-cell Receptor Signaling. Clin Cancer Res Off J Am Assoc Cancer Res. 2018 15;24(4):870–81.

59. Guillerey C, Harjunpää H, Carrié N, Kassem S, Teo T, Miles K, Krumeich S, Weulersse M, Cuisinier M, Stannard K, Yu Y, Minnie Sa, Hill Gr, Dougall Wc, Avet-

Loiseau H, Teng Mwl, Nakamura K, Martinet L, Smyth Mj. TIGIT immune checkpoint blockade restores CD8 + T-cell immunity against multiple myeloma. Blood [Internet]. 2018 Oct 18 [cited 2020 Oct 14];132(16):1689–94. Available from: https://pubmed.ncbi.nlm.nih.gov/29986909/

60. Tan J, Yu Z, Huang J, Chen Y, Huang S, Yao D, Xu L, Lu Y, Chen S, Li Y. Increased PD-1+Tim-3+ exhausted T cells in bone marrow may influence the clinical outcome of patients with AML. Biomark Res [Internet]. 2020 Feb 13 [cited 2021 Mar 5];8(1):6. Available from: https://doi.org/10.1186/s40364-020-0185-8

61. Okoye I, Xu L, Motamedi M, Parashar P, Walker JW, Elahi S. Galectin-9 expression defines exhausted T cells and impaired cytotoxic NK cells in patients with virus-associated solid tumors. J Immunother Cancer [Internet]. 2020 Dec [cited 2021 Mar 5];8(2):e001849. Available from: https://jitc.bmj.com/lookup/doi/10.1136/jitc-2020-001849

62. Krackhardt AM, Witzens M, Harig S, Hodi FS, Zauls AJ, Chessia M, Barrett P, Gribben JG. Identification of tumor-associated antigens in chronic lymphocytic leukemia by SEREX. Blood [Internet]. 2002 Sep 15 [cited 2020 Oct 14];100(6):2123–31. Available from: https://ashpublications.org/blood/article/100/6/2123/16598/Identification-of-tumor-associated-antigens-in

63. Ca K, L G, Np R. Sorting through subsets: which T-cell populations mediate highly effective adoptive immunotherapy? J Immunother Hagerstown Md 1997 [Internet]. 2012 Dec [cited 2020 Oct 14];35(9):651–60. Available from: https://pubmed.ncbi.nlm.nih.gov/23090074/

64. Bonnon C, Wendeler MW, Paccaud J-P, Hauri H-P. Selective export of human GPI-anchored proteins from the endoplasmic reticulum. J Cell Sci [Internet]. 2010 May 15 [cited 2020 Oct 14];123(10):1705–15. Available from: https://jcs.biologists.org/content/123/10/1705

65. Satoa, Ken, Nakano, Akohiko. Mechanisms of COPII vesicle formation and protein sorting - PubMed. FEBS Lett [Internet]. [cited 2020 Oct 14];581(11):2076–82. Available from: https://pubmed.ncbi.nlm.nih.gov/17316621/

66. A L, De S, H P, D Z, Sa FM. Inhibitory Receptor Expression Depends More Dominantly on Differentiation and Activation than "Exhaustion" of Human CD8 T Cells [Internet]. Vol. 4, Frontiers in immunology. Front Immunol; 2013 [cited 2020 Oct 14]. Available from: https://pubmed.ncbi.nlm.nih.gov/24391639/

67. Xie F, Xu M, Lu J, Mao L, Wang S. The role of exosomal PD-L1 in tumor progression and immunotherapy. Mol Cancer [Internet]. 2019 Oct 23 [cited 2020 Oct 13];18(1):146. Available from: https://doi.org/10.1186/s12943-019-1074-3

68. Sabzevary-Ghahfarokhi M, Shirzad H, Rafieian-Kopaei M, Ghatreh-Samani M, Shohan M. The Role of Inflammatory Cytokines in Creating T Cell Exhaustion in Cancer. Cancer Biother Radiopharm. 2018 Sep;33(7):267–73.

69. Alhakeem SS, McKenna MK, Oben KZ, Noothi SK, Rivas JR, Hildebrandt GC, Fleischman RA, Rangnekar VM, Muthusamy N, Bondada S. Chronic Lymphocytic Leukemia–Derived IL-10 Suppresses Antitumor Immunity. J Immunol [Internet]. 2018 Jun 15 [cited 2020 Oct 14];200(12):4180–9. Available from: https://www.jimmunol.org/content/200/12/4180

70. Kitabayashi A, Hirokawa M, Miura AB. The role of interleukin-10 (IL-10) in chronic B-lymphocytic leukemia: IL-10 prevents leukemic cells from apoptotic cell death. Int J Hematol. 1995 Aug;62(2):99–106.

71. Schröttner P, Leick M, Burger M. The role of chemokines in B cell chronic lymphocytic leukaemia: pathophysiological aspects and clinical impact. Ann Hematol. 2010 May;89(5):437–46.

72. Ghamlouch H, Ouled-Haddou H, Damaj G, Royer B, Gubler B, Marolleau J-P. A Combination of Cytokines Rescues Highly Purified Leukemic CLL B-Cells from Spontaneous Apoptosis In Vitro. PLoS ONE [Internet]. 2013 Mar 26 [cited 2020 Oct 14];8(3). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3608602/

73. Schulz A, Toedt G, Zenz T, Stilgenbauer S, Lichter P, Seiffert M. Inflammatory cytokines and signaling pathways are associated with survival of primary chronic lymphocytic leukemia cells in vitro: a dominant role of CCL2. Haematologica. 2011 Mar;96(3):408–16.

74. Baba T, Mukaida N. Role of macrophage inflammatory protein (MIP)-1α/CCL3 in leukemogenesis. Mol Cell Oncol [Internet]. 2014 Jul 15 [cited 2020 Oct 14];1(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4905173/

75. Wierda WG, Johnson MM, Do K-A, Manshouri T, Dey A, O'Brien S, Giles FJ, Kantarjian H, Thomas D, Faderl S, Lerner S, Keating M, Albitar M. Plasma interleukin 8 level predicts for survival in chronic lymphocytic leukaemia. Br J Haematol. 2003 Feb;120(3):452–6.

76. Somasundaram A, Cillo A, Oliveri L, Herman J, Kirkwood J, Ferris RL, Bruno TC, Vignali D. IL-6, IL-8 drive LAG3/PD1 immune suppression on effector and naïve, peripheral blood CD8+ T cells in cancer patients. J Immunol. 2019 May 1;202(1 Supplement):195.9-195.9.

77. Bellomo G, Allegra A, Alonci A, Quartarone E, Pizzoleo MA, Cincotta M, Del Fabro V, Guglielmo S, Musolino C. Serum levels of interleukin-16 in lymphoid malignancies. Leuk Lymphoma. 2007 Jun;48(6):1225–7.

78. Cruikshank WW, Kornfeld H, Center DM. Interleukin-16. J Leukoc Biol [Internet]. 2000 [cited 2020 Oct 24];67(6):757–66. Available from: https://jlb.onlinelibrary.wiley.com/doi/abs/10.1002/jlb.67.6.757

79. Atanackovic D, Hildebrandt Y, Templin J, Cao Y, Keller C, Panse J, Meyer S, Reinhard H, Bartels K, Lajmi N, Sezer O, Zander AR, Marx AH, Uhlig R, Zustin J,

Bokemeyer C, Kröger N. Role of interleukin 16 in multiple myeloma. J Natl Cancer Inst. 2012 Jul 3;104(13):1005–20.

Chapter 3

1. Engel M, Hoffmann T, Wagner L, Wermann M, Heiser U, Kiefersauer R, et al. The crystal structure of dipeptidyl peptidase IV (CD26) reveals its functional regulation and enzymatic mechanism. Proc Natl Acad Sci U S A. 2003;100:5063–8.

2. Yaron A, Naider F, Scharpe S. Proline-Dependent Structural and Biological Properties of Peptides and Proteins. Crit Rev Biochem Mol Biol [Internet]. Taylor & Francis; 1993 [cited 2022 Jun 6];28:31–81. Available from: https://doi.org/10.3109/10409239309082572

3. Ahrén B, Hughes TE. Inhibition of dipeptidyl peptidase-4 augments insulin secretion in response to exogenously administered glucagon-like peptide-1, glucose-dependent insulinotropic polypeptide, pituitary adenylate cyclase-activating polypeptide, and gastrin-releasing peptide in mice. Endocrinology. 2005;146:2055–9.

4. Chitadze G, Wehkamp U, Janssen O, Brüggemann M, Lettau M. The Serine Protease CD26/DPP4 in Non-Transformed and Malignant T Cells. Cancers [Internet]. 2021 [cited 2022 Jun 8];13:5947. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8657226/

5. Abbott CA, Baker E, Sutherland GR, McCaughan GW. Genomic organization, exact localization, and tissue expression of the human CD26 (dipeptidyl peptidase IV) gene. Immunogenetics. 1994;40:331–8.

6. Morimoto C, Torimoto Y, Levinson G, Rudd CE, Schrieber M, Dang NH, et al. 1F7, a novel cell surface molecule, involved in helper function of CD4 cells. J Immunol Baltim Md 1950. 1989;143:3430–9.

7. Bühling F, Junker U, Reinhold D, Neubert K, Jäger L, Ansorge S. Functional role of CD26 on human B lymphocytes. Immunol Lett. 1995;45:47–51.

8. Bühling F, Kunz D, Reinhold D, Ulmer AJ, Ernst M, Flad HD, et al. Expression and functional role of dipeptidyl peptidase IV (CD26) on human natural killer cells. Nat Immun. 1994;13:270–9.

9. Gliddon DR, Howard CJ. CD26 is expressed on a restricted subpopulation of dendritic cells in vivo. Eur J Immunol. 2002;32:1472–81.

10. Shah Z, Kampfrath T, Deiuliis JA, Zhong J, Pineda C, Ying Z, et al. Long-term dipeptidyl-peptidase 4 inhibition reduces atherosclerosis and inflammation via effects on monocyte recruitment and chemotaxis. Circulation. 2011;124:2338–49.

