Prognostic and predictive effects of body composition in advanced malignant melanoma

by

Susie Youn

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science
in
Clinical Epidemiology

School of Public Health
University of Alberta

Abstract

Body composition, or the breakdown of the body into components such as skeletal muscle and adipose tissue, is increasingly recognized as an important factor impacting morbidity and survival in a variety of diseases. Body composition analysis provides both valuable prognostic information and identifies patients who may benefit from nutritional intervention and/or rehabilitation.

The majority of body composition studies in cancer patients have been performed in those with intra-abdominal or intra-thoracic malignancies, as many body composition analysis techniques require cross-sectional imaging of the chest or abdomen. Relatively little body composition research has been done in melanoma, though patients with signs of advanced disease (stage III or IV) typically have whole-body or abdominal imaging performed as part of their staging. Baseline body composition and prevalence of factors such as sarcopenia (skeletal muscle depletion) and myosteatosis (reduced skeletal muscle density) have not been well-characterized in melanoma patients, particularly in patients with locoregional disease (stage III). This thesis includes a systematic review of current body composition research in melanoma. Existing research in melanoma has focused mostly on patients with metastatic disease and has been limited by the use of differing techniques of body composition analysis, some of which are not well-supported by the majority of body composition literature.

Medical management of advanced melanoma has significantly changed in recent years with the emergence of immunotherapies, which have been shown to significantly improve survival in patients with metastatic disease. A growing number of studies are evaluating body composition in these patients as both a prognostic marker and predictor of immunotherapy toxicity. We performed a retrospective analysis of body composition in patients with metastatic

melanoma receiving the immunotherapy agent nivolumab and found that myosteatosis was a strong predictor of decreased survival in these patients.

Finally, we sought to characterize baseline body composition and its impacts on survival in patients with resectable stage III disease. A retrospective analysis was performed of a cohort of patients with resected stage III melanoma presenting to a cancer care center in Alberta, Canada from 2007-2017. Peri-operative computed tomography (CT) scans were analyzed at the third lumbar vertebrae to measure surface area of skeletal muscle and adipose tissue. Sarcopenia and myosteatosis were defined using previously published cut-offs commonly used in body composition literature. In addition, we determined cohort-specific cut-offs that significantly impacted overall survival (OS) using optimal stratification. Sarcopenia and myosteatosis defined using cohort-specific cut-offs were predictive of OS, melanoma-specific survival (MSS), and recurrence-free survival (RFS) in a multivariate model accounting for other known prognostic factors in melanoma.

An exploratory analysis was undertaken of adipose tissue in this cohort of patients with stage III melanoma. Prognostic and predictive effects of adipose tissue are not well-understood, though emerging evidence in other cancer types suggests that an excess of visceral fat is associated with decreased survival. Unlike skeletal muscle, no cut-offs of adipose tissue associated with decreased survival have been widely adopted in the literature. We applied several methods of stratifying and analyzing measurements of adipose tissue. Though significant cut-offs of visceral adipose tissue index that impacted OS were identified using optimal stratification, these associations were only borderline significant in multivariate analyses. These results suggest a need for further research to elucidate the role of adipose tissue in melanoma.

This thesis demonstrates that body composition, which has been identified as a significant prognostic marker in other cancer types, has a similar role in patients with advanced melanoma. Though melanoma lacks some of the more traditional risk factors for malnutrition associated with gastrointestinal malignancies, patients with resectable stage III melanoma in fact have comparable rates of sarcopenia and myosteatosis. These factors have strong negative effects on survival, independent of other known prognostic factors in melanoma. Our findings are a novel contribution to body composition research in melanoma patients, a relatively understudied field, and underscore the significant role of body composition in cancer progression and prognosis.

Preface

This thesis is an original work by Susie Youn. The research project, of which this thesis is a part, received ethics approval from the Health Research Ethics Board of Alberta Cancer Committee, "Lean Body Mass and Skeletal Muscle Density in Resected Melanoma", HREBA.CC-19-0199, July 10, 2019.