11. Shahbaz S, Xu L, Osman M, Sligl W, Shields J, Joyce M, et al. Erythroid precursors and progenitors suppress adaptive immunity and get invaded by SARS-CoV-2. Stem Cell Rep. 2021;16:1165–81.

12. Dang NH, Torimoto Y, Shimamura K, Tanaka T, Daley JF, Schlossman SF, et al. 1F7 (CD26): a marker of thymic maturation involved in the differential regulation of the CD3 and CD2 pathways of human thymocyte activation. J Immunol Baltim Md 1950. 1991;147:2825–32.

13. Yan S, Marguet D, Dobers J, Reutter W, Fan H. Deficiency of CD26 results in a change of cytokine and immunoglobulin secretion after stimulation by pokeweed mitogen. Eur J Immunol. 2003;33:1519–27.

14. Pinheiro MM, Stoppa CL, Valduga CJ, Okuyama CE, Gorjão R, Pereira RMS, et al. Sitagliptin inhibit human lymphocytes proliferation and Th1/Th17 differentiation in vitro. Eur J Pharm Sci Off J Eur Fed Pharm Sci. 2017;100:17–24.

15. Yegutkin GG. Nucleotide- and nucleoside-converting ectoenzymes: Important modulators of purinergic signalling cascade. Biochim Biophys Acta. 2008;1783:673–94.

16. Dong RP, Kameoka J, Hegen M, Tanaka T, Xu Y, Schlossman SF, et al. Characterization of adenosine deaminase binding to human CD26 on T cells and its biologic role in immune response. J Immunol [Internet]. American Association of Immunologists; 1996 [cited 2022 Jun 6];156:1349–55. Available from: https://www.jimmunol.org/content/156/4/1349

17. Kameoka J, Tanaka T, Nojima Y, Schlossman SF, Morimoto C. Direct association of adenosine deaminase with a T cell activation antigen, CD26. Science. 1993;261:466–9.

18. Martín M, Huguet J, Centelles JJ, Franco R. Expression of ecto-adenosine deaminase and CD26 in human T cells triggered by the TCR-CD3 complex. Possible role of adenosine deaminase as costimulatory molecule. J Immunol Baltim Md 1950. 1995;155:4630–43.

19. Ohnuma K, Uchiyama M, Yamochi T, Nishibashi K, Hosono O, Takahashi N, et al. Caveolin-1 triggers T-cell activation via CD26 in association with CARMA1. J Biol Chem. 2007;282:10117–31.

20. Rai AK, Thakur CP, Kumar P, Mitra DK. Impaired expression of CD26 compromises T-cell recruitment in human visceral leishmaniasis. Eur J Immunol [Internet]. 2012 [cited 2022 Jun 8];42:2782–91. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/eji.201141912

21. Am L, P P, C D, G B, K S, K A, et al. Kinetic investigation of chemokine truncation by CD26/dipeptidyl peptidase IV reveals a striking selectivity within the chemokine family. J Biol Chem [Internet]. J Biol Chem; 2001 [cited 2022 Jun 6];276. Available from: https://pubmed.ncbi.nlm.nih.gov/11390394/

22. Mortier A, Gouwy M, Van Damme J, Proost P, Struyf S. CD26/dipeptidylpeptidase IV—chemokine interactions: double-edged regulation of inflammation and tumor biology. J Leukoc Biol [Internet]. 2016 [cited 2022 Jun 29];99:955–69. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7166560/

23. Cheng H-C, Abdel-Ghany M, Pauli BU. A novel consensus motif in fibronectin mediates dipeptidyl peptidase IV adhesion and metastasis. J Biol Chem. 2003;278:24600–7.

24. Brezinschek RI, Lipsky PE, Galea P, Vita R, Oppenheimer-Marks N. Phenotypic characterization of CD4+ T cells that exhibit a transendothelial migratory capacity. J Immunol Baltim Md 1950. 1995;154:3062–77.

25. Bengsch B, Seigel B, Flecken T, Wolanski J, Blum HE, Thimme R. Human Th17 cells express high levels of enzymatically active dipeptidylpeptidase IV (CD26). J Immunol Baltim Md 1950. 2012;188:5438–47.

26. Bailey SR, Nelson MH, Majchrzak K, Bowers JS, Wyatt MM, Smith AS, et al. Human CD26high T cells elicit tumor immunity against multiple malignancies via enhanced migration and persistence. Nat Commun [Internet]. Nature Publishing Group; 2017 [cited 2022 Jun 15];8:1961. Available from: https://www.nature.com/articles/s41467-017-01867-9

27. Nelson MH, Knochelmann HM, Bailey SR, Huff LW, Bowers JS, Majchrzak-Kuligowska K, et al. Identification of human CD4+ T cell populations with distinct antitumor activity. Sci Adv [Internet]. 2020 [cited 2022 Jun 8];6:eaba7443. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7458458/

28. Hatano R, Ohnuma K, Yamamoto J, Dang NH, Morimoto C. CD26-mediated co-stimulation in human CD8(+) T cells provokes effector function via pro-inflammatory cytokine production. Immunology. 2013;138:165–72.

29. Yamada Y, Jang J, Meester ID, Inci I, Weder W, Jungraithmayr W. Acute Allograft Rejection Is Attenuated By CD26-Inhibition Through IL-17 Suppression in Mouse Lung Transplants. J Heart Lung Transplant [Internet]. Elsevier; 2015 [cited 2022 Jun 8];34:S52. Available from: https://www.jhltonline.org/article/S1053-2498(15)00157-6/fulltext

30. Yamada Y, Jang J-H, Meester ID, Baerts L, Vliegen G, Inci I, et al. CD26 costimulatory blockade improves lung allograft rejection and is associated with enhanced interleukin-10 expression. J Heart Lung Transplant [Internet]. Elsevier; 2016 [cited 2022 Jun 8];35:508–17. Available from: https://www.jhltonline.org/article/S1053-2498(15)01519-3/fulltext

31. Zhao X, Zhang K, Daniel P, Wisbrun N, Fuchs H, Fan H. Delayed allogeneic skin graft rejection in CD26-deficient mice. Cell Mol Immunol [Internet]. 2019 [cited 2022 Jun 8];16:557–67. Available from: https://europepmc.org/articles/PMC6804736

32. Jungraithmayr W, Enz N. CD26 – The emerging role of a costimulatory molecule in allograft rejection. Cell Mol Immunol [Internet]. Nature Publishing Group; 2020 [cited 2022 Jun 8];17:1208–9. Available from: https://www.nature.com/articles/s41423-018-0028-9

33. Ibegbu CC, Xu Y-X, Fillos D, Radziewicz H, Grakoui A, Kourtis AP. Differential expression of CD26 on virus-specific CD8(+) T cells during active, latent and resolved infection. Immunology. 2009;126:346–53.

34. Vanham G, Kestens L, De Meester I, Vingerhoets J, Penne G, Vanhoof G, et al. Decreased expression of the memory marker CD26 on both CD4+ and CD8+ T lymphocytes of HIV-infected subjects. J Acquir Immune Defic Syndr. 1993;6:749–57.

35. Morrison ME, Vijayasaradhi S, Engelstein D, Albino AP, Houghton AN. A marker for neoplastic progression of human melanocytes is a cell surface ectopeptidase. J Exp Med. 1993;177:1135–43.

36. Sharma PK, Wong EB, Napier RJ, Bishai WR, Ndung'u T, Kasprowicz VO, et al. High expression of CD26 accurately identifies human bacteria-reactive MR1-restricted MAIT cells. Immunology. 2015;145:443–53.

37. Treiner E, Duban L, Bahram S, Radosavljevic M, Wanner V, Tilloy F, et al. Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. Nature. 2003;422:164–9.

38. Porcelli S, Yockey CE, Brenner MB, Balk SP. Analysis of T cell antigen receptor (TCR) expression by human peripheral blood CD4-8- alpha/beta T cells demonstrates preferential use of several V beta genes and an invariant TCR alpha chain. J Exp Med. 1993;178:1–16.

39. Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, Liu L, et al. MR1 presents microbial vitamin B metabolites to MAIT cells. Nature. 2012;491:717–23.

40. Dusseaux M, Martin E, Serriari N, Péguillet I, Premel V, Louis D, et al. Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting T cells. Blood. 2011;117:1250–9.

41. Fergusson JR, Smith KE, Fleming VM, Rajoriya N, Newell EW, Simmons R, et al. CD161 Defines a Transcriptional and Functional Phenotype across Distinct Human T Cell Lineages. Cell Rep [Internet]. 2014 [cited 2022 Jun 1];9:1075–88. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2211124714008286

42. Seifert M, Sellmann L, Bloehdorn J, Wein F, Stilgenbauer S, Dürig J, et al. Cellular origin and pathophysiology of chronic lymphocytic leukemia. J Exp Med. 2012;209:2183–98.

43. Allegra A, Tonacci A, Musolino C, Pioggia G, Gangemi S. Secondary Immunodeficiency in Hematological Malignancies: Focus on Multiple Myeloma and

Chronic Lymphocytic Leukemia. Front Immunol [Internet]. 2021 [cited 2022 Jun 17];12. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2021.738915

44. Riches JC, Davies JK, McClanahan F, Fatah R, Iqbal S, Agrawal S, et al. T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. Blood [Internet]. 2013 [cited 2022 Jun 15];121:1612–21. Available from: https://doi.org/10.1182/blood-2012-09-457531

45. Bozorgmehr N, Okoye I, Oyegbami O, Xu L, Fontaine A, Cox-Kennett N, et al. Expanded antigen-experienced CD160+CD8+effector T cells exhibit impaired effector functions in chronic lymphocytic leukemia. J Immunother Cancer. 2021;9:e002189.

46. Wang Y, Achenbach SJ, Rabe KG, Shanafelt TD, Call TG, Ding W, et al. Cause of death in patients with newly diagnosed chronic lymphocytic leukemia (CLL) stratified by the CLL-International Prognostic Index. Blood Cancer J [Internet]. Nature Publishing Group; 2021 [cited 2022 Jun 10];11:1–4. Available from: https://www.nature.com/articles/s41408-021-00532-1

47. Molica S. Second neoplasms in chronic lymphocytic leukemia: incidence and pathogenesis with emphasis on the role of different therapies. Leuk Lymphoma. 2005;46:49–54.