A version of chapter 2 of this thesis, "Prognostic and predictive value of body composition in melanoma: a systematic review", is currently under review at *Clinical Nutrition ESPEN*, submitted January 22, 2021. Youn S., Jogiat U., Baracos V.E., McCall M., Eurich D.T., Sawyer M.B.
SY and UJ were responsible for study design and qualitative analysis. VB, MM, and DE contributed to manuscript edits. MS was the supervisory author and contributed to study design and manuscript composition.

A version of chapter 3 of this thesis has been published as:
Youn S., Reif R., Chu M.P., Smylie M., Walker J., Eurich D.T., Ghosh S., Sawyer M.B. Myosteatosis is prognostic in metastatic melanoma treated with nivolumab. *Clinical Nutrition ESPEN* 2021. https://doi.org/10.1016/j.clnesp.2021.01.009
SY was responsible for study design, analysis, and manuscript composition. RR contributed to data collection. MC, MS, JW, and DE assisted with manuscript edits. SG assisted with statistical analyses. MS was the supervisory author and was involved with concept formation and manuscript composition.

Acknowledgements

I would like to acknowledge the Clinician Investigator Program, funded by Alberta Health Services, which allowed me to complete my graduate studies in the course of my surgical residency.

I would like to thank my supervisors, Dr. Michael McCall and Dr. Dean Eurich, for all their help and insight throughout my research. Dr. McCall provided career advice and a clinical perspective on my research. Dr. Eurich was always available to answer questions regarding the public health aspects of my project and provided many valuable edits to my manuscripts. I would also like to thank Dr. Vickie Baracos for her teaching and for providing new perspectives to my research.

This thesis could not have been completed without the expertise and support of my primary supervisor, Dr. Michael Sawyer. Despite his incredibly busy schedule, he has always been available to answer my many questions, advocate for my research, and formulate new, thought-provoking questions.

Finally, on a personal note, I would like to thank my partner, Alex Donovan, and my family for all their support throughout the completion of this project.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AE – adverse event

AJCC – American Joint Committee on Cancer

BMI – body mass index

CI – confidence interval

CLND – completion lymph node dissection

CT – computed tomography

CTLA-4 - cytotoxic T-lymphocyte-associated antigen 4

DFS – disease-free survival

DLT – dose-limiting toxicity

ECOG PS - Eastern Cooperative Oncology Group Performance Status

HU – Hounsfield units

HR – hazard ratio

IQR – interquartile range

LDH – lactate dehydrogenase

MM – metastatic melanoma

MRI – magnetic resonance imaging

MSA – muscle surface area

MSLT – Multicenter Selective Lymphadenectomy Trials

MSS – melanoma-specific survival

NCI-CTCAE - National Cancer Institute Common Terminology Criteria for Adverse Events

OS – overall survival

PD-1 - anti-programmed death 1

PET-CT – position emission tomography-computed tomography

PFS – progression-free survival

QUIPS – quality in prognostic factor studies

RFS – recurrence-free survival

SAT – subcutaneous adipose tissue

SMD – skeletal muscle density

SMG – skeletal muscle gauge

SMI – skeletal muscle index

TATI – total adipose tissue index

VAT – visceral adipose tissue

VFI – visceral fat index

Chapter 1: Introduction

1.1 Statement of the problem

1.1.1 Melanoma epidemiology, incidence, and survival

Malignant melanoma (called melanoma herein) is the deadliest of skin cancers and the sixth most commonly diagnosed cancer in Western countries.[1] Melanoma incidence is increasing worldwide. In Canada, melanoma incidence increased by 2% per year in men and by 1.5% per year in women between 1986 and 2010.[2] An estimated 8000 new cases of melanoma were diagnosed in Canada in 2020.[3] Rising incidence is thought to be driven by an aging population and trends in sunlight and ultraviolet radiation exposure, the strongest modifiable risk factors for developing melanoma.

Surgery remains the standard of care for melanoma, and patients with early-stage disease have a favorable prognosis. However, survival rates drop significantly with more advanced disease. Estimated 5-year relative survival for patients with regional (stage III) and distant (stage IV) disease is 66 and 27%, respectively.[4] The most recent edition of melanoma staging criteria released by the American Joint Committee on Cancer (AJCC 8th edition) accounts for tumor characteristics, such as tumor thickness and presence of ulceration, and extent of nodal involvement as key factors influencing prognosis.[5] Other factors thought to impact melanoma survival are age, sex, and potentially tumor location. Similar to many other cancer types, advanced age is associated with decreased survival. Women tend to have improved survival compared to men, though it is unclear whether this is due to earlier detection or to biological differences between sexes.[6,7] Tumors at specific anatomic sites, such as head and neck tumors, may be associated with worse survival compared to tumors in other locations.[8]

Management of advanced melanoma (stage III and IV disease) has drastically changed in the past decade as a result of several landmark trials. The Multicenter Selective Lymphadenectomy Trials (MSLT) evaluated the role of sentinel lymph node biopsy and completion lymph node dissection (CLND) in surgical management of melanoma. MSLT-I demonstrated benefits of routinely performing sentinel lymph node sampling for staging purposes in patients with tumors staged T1b and higher.[9,10] The follow-up trial, MSLT-II,[11] and another study, the Dermatologic Cooperative Oncology Group-selective lymphadenectomy Trials (DeCOG-SLT),[12] demonstrated no survival benefits to routine CLND in patients with positive sentinel lymph nodes. These trials have led to a more selective use of CLND, a surgery associated with significant morbidity.

Another significant change in melanoma management occurred with the development of immunotherapy drugs for treatment of metastatic melanoma. These therapies function by blocking tumor cell inhibition of host immune response, thereby enabling the immune system to target tumor cells.[13] Ipilimumab, an antibody that blocks cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), was among the first immunotherapy agents to be studied, followed by nivolumab, an anti-programmed death 1 (PD-1) antibody. Both agents have been shown to significantly improve overall survival (OS) in stage IV melanoma, a disease which previously carried a grim prognosis and had no known effective treatment.[14–17] Recently, ipilimumab and nivolumab have also been shown to improve survival in high-risk, resected stage III melanoma.[18,19] However, these therapies are associated with significant toxicity,[20] and there is no known way of predicting which patients will experience toxicity or preventing toxicity without compromising treatment effectiveness.

Outside the factors mentioned above, few predictive and prognostic markers have been identified in patients with advanced melanoma. A potential prognostic factor that has recently garnered interest is body mass index (BMI), with several studies demonstrating a so-called "obesity paradox" wherein patients with a higher BMI seem to have improved outcomes.[21–24] These studies have been conducted mostly in patients with metastatic disease receiving systemic therapy. Results have been somewhat conflicting, with one study suggesting that the survival benefit may be limited to men with higher serum creatinine levels suggestive of increased skeletal muscle.[23] Such findings suggest a need for research focused on body composition, rather than anthropometric measures, as a potential prognostic marker in advanced melanoma.

1.1.2 An overview of body composition research in cancer

There is growing emphasis on malnutrition as not just a side effect of advanced cancer, but as a prognostic marker and potential driver of cancer progression. Body composition analysis, which provides a detailed breakdown of tissue compartments such as skeletal muscle and adipose tissue, has emerged as a promising, non-invasive method of assessing nutritional status. Technologies are now available that enable quick and accurate analysis of routinely performed diagnostic imaging.[25,26] These techniques are of growing importance with rising rates of overweight and obese patients, in whom signs of skeletal muscle loss may not be apparent on physical exam.