48. Christopoulos P, Pfeifer D, Bartholomé K, Follo M, Timmer J, Fisch P, et al. Definition and characterization of the systemic T-cell dysregulation in untreated indolent B-cell lymphoma and very early CLL. Blood. 2011;117:3836–46.

49. Landego I, Hewitt D, Hibbert I, Dhaliwal D, Pieterse W, Grenier D, et al. PD-1 inhibition in malignant melanoma and lack of clinical response in chronic lymphocytic leukemia in the same patients: a case series. Curr Oncol [Internet]. 2020 [cited 2022 Jun 29];27:169–72. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7339846/

50. Mancikova V, Smida M. Current State of CAR T-Cell Therapy in Chronic Lymphocytic Leukemia. Int J Mol Sci [Internet]. 2021 [cited 2022 Jun 11];22:5536. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8197365/

51. Lee S. Stages of chronic lymphocytic leukemia (CLL) [Internet]. Can. Cancer Soc. [cited 2022 Jun 1]. Available from: https://cancer.ca/en/cancer-information/cancer-types/chronic-lymphocytic-leukemia-cll/staging

52. Bozorgmehr N, Mashhouri S, Perez Rosero E, Xu L, Shahbaz S, Sligl W, et al. Galectin-9, a Player in Cytokine Release Syndrome and a Surrogate Diagnostic Biomarker in SARS-CoV-2 Infection. mBio [Internet]. 2021 [cited 2023 Jan 6];12:e00384-21. Available from: https://journals.asm.org/doi/10.1128/mBio.00384-21

53. Shahbaz S, Dunsmore G, Koleva P, Xu L, Houston S, Elahi S. Galectin-9 and VISTA Expression Define Terminally Exhausted T Cells in HIV-1 Infection. J Immunol

[Internet]. American Association of Immunologists; 2020 [cited 2022 Jun 29]; Available from: https://www.jimmunol.org/content/early/2020/03/20/jimmunol.1901481

54. Elahi S, Shahbaz S, Houston S. Selective Upregulation of CTLA-4 on CD8+ T Cells Restricted by HLA-B*35Px Renders them to an Exhausted Phenotype in HIV-1 infection. PLOS Pathog [Internet]. Public Library of Science; 2020 [cited 2022 Jul 29];16:e1008696. Available from: https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1008696

55. Shahbaz S, Okoye I, Blevins G, Elahi S. Elevated ATP via enhanced miRNA-30b, 30c, and 30e downregulates the expression of CD73 in CD8+ T cells of HIV-infected individuals. PLOS Pathog [Internet]. Public Library of Science; 2022 [cited 2022 Jun 29];18:e1010378. Available from: https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1010378

56. Dunsmore G, Rosero EP, Shahbaz S, Santer DM, Jovel J, Lacy P, et al. Neutrophils promote T-cell activation through the regulated release of CD44-bound Galectin-9 from the cell surface during HIV infection. PLOS Biol [Internet]. Public Library of Science; 2021 [cited 2022 Jun 29];19:e3001387. Available from: https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.3001387

57. Li M, Yao D, Zeng X, Kasakovski D, Zhang Y, Chen S, et al. Age related human T cell subset evolution and senescence. Immun Ageing [Internet]. 2019 [cited 2022 Jun 1];16:24. Available from: https://doi.org/10.1186/s12979-019-0165-8

58. Sulda ML, Abbott CA, Macardle PJ, Hall RK, Kuss BJ. Expression and prognostic assessment of dipeptidyl peptidase IV and related enzymes in B-cell chronic lymphocytic leukemia. Cancer Biol Ther. 2010;10:180–9.

59. Bauvois B, De Meester I, Dumont J, Rouillard D, Zhao HX, Bosmans E. Constitutive expression of CD26/dipeptidylpeptidase IV on peripheral blood B lymphocytes of patients with B chronic lymphocytic leukaemia. Br J Cancer. 1999;79:1042–8.

60. Sallusto F, Lenig D, Förster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature. 1999;401:708–12.

61. Shahbaz S, Xu L, Sligl W, Osman M, Bozorgmehr N, Mashhouri S, et al. The Quality of SARS-CoV-2-Specific T Cell Functions Differs in Patients with Mild/Moderate versus Severe Disease, and T Cells Expressing Coinhibitory Receptors Are Highly Activated. J Immunol Baltim Md 1950. 2021;207:1099–111.

62. Lugli E, Gattinoni L. Harnessing Stem Cell-Like Memory T Cells for Adoptive Cell Transfer Therapy of Cancer. In: Ascierto PA, Stroncek DF, Wang E, editors. Dev T Cell Based Cancer Immunother [Internet]. Cham: Springer International Publishing; 2015 [cited 2022 Oct 11]. p. 183–209. Available from: https://doi.org/10.1007/978-3-319-21167-1_8

63. Mahnke YD, Brodie TM, Sallusto F, Roederer M, Lugli E. The who's who of T-cell differentiation: Human memory T-cell subsets. Eur J Immunol [Internet]. 2013 [cited 2022 Jun 1];43:2797–809. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/eji.201343751

64. Song D-G, Ye Q, Poussin M, Harms GM, Figini M, Powell DJ. CD27 costimulation augments the survival and antitumor activity of redirected human T cells in vivo. Blood. 2012;119:696–706.

65. Gherardin NA, Souter MN, Koay H-F, Mangas KM, Seemann T, Stinear TP, et al. Human blood MAIT cell subsets defined using MR1 tetramers. Immunol Cell Biol. 2018;96:507–25.

66. Allard B, Allard D, Buisseret L, Stagg J. The adenosine pathway in immuno-oncology. Nat Rev Clin Oncol [Internet]. Nature Publishing Group; 2020 [cited 2022 Jun 1];17:611–29. Available from: https://www.nature.com/articles/s41571-020-0382-2

67. Raskov H, Orhan A, Christensen JP, Gögenur I. Cytotoxic CD8+ T cells in cancer and cancer immunotherapy. Br J Cancer [Internet]. Nature Publishing Group; 2021 [cited 2022 Jun 1];124:359–67. Available from: https://www.nature.com/articles/s41416-020-01048-4

68. Elahi S, Dinges WL, Lejarcegui N, Laing KJ, Collier AC, Koelle DM, et al. Protective HIV-specific CD8+ T cells evade Treg cell suppression. Nat Med. 2011;17:989–95.

69. Trapani JA, Smyth MJ. Functional significance of the perforin/granzyme cell death pathway. Nat Rev Immunol. 2002;2:735–47.

70. Lopez JA, Susanto O, Jenkins MR, Lukoyanova N, Sutton VR, Law RHP, et al. Perforin forms transient pores on the target cell plasma membrane to facilitate rapid access of granzymes during killer cell attack. Blood. 2013;121:2659–68.

71. Peters PJ, Borst J, Oorschot V, Fukuda M, Krähenbühl O, Tschopp J, et al. Cytotoxic T lymphocyte granules are secretory lysosomes, containing both perforin and granzymes. J Exp Med. 1991;173:1099–109.

72. Mousset CM, Hobo W, Woestenenk R, Preijers F, Dolstra H, van der Waart AB. Comprehensive Phenotyping of T Cells Using Flow Cytometry. Cytom Part J Int Soc Anal Cytol. 2019;95:647–54.

73. Sekine T, Perez-Potti A, Nguyen S, Gorin J-B, Wu VH, Gostick E, et al. TOX is expressed by exhausted and polyfunctional human effector memory CD8+ T cells. Sci Immunol. 2020;5:eaba7918.

74. Slaney CY, Kershaw MH, Darcy PK. Trafficking of T cells into tumors. Cancer Res. 2014;74:7168–74.

75. Stein JV, Nombela-Arrieta C. Chemokine control of lymphocyte trafficking: a general overview. Immunology [Internet]. 2005 [cited 2022 Jun 2];116:1–12. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1802414/

76. Wang C, Kang SG, Lee J, Sun Z, Kim CH. The roles of CCR6 in migration of Th17 cells and regulation of effector T-cell balance in the gut. Mucosal Immunol [Internet]. Nature Publishing Group; 2009 [cited 2022 Jun 2];2:173–83. Available from: https://www.nature.com/articles/mi200884

77. Hamann A, Andrew DP, Jablonski-Westrich D, Holzmann B, Butcher EC. Role of alpha 4-integrins in lymphocyte homing to mucosal tissues in vivo. J Immunol [Internet]. American Association of Immunologists; 1994 [cited 2022 Jun 2];152:3282–93. Available from: https://www.jimmunol.org/content/152/7/3282

78. Baekkevold ES, Yamanaka T, Palframan RT, Carlsen HS, Reinholt FP, von Andrian UH, et al. The CCR7 ligand elc (CCL19) is transcytosed in high endothelial venules and mediates T cell recruitment. J Exp Med. 2001;193:1105–12.

79. Picker LJ, Michie SA, Rott LS, Butcher EC. A unique phenotype of skin-associated lymphocytes in humans. Preferential expression of the HECA-452 epitope by benign and malignant T cells at cutaneous sites. Am J Pathol. 1990;136:1053–68.

80. Mackay LK, Braun A, Macleod BL, Collins N, Tebartz C, Bedoui S, et al. Cutting edge: CD69 interference with sphingosine-1-phosphate receptor function regulates peripheral T cell retention. J Immunol Baltim Md 1950. 2015;194:2059–63.

81. Remmerswaal EBM, Hombrink P, Nota B, Pircher H, Ten Berge IJM, van Lier RAW, et al. Expression of IL-7Rα and KLRG1 defines functionally distinct CD8+ T-cell populations in humans. Eur J Immunol. 2019;49:694–708.

82. Mezawa Y, Daigo Y, Takano A, Miyagi Y, Yokose T, Yamashita T, et al. CD26 expression is attenuated by TGF-β and SDF-1 autocrine signaling on stromal myofibroblasts in human breast cancers. Cancer Med. 2019;8:3936–48.

83. Kashio Y, Nakamura K, Abedin MJ, Seki M, Nishi N, Yoshida N, et al. Galectin-9 Induces Apoptosis Through the Calcium-Calpain-Caspase-1 Pathway. J Immunol [Internet]. American Association of Immunologists; 2003 [cited 2022 Jul 20];170:3631–6. Available from: https://www.jimmunol.org/content/170/7/3631

84. Lhuillier C, Barjon C, Niki T, Gelin A, Praz F, Morales O, et al. Impact of Exogenous Galectin-9 on Human T Cells. J Biol Chem [Internet]. 2015 [cited 2022 Jul 20];290:16797–811. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4505427/

85. Okoye I, Xu L, Motamedi M, Parashar P, Walker JW, Elahi S. Galectin-9 expression defines exhausted T cells and impaired cytotoxic NK cells in patients with virus-associated solid tumors. J Immunother Cancer. 2020;8:e001849.

86. Rahimpour A, Koay HF, Enders A, Clanchy R, Eckle SBG, Meehan B, et al. Identification of phenotypically and functionally heterogeneous mouse mucosal-associated invariant T cells using MR1 tetramers. J Exp Med. 2015;212:1095–108.

87. Okoye IS, Houghton M, Tyrrell L, Barakat K, Elahi S. Coinhibitory Receptor Expression and Immune Checkpoint Blockade: Maintaining a Balance in CD8+ T Cell Responses to Chronic Viral Infections and Cancer. Front Immunol. 2017;8:1215.

88. Hendriks J, Xiao Y, Borst J. CD27 promotes survival of activated T cells and complements CD28 in generation and establishment of the effector T cell pool. J Exp Med. 2003;198:1369–80.

89. Ochsenbein AF, Riddell SR, Brown M, Corey L, Baerlocher GM, Lansdorp PM, et al. CD27 Expression Promotes Long-Term Survival of Functional Effector–Memory CD8+Cytotoxic T Lymphocytes in HIV-infected Patients. J Exp Med [Internet]. 2004 [cited 2022 Jun 29];200:1407–17. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2211945/

90. Leng T, Akther HD, Hackstein C-P, Powell K, King T, Friedrich M, et al. TCR and Inflammatory Signals Tune Human MAIT Cells to Exert Specific Tissue Repair and Effector Functions. Cell Rep. 2019;28:3077-3091.e5.

91. Kahan SM, Bakshi RK, Ingram JT, Hendrickson RC, Lefkowitz EJ, Crossman DK, et al. Intrinsic IL-2 production by effector CD8 T cells affects IL-2 signaling and promotes fate decisions, stemness, and protection. Sci Immunol [Internet]. American Association for the Advancement of Science; 2022 [cited 2022 Jun 14];7:eabl6322. Available from: https://www.science.org/doi/10.1126/sciimmunol.abl6322

92. Bouwman AC, van Daalen KR, Crnko S, ten Broeke T, Bovenschen N. Intracellular and Extracellular Roles of Granzyme K. Front Immunol [Internet]. 2021 [cited 2022 Jun 14];12. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2021.677707

93. Lückel C, Picard FelixSR, Huber M. Tc17 biology and function: Novel concepts. Eur J Immunol [Internet]. 2020 [cited 2022 Jun 17];50:1257–67. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/eji.202048627

94. Leeansyah E, Boulouis C, Kwa ALH, Sandberg JK. Emerging Role for MAIT Cells in Control of Antimicrobial Resistance. Trends Microbiol [Internet]. 2021 [cited 2022 Jun 15];29:504–16. Available from: https://www.sciencedirect.com/science/article/pii/S0966842X20303140

95. Godfrey DI, Koay H-F, McCluskey J, Gherardin NA. The biology and functional importance of MAIT cells. Nat Immunol [Internet]. Nature Publishing Group; 2019 [cited 2022 Jun 17];20:1110–28. Available from: https://www.nature.com/articles/s41590-019-0444-8

96. Murata K, Tsukahara T, Emori M, Shibayama Y, Mizushima E, Matsumiya H, et al. Identification of a novel human memory T-cell population with the characteristics of

stem-like chemo-resistance. OncoImmunology [Internet]. Taylor & Francis; 2016 [cited 2022 Jun 17];5:e1165376. Available from: https://doi.org/10.1080/2162402X.2016.1165376

97. Walling BL, Kim M. LFA-1 in T Cell Migration and Differentiation. Front Immunol [Internet]. 2018 [cited 2022 Jun 29];9. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2018.00952

98. Pangrazzi L, Reidla J, Carmona Arana JA, Naismith E, Miggitsch C, Meryk A, et al. CD28 and CD57 define four populations with distinct phenotypic properties within human CD8+ T cells. Eur J Immunol [Internet]. 2020 [cited 2022 Jun 29];50:363–79. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7079235/

99. Mou D, Espinosa J, Lo DJ, Kirk AD. CD28 Negative T cells: is their loss our gain? Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg [Internet]. 2014 [cited 2022 Jun 17];14:2460–6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4886707/

100. Strioga M, Pasukoniene V, Characiejus D. CD8+ CD28− and CD8+ CD57+ T cells and their role in health and disease. Immunology [Internet]. 2011 [cited 2022 Jun 17];134:17–32. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3173691/

101. Pang R, Law WL, Chu ACY, Poon JT, Lam CSC, Chow AKM, et al. A subpopulation of CD26+ cancer stem cells with metastatic capacity in human colorectal cancer. Cell Stem Cell. 2010;6:603–15.

102. Barreira da Silva R, Laird ME, Yatim N, Fiette L, Ingersoll MA, Albert ML. Dipeptidylpeptidase 4 inhibition enhances lymphocyte trafficking, improving both naturally occurring tumor immunity and immunotherapy. Nat Immunol. 2015;16:850–8.

103. Wang H, Liu X, Long M, Huang Y, Zhang L, Zhang R, et al. NRF2 activation by antioxidant antidiabetic agents accelerates tumor metastasis. Sci Transl Med. 2016;8:334ra51.

104. Detel D, Baticic L, Varljen J. The influence of age on intestinal dipeptidyl peptidase IV (DPP IV/CD26), disaccharidases, and alkaline phosphatase enzyme activity in C57BL/6 mice. Exp Aging Res. 2008;34:49–62

Chapter 4

1. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumors. Nat Rev Immunol [Internet]. 2012 [cited 2023 Feb 2];12:253–68. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3587148/

2. Zhao L, He R, Long H, Guo B, Jia Q, Qin D, et al. Late-stage tumors induce anemia and immunosuppressive extramedullary erythroid progenitor cells. Nat Med [Internet]. 2018 [cited 2023 Mar 11];24:1536–44. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6211844/

3. Pathria P, Louis TL, Varner JA. Targeting Tumor-Associated Macrophages in Cancer. Trends Immunol. 2019;40:310–27.

4. Wu W-C, Sun H-W, Chen H-T, Liang J, Yu X-J, Wu C, et al. Circulating hematopoietic stem and progenitor cells are myeloid-biased in cancer patients. Proc Natl Acad Sci U S A. 2014;111:4221–6.

5. Elahi S. Neglected Cells: Immunomodulatory Roles of CD71+ Erythroid Cells. Trends Immunol. 2019;40:181–5.

6. Anderson NM, Simon MC. The tumor microenvironment. Curr Biol [Internet]. 2020 [cited 2023 Feb 9];30:R921–5. Available from: https://www.sciencedirect.com/science/article/pii/S0960982220309337

7. Okoye IS, Houghton M, Tyrrell L, Barakat K, Elahi S. Coinhibitory Receptor Expression and Immune Checkpoint Blockade: Maintaining a Balance in CD8+ T Cell Responses to Chronic Viral Infections and Cancer. Front Immunol [Internet]. 2017 [cited 2023 Feb 9];8. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2017.01215

8. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. Nat Rev Immunol [Internet]. Nature Publishing Group; 2020 [cited 2023 Feb 9];20:651–68. Available from: https://www.nature.com/articles/s41577-020-0306-5

9. Ribas A, Wolchok JD. Cancer Immunotherapy Using Checkpoint Blockade. Science [Internet]. 2018 [cited 2023 Feb 9];359:1350–5. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7391259/

10. Groth C, Hu X, Weber R, Fleming V, Altevogt P, Utikal J, et al. Immunosuppression mediated by myeloid-derived suppressor cells (MDSCs) during tumor progression. Br J Cancer [Internet]. Nature Publishing Group; 2019 [cited 2023 Feb 2];120:16–25. Available from: https://www.nature.com/articles/s41416-018-0333-1

11. Bronte V, Serafini P, Apolloni E, Zanovello P. Tumor-induced immune dysfunctions caused by myeloid suppressor cells. J Immunother Hagerstown Md 1997. 2001;24:431–46.

12. Bronte V, Brandau S, Chen S-H, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. Nat Commun [Internet]. 2016 [cited 2023 Feb 13];7:12150. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4935811/

13. Hou A, Hou K, Huang Q, Lei Y, Chen W. Targeting Myeloid-Derived Suppressor Cell, a Promising Strategy to Overcome Resistance to Immune Checkpoint Inhibitors. Front Immunol. 2020;11:783.

14. Li X, Zhong J, Deng X, Guo X, Lu Y, Lin J, et al. Targeting Myeloid-Derived Suppressor Cells to Enhance the Anti-tumor Efficacy of Immune Checkpoint Blockade Therapy. Front Immunol [Internet]. 2021 [cited 2023 Feb 27];12. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2021.754196

15. Otter S, Whitaker S, Chatterjee J, Stewart A. The Human Papillomavirus as a Common Pathogen in Oropharyngeal, Anal and Cervical Cancers. Clin Oncol [Internet]. 2019 [cited 2023 Feb 9];31:81–90. Available from: https://www.sciencedirect.com/science/article/pii/S0936655518304655

16. Lechner M, Liu J, Masterson L, Fenton TR. HPV-associated oropharyngeal cancer: epidemiology, molecular biology, and clinical management. Nat Rev Clin Oncol [Internet]. Nature Publishing Group; 2022 [cited 2023 Mar 5];19:306–27. Available from: https://www.nature.com/articles/s41571-022-00603-7

17. Gao P, Lazare C, Cao C, Meng Y, Wu P, Zhi W, et al. Immune checkpoint inhibitors in the treatment of virus-associated cancers. J Hematol OncolJ Hematol Oncol [Internet]. 2019 [cited 2023 Feb 9];12:58. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6558794/

18. Liu C, Lu J, Tian H, Du W, Zhao L, Feng J, et al. Increased expression of PD-L1 by the human papillomavirus 16 E7 oncoprotein inhibits anticancer immunity. Mol Med Rep. 2017;15:1063–70.