The clinical significance of information extracted from body composition analysis is an area of ongoing study. Several parameters determined from measurements of skeletal muscle on cross-sectional imaging have emerged as reliable prognostic markers in a variety of cancer patients. A radiologic method of identifying patients with sarcopenia, or skeletal muscle

depletion, is to measure muscle surface area at a specific anatomic level and normalize this area for height to determine a skeletal muscle index (SMI).[26,27] Sarcopenia, or low SMI, has been associated with a variety of poor outcomes in cancer, including increased rates of post-operative complications, chemotherapy toxicity, and decreased survival.[28] Another measure of skeletal muscle on computed tomography (CT) imaging is its average radio-attenuation in Hounsfield units (HU), which has been shown to correlate with the degree of fatty infiltration into muscle.[29] Decreased skeletal muscle density (SMD), also termed myosteatosis, is thought to be distinct from sarcopenia but has similarly been associated with a worse prognosis in cancer.[30] Mechanisms behind these associations are unclear, with some hypothesizing that myosteatosis reflects a state of heightened systemic inflammation.[31,32]

Measurements of adipose tissue from body composition analysis are another area of ongoing research. Compared to skeletal muscle, prognostic implications of decreased or increased amounts of adipose tissue have not been as well-characterized. From a physiologic standpoint, it is apparent that adipose tissue functions as an endocrine organ which may influence cancer development and progression, and furthermore that the various adipose tissue compartments (visceral, subcutaneous, and intermuscular) are biologically distinct.[33,34] Exploratory studies in colorectal cancer patients suggest that patients with increased visceral adipose tissue, or visceral obesity, have worse outcomes.[35–37] However, the optimal method of measuring and analyzing adipose tissue in body composition research is unknown.

A further application of body composition analysis in cancer patients, besides correlating imaging findings with survival and recurrence rates, is in pharmacokinetics of anti-cancer treatments. Most systemic cancer treatments are dosed by body weight or body surface area, but such dosing methods have been questioned for their ability to reduce treatment toxicity.[38] In

body composition analysis, the body can be divided into lean and adipose tissue compartments, which function as drug distribution volumes for non-lipophilic and lipophilic drugs, respectively.[39] Patients with high levels of adipose tissue but relatively low skeletal muscle, also termed sarcopenic obese, could theoretically receive excessive doses of chemotherapy or immunotherapy drugs that distribute in the lean tissue compartment. Sarcopenia and sarcopenic obesity have been linked to increased risk of treatment toxicity in multiple cancer types.[40] Thus, body composition may explain why some patients are more likely to experience toxicity and, in the future, serve as a method of providing personalized cancer therapy dosing.

While thousands of body composition studies can be found in the literature, caution should be applied when interpreting results due to a lack of consensus regarding optimal methods of analyzing imaging and defining clinically significant cut-offs. Various modalities including CT, magnetic resonance imaging (MRI), dual X-ray absorptiometry, and bioelectrical impedance analysis can be used in body composition analysis. Further methodologic variation occurs within each imaging type; for example, studies using abdominal CTs may analyze images from different anatomic levels or focus on specific muscle groups. Identifying clinically significant thresholds of skeletal muscle loss can be difficult, as multiple factors including age, sex, BMI, ethnicity, and comorbidities contribute to variation in skeletal muscle mass.[41] Many studies attempt to address these sources of variation in SMI by establishing sex- and/or BMI-specific skeletal muscle cut-offs within their cohort; alternatively, some studies may apply cut-offs established in another study, with Martin's[42] and Prado's[27] cut-offs among the most common in Western literature. Finally, statistical methods of determining skeletal muscle cut-offs may vary, with some studies using optimal stratification to identify statistically significant cut-offs and others simply identifying patients below a certain percentile as having sarcopenia.