19. Kuss-Duerkop SK, Westrich JA, Pyeon D. DNA Tumor Virus Regulation of Host DNA Methylation and Its Implications for Immune Evasion and Oncogenesis. Viruses. 2018;10:82.

20. Qi L, Li N, Lin A, Wang X, Cong J. Efficacy and safety of pembrolizumab on cervical cancer: A systematic review and single-arm meta-analysis. Front Oncol [Internet]. 2022 [cited 2023 Feb 9];12:910486. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9399507/

21. Li X, Su X, Liu R, Pan Y, Fang J, Cao L, et al. HDAC inhibition potentiates the anti-tumor activity of macrophages and enhances anti-PD-L1-mediated tumor suppression. Oncogene [Internet]. Nature Publishing Group; 2021 [cited 2023 Feb 14];40:1836–50. Available from: https://www.nature.com/articles/s41388-020-01636-x

22. Duenas-Gonzalez A, Candelaria M, Perez-Plascencia C, Perez-Cardenas E, de la Cruz-Hernandez E, Herrera LA. Valproic acid as epigenetic cancer drug: preclinical, clinical and transcriptional effects on solid tumors. Cancer Treat Rev. 2008;34:206–22.

23. Suraweera A, O'Byrne KJ, Richard DJ. Combination Therapy With Histone Deacetylase Inhibitors (HDACi) for the Treatment of Cancer: Achieving the Full Therapeutic Potential of HDACi. Front Oncol [Internet]. 2018 [cited 2022 Sep 3];8. Available from: https://www.frontiersin.org/articles/10.3389/fonc.2018.00092

24. Smith KT, Workman JL. Histone deacetylase inhibitors: anticancer compounds. Int J Biochem Cell Biol. 2009;41:21–5.

25. Cinatl J, Kotchetkov R, Blaheta R, Driever PH, Vogel JU, Cinatl J. Induction of differentiation and suppression of malignant phenotype of human neuroblastoma BE(2)-C cells by valproic acid: enhancement by combination with interferon-alpha. Int J Oncol. 2002;20:97–106.

26. Kawagoe R, Kawagoe H, Sano K. Valproic acid induces apoptosis in human leukemia cells by stimulating both caspase-dependent and -independent apoptotic signaling pathways. Leuk Res. 2002;26:495–502.

27. Feng S, Yang Y, Lv J, Sun L, Liu M. Valproic acid exhibits different cell growth arrest effects in three HPV-positive/negative cervical cancer cells and possibly via inducing Notch1 cleavage and E6 downregulation. Int J Oncol. 2016;49:422–30.

28. Mora-García M de L, Duenas-González A, Hernández-Montes J, De la Cruz-Hernández E, Pérez-Cárdenas E, Weiss-Steider B, et al. Up-regulation of HLA class-I antigen expression and antigen-specific CTL response in cervical cancer cells by the demethylating agent hydralazine and the histone deacetylase inhibitor valproic acid. J Transl Med [Internet]. 2006 [cited 2023 Feb 9];4:55. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1781077/

29. Soria-Castro R, Schcolnik-Cabrera A, Rodríguez-López G, Campillo-Navarro M, Puebla-Osorio N, Estrada-Parra S, et al. Exploring the Drug Repurposing Versatility of Valproic Acid as a Multifunctional Regulator of Innate and Adaptive Immune Cells. J Immunol Res. 2019;2019:9678098.

30. Okoye I, Xu L, Motamedi M, Parashar P, Walker JW, Elahi S. Galectin-9 expression defines exhausted T cells and impaired cytotoxic NK cells in patients with virus-associated solid tumors. J Immunother Cancer. 2020;8:e001849.
31. Seymour L, Bogaerts J, Perrone A, Ford R, Schwartz LH, Mandrekar S, et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. Lancet Oncol [Internet]. 2017 [cited 2023 Jan 23];18:e143–52. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5648544/

32. Shahbaz S, Jovel J, Elahi S. Differential transcriptional and functional properties of regulatory T cells in HIV-infected individuals on antiretroviral therapy and long-term non-progressors. Clin Transl Immunol. 2021;10:e1289.

33. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun. 2019;10:1523.

34. Newman AM, Steen CB, Liu CL, Gentles AJ, Chaudhuri AA, Scherer F, et al. Determining cell-type abundance and expression from bulk tissues with digital cytometry. Nat Biotechnol [Internet]. 2019 [cited 2023 Jan 23];37:773–82. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6610714/

35. Nadel BB, Oliva M, Shou BL, Mitchell K, Ma F, Montoya DJ, et al. Systematic evaluation of transcriptomics-based deconvolution methods and references using thousands of clinical samples. Brief Bioinform. 2021;22:bbab265.

36. Bozorgmehr N, Okoye I, Oyegbami O, Xu L, Fontaine A, Cox-Kennett N, et al. Expanded antigen-experienced CD160+CD8+effector T cells exhibit impaired effector functions in chronic lymphocytic leukemia. J Immunother Cancer. 2021;9:e002189.

37. Bozorgmehr N, Hnatiuk M, Peters AC, Elahi S. Depletion of polyfunctional CD26highCD8+ T cells repertoire in chronic lymphocytic leukemia. Exp Hematol Oncol. 2023;12:13.

38. AHS Cancer Control Alberta. The LATENT Trial: Lytic Activation To Enhance Neoantigen-directed Therapy A Study to Evaluate the Feasibility and Efficacy of the Combined Use of Avelumab With Valproic Acid for the Treatment of Virus-associated Cancer [Internet]. clinicaltrials.gov; 2019 Jul. Report No.: NCT03357757. Available from: https://clinicaltrials.gov/ct2/show/NCT03357757

39. Grunstein M. Histone acetylation in chromatin structure and transcription. Nature. 1997;389:349–52.

40. O'Shea JJ, Schwartz DM, Villarino AV, Gadina M, McInnes IB, Laurence A. The JAK-STAT Pathway: Impact on Human Disease and Therapeutic Intervention. Annu Rev Med [Internet]. 2015 [cited 2023 Feb 6];66:311–28. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5634336/

41. Addison CL, Daniel TO, Burdick MD, Liu H, Ehlert JE, Xue YY, et al. The CXC chemokine receptor 2, CXCR2, is the putative receptor for ELR+ CXC chemokine-induced angiogenic activity. J Immunol Baltim Md 1950. 2000;165:5269–77.

42. Stothers C, Luan L, Fensterheim B, Bohannon J. Hypoxia-inducible factor-1α regulation of myeloid cells. J Mol Med Berl Ger [Internet]. 2018 [cited 2023 Mar 1];96:1293–306. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6292431/

43. Xie Z, Ago Y, Okada N, Tachibana M. Valproic acid attenuates immunosuppressive function of myeloid-derived suppressor cells. J Pharmacol Sci. 2018;137:359–65.

44. Bar N, Costa F, Das R, Duffy A, Samur M, McCachren S, et al. Differential effects of PD-L1 versus PD-1 blockade on myeloid inflammation in human cancer. JCI Insight [Internet]. American Society for Clinical Investigation; 2020 [cited 2023 Feb 21];5. Available from: https://insight.jci.org/articles/view/129353

45. Fairbanks LD, Bofill M, Ruckemann K, Simmonds HA. Importance of ribonucleotide availability to proliferating T-lymphocytes from healthy humans. Disproportionate expansion of pyrimidine pools and contrasting effects of de novo synthesis inhibitors. J Biol Chem. 1995;270:29682–9.

46. Bertheloot D, Latz E, Franklin BS. Necroptosis, pyroptosis and apoptosis: an intricate game of cell death. Cell Mol Immunol [Internet]. Nature Publishing Group; 2021 [cited 2023 Feb 2];18:1106–21. Available from: https://www.nature.com/articles/s41423-020-00630-3

47. Dumitru CA, Moses K, Trellakis S, Lang S, Brandau S. Neutrophils and granulocytic myeloid-derived suppressor cells: immunophenotyping, cell biology and clinical relevance in human oncology. Cancer Immunol Immunother CII. 2012;61:1155–67.

48. Li K, Shi H, Zhang B, Ou X, Ma Q, Chen Y, et al. Myeloid-derived suppressor cells as immunosuppressive regulators and therapeutic targets in cancer. Signal Transduct Target Ther [Internet]. Nature Publishing Group; 2021 [cited 2023 Mar 6];6:1–25. Available from: https://www.nature.com/articles/s41392-021-00670-9

49. Propper DJ, Balkwill FR. Harnessing cytokines and chemokines for cancer therapy. Nat Rev Clin Oncol [Internet]. Nature Publishing Group; 2022 [cited 2023 Feb 22];19:237–53. Available from: https://www.nature.com/articles/s41571-021-00588-9

50. Bozorgmehr N, Mashhouri S, Perez Rosero E, Xu L, Shahbaz S, Sligl W, et al. Galectin-9, a Player in Cytokine Release Syndrome and a Surrogate Diagnostic Biomarker in SARS-CoV-2 Infection. mBio. 2021;12:e00384-21.

51. Zhao S, Wu D, Wu P, Wang Z, Huang J. Serum IL-10 Predicts Worse Outcome in Cancer Patients: A Meta-Analysis. PLoS ONE [Internet]. 2015 [cited 2023 Mar 7];10:e0139598. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4595202/

52. Gelfo V, Romaniello D, Mazzeschi M, Sgarzi M, Grilli G, Morselli A, et al. Roles of IL-1 in Cancer: From Tumor Progression to Resistance to Targeted Therapies. Int J Mol Sci [Internet]. 2020 [cited 2023 Mar 7];21:6009. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7503335/

53. STATHOPOULOS J, ARMAKOLAS A, STATHOPOULOS GP, GOMATOS IP. Plasma VEGF levels in breast cancer patients with and without metastases. Oncol Lett [Internet]. 2010 [cited 2023 Mar 7];1:739–41. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3436353/

54. Vetsika E-K, Koukos A, Kotsakis A. Myeloid-Derived Suppressor Cells: Major Figures that Shape the Immunosuppressive and Angiogenic Network in Cancer. Cells

[Internet]. 2019 [cited 2023 Mar 7];8:1647. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6953061/

55. Yao Z, Zhao M, Gao G, Yang J, Wang Z, Liu Y. Prognostic Role of IL-18 in Various Human Cancers and Radiation Injuries: A Meta-Analysis. Dose-Response [Internet]. 2020 [cited 2023 Feb 22];18:1559325820931360. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7323287/

56. Xu Q, Ma H, Chang H, Feng Z, Zhang C, Yang X. The interaction of interleukin-8 and PTEN inactivation promotes the malignant progression of head and neck squamous cell carcinoma via the STAT3 pathway. Cell Death Dis [Internet]. Nature Publishing Group; 2020 [cited 2023 Feb 13];11:1–14. Available from: https://www.nature.com/articles/s41419-020-2627-5

57. Ben-Hamo R, Jacob Berger A, Gavert N, Miller M, Pines G, Oren R, et al. Predicting and affecting response to cancer therapy based on pathway-level biomarkers. Nat Commun [Internet]. Nature Publishing Group; 2020 [cited 2023 Feb 17];11:3296. Available from: https://www.nature.com/articles/s41467-020-17090-y

58. Nakamura K, Kassem S, Cleynen A, Chrétien M-L, Guillerey C, Putz EM, et al. Dysregulated IL-18 Is a Key Driver of Immunosuppression and a Possible Therapeutic Target in the Multiple Myeloma Microenvironment. Cancer Cell. 2018;33:634-648.e5.

59. Alfaro C, Teijeira A, Oñate C, Pérez G, Sanmamed MF, Andueza MP, et al. Tumor-Produced Interleukin-8 Attracts Human Myeloid-Derived Suppressor Cells and Elicits Extrusion of Neutrophil Extracellular Traps (NETs). Clin Cancer Res [Internet]. 2016 [cited 2023 Feb 24];22:3924–36. Available from: https://doi.org/10.1158/1078-0432.CCR-15-2463

60. Veglia F, Sanseviero E, Gabrilovich DI. Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity. Nat Rev Immunol [Internet]. Nature Publishing Group; 2021 [cited 2023 Feb 24];21:485–98. Available from: https://www.nature.com/articles/s41577-020-00490-y

61. Feng F, Zheng G, Wang Q, Liu S, Liu Z, Xu G, et al. Low lymphocyte count and high monocyte count predicts poor prognosis of gastric cancer. BMC Gastroenterol [Internet]. 2018 [cited 2023 Feb 26];18:148. Available from: https://doi.org/10.1186/s12876-018-0877-9

62. Bizzarri C, Beccari AR, Bertini R, Cavicchia MR, Giorgini S, Allegretti M. ELR+ CXC chemokines and their receptors (CXC chemokine receptor 1 and CXC chemokine receptor 2) as new therapeutic targets. Pharmacol Ther [Internet]. 2006 [cited 2023 Feb 13];112:139–49. Available from: https://www.sciencedirect.com/science/article/pii/S0163725806000556

63. Yuen KC, Liu L-F, Gupta V, Madireddi S, Keerthivasan S, Li C, et al. High systemic and tumor-associated IL-8 correlates with reduced clinical benefit of PD-L1 blockade. Nat Med. 2020;26:693–8.

64. Lechner MG, Liebertz DJ, Epstein AL. CHARACTERIZATION OF CYTOKINE-INDUCED MYELOID-DERIVED SUPPRESSOR CELLS FROM NORMAL HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS. J Immunol Baltim Md 1950 [Internet]. 2010 [cited 2023 Feb 2];185:2273–84. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2923483/

65. Tobin RP, Jordan KR, Kapoor P, Spongberg E, Davis D, Vorwald VM, et al. IL-6 and IL-8 Are Linked With Myeloid-Derived Suppressor Cell Accumulation and Correlate With Poor Clinical Outcomes in Melanoma Patients. Front Oncol [Internet]. 2019 [cited 2023 Feb 13];9:1223. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6857649/

66. Zhang Y, Huang H, Coleman M, Ziemys A, Gopal P, Kazmi SM, et al. VEGFR2 activity on myeloid cells mediates immune suppression in the tumor microenvironment. JCI Insight [Internet]. American Society for Clinical Investigation; 2021 [cited 2023 Mar 3];6. Available from: https://insight.jci.org/articles/view/150735

67. Yang L, Liu Q, Zhang X, Liu X, Zhou B, Chen J, et al. DNA of neutrophil extracellular traps promotes cancer metastasis via CCDC25. Nature [Internet]. Nature Publishing Group; 2020 [cited 2023 Feb 26];583:133–8. Available from: https://www.nature.com/articles/s41586-020-2394-6

68. He X-Y, Ng D, Egeblad M. Caught in a Web: Emerging Roles of Neutrophil Extracellular Traps in Cancer. Annu Rev Cancer Biol [Internet]. 2022 [cited 2023 Feb 27];6:223–43. Available from: https://doi.org/10.1146/annurev-cancerbio-080421-015537

69. Nencioni A, Beck J, Werth D, Grünebach F, Patrone F, Ballestrero A, et al. Histone deacetylase inhibitors affect dendritic cell differentiation and immunogenicity. Clin Cancer Res Off J Am Assoc Cancer Res. 2007;13:3933–41.

70. Hashimoto A, Fukumoto T, Zhang R, Gabrilovich D. Selective targeting of different populations of myeloid-derived suppressor cells by histone deacetylase inhibitors. Cancer Immunol Immunother CII. 2020;69:1929–36.

71. Li J, Bolyard C, Xin G, Li Z. Targeting Metabolic Pathways of Myeloid Cells Improves Cancer Immunotherapy. Front Cell Dev Biol [Internet]. 2021 [cited 2023 Feb 27];9. Available from: https://www.frontiersin.org/articles/10.3389/fcell.2021.747863

72. Papaspyridonos M, Matei I, Huang Y, do Rosario Andre M, Brazier-Mitouart H, Waite JC, et al. Id1 suppresses anti-tumour immune responses and promotes tumour progression by impairing myeloid cell maturation. Nat Commun [Internet]. Nature Publishing Group; 2015 [cited 2023 Feb 2];6:6840. Available from: https://www.nature.com/articles/ncomms7840

73. Kazi JU, Rönnstrand L. FMS-like Tyrosine Kinase 3/FLT3: From Basic Science to Clinical Implications. Physiol Rev [Internet]. American Physiological Society; 2019 [cited 2023 Feb 14];99:1433–66. Available from: https://journals.physiology.org/doi/full/10.1152/physrev.00029.2018

74. Lv J, Du C, Wei W, Wu Z, Zhao G, Li Z, et al. The Antiepileptic Drug Valproic Acid Restores T Cell Homeostasis and Ameliorates Pathogenesis of Experimental Autoimmune Encephalomyelitis. J Biol Chem [Internet]. 2012 [cited 2023 Feb 2];287:28656–65. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3436564/

75. Cui Y, Cai J, Wang W, Wang S. Regulatory Effects of Histone Deacetylase Inhibitors on Myeloid-Derived Suppressor Cells. Front Immunol [Internet]. 2021 [cited 2023 Feb 14];12:690207. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8208029/

76. Adeshakin AO, Yan D, Zhang M, Wang L, Adeshakin FO, Liu W, et al. Blockade of myeloid-derived suppressor cell function by valproic acid enhanced anti-PD-L1 tumor immunotherapy. Biochem Biophys Res Commun [Internet]. 2020 [cited 2023 Feb 14];522:604–11. Available from: https://www.sciencedirect.com/science/article/pii/S0006291X19322831

77. Arts RJW, Joosten LAB, van der Meer JWM, Netea MG. TREM-1: intracellular signaling pathways and interaction with pattern recognition receptors. J Leukoc Biol. 2013;93:209–15.

78. Cesaro T, Michiels T. Inhibition of PKR by Viruses. Front Microbiol [Internet]. 2021 [cited 2023 Feb 6];12:757238. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8573351/

79. Noman MZ, Desantis G, Janji B, Hasmim M, Karray S, Dessen P, et al. PD-L1 is a novel direct target of HIF-1α, and its blockade under hypoxia enhanced MDSC-mediated T cell activation. J Exp Med [Internet]. 2014 [cited 2023 Feb 27];211:781–90. Available from: https://doi.org/10.1084/jem.20131916

80. Jachetti E, Sangaletti S, Chiodoni C, Ferrara R, Colombo MP. Modulation of PD-1/PD-L1 axis in myeloid-derived suppressor cells by anti-cancer treatments. Cell Immunol [Internet]. 2021 [cited 2023 Feb 27];362:104301. Available from: https://www.sciencedirect.com/science/article/pii/S0008874921000204

Chapter 5

1. Zamora AE, Crawford JC, Thomas PG. Hitting the target: how T cells detect and eliminate tumors. J Immunol Baltim Md 1950 [Internet]. 2018 [cited 2023 Mar

31];200:392–9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6116355/

2. Martínez-Lostao L, Anel A, Pardo J. How Do Cytotoxic Lymphocytes Kill Cancer Cells? Clin Cancer Res [Internet]. 2015 [cited 2023 Mar 31];21:5047–56. Available from: https://doi.org/10.1158/1078-0432.CCR-15-0685

3. Bolitho P, Voskoboinik I, Trapani JA, Smyth MJ. Apoptosis induced by the lymphocyte effector molecule perforin. Curr Opin Immunol. 2007;19:339–47.

4. Hashimoto M, Kamphorst AO, Im SJ, Kissick HT, Pillai RN, Ramalingam SS, et al. CD8 T Cell Exhaustion in Chronic Infection and Cancer: Opportunities for Interventions. Annu Rev Med [Internet]. 2018 [cited 2023 Mar 31];69:301–18. Available from: https://doi.org/10.1146/annurev-med-012017-043208

5. Clemente CG, Mihm MC, Bufalino R, Zurrida S, Collini P, Cascinelli N. Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. Cancer. 1996;77:1303–10.

6. Beatty GL, Gladney WL. Immune escape mechanisms as a guide for cancer immunotherapy. Clin Cancer Res Off J Am Assoc Cancer Res [Internet]. 2015 [cited 2023 Mar 31];21:687–92. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4334715/

7. Farhood B, Najafi M, Mortezaee K. CD8+ cytotoxic T lymphocytes in cancer immunotherapy: A review. J Cell Physiol. 2019;234:8509–21.

8. Blank CU, Haining WN, Held W, Hogan PG, Kallies A, Lugli E, et al. Defining "T cell exhaustion." Nat Rev Immunol. 2019;19:665–74.

9. Wherry EJ. T cell exhaustion. Nat Immunol. 2011;12:492–9.

10. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. Nat Rev Immunol. 2015;15:486–99.

11. Montironi C, Muñoz-Pinedo C, Eldering E. Hematopoietic versus Solid Cancers and T Cell Dysfunction: Looking for Similarities and Distinctions. Cancers [Internet]. 2021 [cited 2023 Mar 31];13:284. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7828769/

12. Okoye IS, Houghton M, Tyrrell L, Barakat K, Elahi S. Coinhibitory Receptor Expression and Immune Checkpoint Blockade: Maintaining a Balance in CD8+ T Cell Responses to Chronic Viral Infections and Cancer. Front Immunol. 2017;8:1215.

13. McLane LM, Abdel-Hakeem MS, Wherry EJ. CD8 T Cell Exhaustion During Chronic Viral Infection and Cancer. Annu Rev Immunol [Internet]. 2019 [cited 2021 Jun 1];37:457–95. Available from: https://doi.org/10.1146/annurev-immunol-041015-055318

14. Andrews LP, Yano H, Vignali DAA. Inhibitory receptors and ligands beyond PD-1, PD-L1 and CTLA-4: breakthroughs or backups. Nat Immunol [Internet]. 2019 [cited 2021 Oct 27];20:1425–34. Available from: https://www.nature.com/articles/s41590-019-0512-0

15. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer [Internet]. Nature Publishing Group; 2012 [cited 2021 May 17];12:252–64. Available from: https://www.nature.com/articles/nrc3239

16. Wilson RAM, Evans TRJ, Fraser AR, Nibbs RJB. Immune checkpoint inhibitors: new strategies to checkmate cancer. Clin Exp Immunol [Internet]. 2018 [cited 2021 Jun 1];191:133–48. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5758374/

17. Vaddepally RK, Kharel P, Pandey R, Garje R, Chandra AB. Review of Indications of FDA-Approved Immune Checkpoint Inhibitors per NCCN Guidelines with the Level of Evidence. Cancers. 2020;12.

18. Koustas E, Sarantis P, Papavassiliou AG, Karamouzis MV. The Resistance Mechanisms of Checkpoint Inhibitors in Solid Tumors. Biomolecules [Internet]. 2020 [cited 2023 Apr 6];10:666. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7277892/

19. Curran EK, Godfrey J, Kline J. Mechanisms of Immune Tolerance in Leukemia and Lymphoma. Trends Immunol. 2017;38:513–25.

20. Suen H, Brown R, Yang S, Weatherburn C, Ho PJ, Woodland N, et al. Multiple myeloma causes clonal T-cell immunosenescence: identification of potential novel targets for promoting tumour immunity and implications for checkpoint blockade. Leukemia [Internet]. Nature Publishing Group; 2016 [cited 2023 Mar 31];30:1716–24. Available from: https://www.nature.com/articles/leu201684

21. Jafferji MS, Yang JC. Adoptive T-Cell Therapy for Solid Malignancies. Surg Oncol Clin N Am. 2019;28:465–79.

22. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell. 2012;21:309–22.

23. Balachandran VP, Łuksza M, Zhao JN, Makarov V, Moral JA, Remark R, et al. Identification of unique neoantigen qualities in long-term survivors of pancreatic cancer. Nature. 2017;551:512–6.

24. O'Donnell JS, Teng MWL, Smyth MJ. Cancer immunoediting and resistance to T cell-based immunotherapy. Nat Rev Clin Oncol. 2019;16:151–67.

25. Martinez M, Moon EK. CAR T Cells for Solid Tumors: New Strategies for Finding, Infiltrating, and Surviving in the Tumor Microenvironment. Front Immunol. 2019;10:128.

26. Cai G, Anumanthan A, Brown JA, Greenfield EA, Zhu B, Freeman GJ. CD160 inhibits activation of human CD4+ T cells through interaction with herpesvirus entry mediator. Nat Immunol. 2008;9:176–85.

27. Tan CL, Peluso MJ, Drijvers JM, Mera CM, Grande SM, Brown KE, et al. CD160 Stimulates CD8+ T Cell Responses and Is Required for Optimal Protective Immunity to Listeria monocytogenes. ImmunoHorizons. 2018;2:238–50.

28. Peretz Y, He Z, Shi Y, Yassine-Diab B, Goulet J-P, Bordi R, et al. CD160 and PD-1 co-expression on HIV-specific CD8 T cells defines a subset with advanced dysfunction. PLoS Pathog. 2012;8:e1002840.

29. Chibueze CE, Yoshimitsu M, Arima N. CD160 expression defines a uniquely exhausted subset of T lymphocytes in HTLV-1 infection. Biochem Biophys Res Commun. 2014;453:379–84.

30. Liu S, Zhang W, Liu K, Wang Y. CD160 expression on CD8+ T cells is associated with active effector responses but limited activation potential in pancreatic cancer. Cancer Immunol Immunother CII. 2020;69:789–97.

31. Del Rio M-L, Nguyen TH, Tesson L, Heslan J-M, Gutierrez-Adan A, Fernandez-Gonzalez R, et al. The impact of CD160 deficiency on alloreactive CD8 T cell responses and allograft rejection. Transl Res J Lab Clin Med. 2022;239:103–23.

32. Gauci M-L, Giustiniani J, Lepelletier C, Garbar C, Thonnart N, Dumaz N, et al. The soluble form of CD160 acts as a tumor mediator of immune escape in melanoma. Cancer Immunol Immunother CII. 2022;71:2731–42.

33. Giustiniani J, Marie-Cardine A, Bensussan A. A soluble form of the MHC class I-specific CD160 receptor is released from human activated NK lymphocytes and inhibits cell-mediated cytotoxicity. J Immunol Baltim Md 1950. 2007;178:1293–300.

34. Ramsay AG, Clear AJ, Fatah R, Gribben JG. Multiple inhibitory ligands induce impaired T-cell immunologic synapse function in chronic lymphocytic leukemia that can be blocked with lenalidomide: establishing a reversible immune evasion mechanism in human cancer. Blood. 2012;120:1412–21.

35. Riches JC, Davies JK, McClanahan F, Fatah R, Iqbal S, Agrawal S, et al. T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. Blood. 2013;121:1612–21.

36. Krackhardt AM, Witzens M, Harig S, Hodi FS, Zauls AJ, Chessia M, et al. Identification of tumor-associated antigens in chronic lymphocytic leukemia by SEREX. Blood [Internet]. American Society of Hematology; 2002 [cited 2020 Oct 14];100:2123–31. Available from: https://ashpublications.org/blood/article/100/6/2123/16598/Identification-of-tumor-associated-antigens-in

37. Ca K, L G, Np R. Sorting through subsets: which T-cell populations mediate highly effective adoptive immunotherapy? J Immunother Hagerstown Md 1997 [Internet]. J Immunother; 2012 [cited 2020 Oct 14];35:651–60. Available from: https://pubmed.ncbi.nlm.nih.gov/23090074/

38. A L, De S, H P, D Z, Sa FM. Inhibitory Receptor Expression Depends More Dominantly on Differentiation and Activation than "Exhaustion" of Human CD8 T Cells [Internet]. Front. Immunol. Front Immunol; 2013 [cited 2020 Oct 14]. Available from: https://pubmed.ncbi.nlm.nih.gov/24391639/

39. Böttcher M, Böttcher-Loschinski R, Kahlfuss S, Aigner M, Gießl A, Mackensen A, et al. CLL-Derived Extracellular Vesicles Impair T-Cell Activation and Foster T-Cell Exhaustion via Multiple Immunological Checkpoints. Cells. 2022;11:2176.

40. Bellomo G, Allegra A, Alonci A, Quartarone E, Pizzoleo MA, Cincotta M, et al. Serum levels of interleukin-16 in lymphoid malignancies. Leuk Lymphoma. 2007;48:1225–7.

41. Atanackovic D, Hildebrandt Y, Templin J, Cao Y, Keller C, Panse J, et al. Role of interleukin 16 in multiple myeloma. J Natl Cancer Inst. 2012;104:1005–20.

42. Barnaba V. T Cell Memory in Infection, Cancer, and Autoimmunity. Front Immunol [Internet]. 2022 [cited 2023 Apr 2];12. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2021.811968

43. Takahashi H, Sakakura K, Ida S, Kawabata-Iwakawa R, Matsuyama T, Tada H, et al. Circulating naïve and effector memory T cells correlate with prognosis in head and neck squamous cell carcinoma. Cancer Sci [Internet]. 2022 [cited 2023 Apr 2];113:53–64. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8748237/

44. Busch DH, Fräßle SP, Sommermeyer D, Buchholz VR, Riddell SR. Role of memory T cell subsets for adoptive immunotherapy. Semin Immunol [Internet]. 2016 [cited 2023 Apr 2];28:28–34. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5027130/

45. Bailey SR, Nelson MH, Majchrzak K, Bowers JS, Wyatt MM, Smith AS, et al. Human CD26high T cells elicit tumor immunity against multiple malignancies via enhanced migration and persistence. Nat Commun [Internet]. Nature Publishing Group; 2017 [cited 2022 Jun 15];8:1961. Available from: https://www.nature.com/articles/s41467-017-01867-9

46. Barreira da Silva R, Laird ME, Yatim N, Fiette L, Ingersoll MA, Albert ML. Dipeptidylpeptidase 4 inhibition enhances lymphocyte trafficking, improving both naturally occurring tumor immunity and immunotherapy. Nat Immunol. 2015;16:850–8.

47. Wang H, Liu X, Long M, Huang Y, Zhang L, Zhang R, et al. NRF2 activation by antioxidant antidiabetic agents accelerates tumor metastasis. Sci Transl Med. 2016;8:334ra51.

48. Hatano R, Ohnuma K, Yamamoto J, Dang NH, Morimoto C. CD26-mediated co-stimulation in human CD8(+) T cells provokes effector function via pro-inflammatory cytokine production. Immunology. 2013;138:165–72.

49. Mousset CM, Hobo W, Woestenenk R, Preijers F, Dolstra H, van der Waart AB. Comprehensive Phenotyping of T Cells Using Flow Cytometry. Cytom Part J Int Soc Anal Cytol. 2019;95:647–54.

50. Gherardin NA, Souter MN, Koay H, Mangas KM, Seemann T, Stinear TP, et al. Human blood MAIT cell subsets defined using MR1 tetramers. Immunol Cell Biol [Internet]. 2018 [cited 2023 Apr 2];96:507–25. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6446826/

51. Kahan SM, Bakshi RK, Ingram JT, Hendrickson RC, Lefkowitz EJ, Crossman DK, et al. Intrinsic IL-2 production by effector CD8 T cells affects IL-2 signaling and promotes fate decisions, stemness, and protection. Sci Immunol [Internet]. American Association for the Advancement of Science; 2022 [cited 2022 Jun 14];7:eabl6322. Available from: https://www.science.org/doi/10.1126/sciimmunol.abl6322

52. Bouwman AC, van Daalen KR, Crnko S, ten Broeke T, Bovenschen N. Intracellular and Extracellular Roles of Granzyme K. Front Immunol [Internet]. 2021 [cited 2022 Jun 14];12. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2021.677707

53. Bozorgmehr N, Mashhouri S, Perez Rosero E, Xu L, Shahbaz S, Sligl W, et al. Galectin-9, a Player in Cytokine Release Syndrome and a Surrogate Diagnostic Biomarker in SARS-CoV-2 Infection. mBio. 2021;12:e00384-21.

54. Lhuillier C, Barjon C, Niki T, Gelin A, Praz F, Morales O, et al. Impact of Exogenous Galectin-9 on Human T Cells. J Biol Chem [Internet]. 2015 [cited 2023 Mar 23];290:16797–811. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4505427/

55. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol [Internet]. 2012 [cited 2023 Feb 2];12:253–68. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3587148/

56. Anderson NM, Simon MC. The tumor microenvironment. Curr Biol [Internet]. 2020 [cited 2023 Feb 9];30:R921–5. Available from: https://www.sciencedirect.com/science/article/pii/S0960982220309337

57. Zhao L, He R, Long H, Guo B, Jia Q, Qin D, et al. Late-stage tumors induce anemia and immunosuppressive extramedullary erythroid progenitor cells. Nat Med

[Internet]. 2018 [cited 2023 Mar 11];24:1536–44. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6211844/

58. Pathria P, Louis TL, Varner JA. Targeting Tumor-Associated Macrophages in Cancer. Trends Immunol. 2019;40:310–27.

59. Wu W-C, Sun H-W, Chen H-T, Liang J, Yu X-J, Wu C, et al. Circulating hematopoietic stem and progenitor cells are myeloid-biased in cancer patients. Proc Natl Acad Sci U S A. 2014;111:4221–6.

60. Elahi S. Neglected Cells: Immunomodulatory Roles of CD71+ Erythroid Cells. Trends Immunol. 2019;40:181–5.

61. Groth C, Hu X, Weber R, Fleming V, Altevogt P, Utikal J, et al. Immunosuppression mediated by myeloid-derived suppressor cells (MDSCs) during tumour progression. Br J Cancer [Internet]. Nature Publishing Group; 2019 [cited 2023 Feb 2];120:16–25. Available from: https://www.nature.com/articles/s41416-018-0333-1

62. Bronte V, Serafini P, Apolloni E, Zanovello P. Tumor-induced immune dysfunctions caused by myeloid suppressor cells. J Immunother Hagerstown Md 1997. 2001;24:431–46.

63. Bronte V, Brandau S, Chen S-H, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. Nat Commun [Internet]. 2016 [cited 2023 Feb 13];7:12150. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4935811/

64. Hou A, Hou K, Huang Q, Lei Y, Chen W. Targeting Myeloid-Derived Suppressor Cell, a Promising Strategy to Overcome Resistance to Immune Checkpoint Inhibitors. Front Immunol. 2020;11:783.

65. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. Nat Rev Immunol [Internet]. Nature Publishing Group; 2020 [cited 2023 Feb 9];20:651–68. Available from: https://www.nature.com/articles/s41577-020-0306-5

66. Ribas A, Wolchok JD. Cancer Immunotherapy Using Checkpoint Blockade. Science [Internet]. 2018 [cited 2023 Feb 9];359:1350–5. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7391259/

67. Kuss-Duerkop SK, Westrich JA, Pyeon D. DNA Tumor Virus Regulation of Host DNA Methylation and Its Implications for Immune Evasion and Oncogenesis. Viruses. 2018;10:82.

68. Liu C, Lu J, Tian H, Du W, Zhao L, Feng J, et al. Increased expression of PD-L1 by the human papillomavirus 16 E7 oncoprotein inhibits anticancer immunity. Mol Med Rep. 2017;15:1063–70.

69. Qi L, Li N, Lin A, Wang X, Cong J. Efficacy and safety of pembrolizumab on cervical cancer: A systematic review and single-arm meta-analysis. Front Oncol [Internet]. 2022 [cited 2023 Feb 9];12:910486. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9399507/

70. Li X, Su X, Liu R, Pan Y, Fang J, Cao L, et al. HDAC inhibition potentiates anti-tumor activity of macrophages and enhances anti-PD-L1-mediated tumor suppression. Oncogene [Internet]. Nature Publishing Group; 2021 [cited 2023 Feb 14];40:1836–50. Available from: https://www.nature.com/articles/s41388-020-01636-x

71. Yuen KC, Liu L-F, Gupta V, Madireddi S, Keerthivasan S, Li C, et al. High systemic and tumor-associated IL-8 correlates with reduced clinical benefit of PD-L1 blockade. Nat Med. 2020;26:693–8.

72. Nakamura K, Kassem S, Cleynen A, Chrétien M-L, Guillerey C, Putz EM, et al. Dysregulated IL-18 Is a Key Driver of Immunosuppression and a Possible Therapeutic Target in the Multiple Myeloma Microenvironment. Cancer Cell. 2018;33:634-648.e5.

73. Yao Z, Zhao M, Gao G, Yang J, Wang Z, Liu Y. Prognostic Role of IL-18 in Various Human Cancers and Radiation Injuries: A Meta-Analysis. Dose-Response [Internet]. 2020 [cited 2023 Feb 22];18:1559325820931360. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7323287/

74. Newman AM, Steen CB, Liu CL, Gentles AJ, Chaudhuri AA, Scherer F, et al. Determining cell-type abundance and expression from bulk tissues with digital cytometry. Nat Biotechnol [Internet]. 2019 [cited 2023 Jan 23];37:773–82. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6610714/

75. Veglia F, Sanseviero E, Gabrilovich DI. Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity. Nat Rev Immunol [Internet]. Nature Publishing Group; 2021 [cited 2023 Feb 24];21:485–98. Available from: https://www.nature.com/articles/s41577-020-00490-y

76. Feng F, Zheng G, Wang Q, Liu S, Liu Z, Xu G, et al. Low lymphocyte count and high monocyte count predicts poor prognosis of gastric cancer. BMC Gastroenterol [Internet]. 2018 [cited 2023 Feb 26];18:148. Available from: https://doi.org/10.1186/s12876-018-0877-9

77. Li J, Bolyard C, Xin G, Li Z. Targeting Metabolic Pathways of Myeloid Cells Improves Cancer Immunotherapy. Front Cell Dev Biol [Internet]. 2021 [cited 2023 Feb 27];9. Available from: https://www.frontiersin.org/articles/10.3389/fcell.2021.747863

78. Soria-Castro R, Schcolnik-Cabrera A, Rodríguez-López G, Campillo-Navarro M, Puebla-Osorio N, Estrada-Parra S, et al. Exploring the Drug Repurposing Versatility of Valproic Acid as a Multifunctional Regulator of Innate and Adaptive Immune Cells. J Immunol Res. 2019;2019:9678098.

79. Adeshakin AO, Yan D, Zhang M, Wang L, Adeshakin FO, Liu W, et al. Blockade of myeloid-derived suppressor cell function by valproic acid enhanced anti-PD-L1 tumor immunotherapy. Biochem Biophys Res Commun [Internet]. 2020 [cited 2023 Feb 14];522:604–11. Available from: https://www.sciencedirect.com/science/article/pii/S0006291X19322831

80. Yang L, Liu Q, Zhang X, Liu X, Zhou B, Chen J, et al. DNA of neutrophil extracellular traps promotes cancer metastasis via CCDC25. Nature [Internet]. Nature Publishing Group; 2020 [cited 2023 Feb 26];583:133–8. Available from: https://www.nature.com/articles/s41586-020-2394-6

81. He X-Y, Ng D, Egeblad M. Caught in a Web: Emerging Roles of Neutrophil Extracellular Traps in Cancer. Annu Rev Cancer Biol [Internet]. 2022 [cited 2023 Feb 27];6:223–43. Available from: https://doi.org/10.1146/annurev-cancerbio-080421-015537

82. Alfaro C, Teijeira A, Oñate C, Pérez G, Sanmamed MF, Andueza MP, et al. Tumor-Produced Interleukin-8 Attracts Human Myeloid-Derived Suppressor Cells and Elicits Extrusion of Neutrophil Extracellular Traps (NETs). Clin Cancer Res [Internet]. 2016 [cited 2023 Feb 24];22:3924–36. Available from: https://doi.org/10.1158/1078-0432.CCR-15-2463

83. Svanberg R, Janum S, Patten PEM, Ramsay AG, Niemann CU. Targeting the tumor microenvironment in chronic lymphocytic leukemia. Haematologica. 2021;106:2312–24.

84. Detel D, Baticic L, Varljen J. The influence of age on intestinal dipeptidyl peptidase IV (DPP IV/CD26), disaccharidases, and alkaline phosphatase enzyme activity in C57BL/6 mice. Exp Aging Res. 2008;34:49–62.