Despite this heterogeneity, several standards should be considered when assessing body composition research. Lumbar CT and MRI images are generally considered the gold standard in body composition analysis, as measurements of skeletal muscle from these images have been shown to correlate with total lean body mass.[25,26] Importantly, these studies measured all skeletal muscle at the third lumbar vertebrae; a trend in body composition research has been to use a single muscle, such as the psoas muscle, as a surrogate for total skeletal muscle. This method has been brought into question with several studies demonstrating poor correlation between psoas muscle and total lumbar skeletal muscle surface area, as well as no significant association of psoas muscle measurements with survival outcomes.[43,44] Again, while there is no universal definition of sarcopenia, SMI cut-offs should generally be stratified by sex and BMI. Potential confounders such as age, sex, and BMI should be considered when assessing impacts of sarcopenia on clinical outcomes.

1.1.3 Current body composition research in melanoma

Research in body composition has experienced huge growth in recent years, particularly in cancer patients in whom there is a strong need to identify predictive and prognostic biomarkers. However, body composition in melanoma remain understudied, with existing research limited to a small number of studies mostly of patients with metastatic disease.[45–49] Though these studies have been performed of a similar patient group, namely patients with stage IV melanoma receiving immunotherapy, comparability is limited by methodological heterogeneity. As such, prognostic impacts of factors such as sarcopenia and myosteatosis in these patients are unclear. Given variable patient responses to immunotherapy, coupled with a

significant toxicity profile, further research is needed to clarify the predictive role of body composition in these patients.

Stage III melanoma patients represent another group in whom impacts of body composition have not been well-studied, though these patients also routinely have CT or PET-CT scans performed as part of their work-up. Baseline body composition and prevalence of factors such as sarcopenia and myosteatosis in these patients is unknown. Identifying patients with skeletal muscle depletion may be of value in selecting patients for pre-operative nutritional therapy or rehabilitation, with the goal of reducing post-operative complications and improving survival. While there have been improvements to the management of stage III melanoma in the past decade, risk factors for recurrence and shortened survival are unclear. Body composition may play a role in prognosticating these patients and may also help identify patients more likely to benefit from further therapy, such as adjuvant immunotherapy.

1.2 Summary

Melanoma is a common cancer whose incidence is rising in Canada. While management of advanced melanoma has improved in recent years, there is still a lack of accurate predictive and prognostic markers in these patients. Based on research done in other cancer types, body composition may have several applications in patients with advanced melanoma, including identifying patients more likely to benefit from novel immunotherapies or patient at higher risk of recurrence after surgical resection. A small number of studies have been performed of body composition in melanoma, but these have largely been limited by small sample sizes and methodological heterogeneity.

1.3 Objectives

The objectives of this thesis were:

1) To summarize and critically evaluate current research regarding body composition in melanoma, in order to identify gaps in the literature and methodological weaknesses that should be avoided by future studies
2) To further evaluate prognostic and/or predictive roles of body composition in metastatic melanoma patients receiving immunotherapy
3) To characterize baseline body composition in stage III melanoma patients and assess whether body composition impacts long-term survival

The first objective was accomplished by performing a systematic review and summative analysis of existing body composition research in melanoma, using previously established criteria for studies of prognostic factors.

The second objective was addressed by conducting a retrospective study assessing skeletal muscle in a cohort of metastatic melanoma patients receiving the immunotherapy agent nivolumab. Impacts of myosteatosis on survival were assessed in addition to performing an exploratory analysis of nivolumab dosing based on muscle surface area.

For the third objective, a retrospective study was undertaken of all patients in Alberta with resected stage III melanoma from 2007-2017. Peri-operative CT scans were analyzed to determine amounts of skeletal muscle and adipose tissue. Impacts of body composition factors on several survival outcomes, including overall survival (OS), melanoma-specific survival (MSS), and recurrence-free survival (RFS), were measured alongside other known and potential prognostic factors in melanoma.

Chapter 2: A systematic review of prognostic and predictive roles of body composition in melanoma

2.1 Introduction

Body composition analysis is a rapidly growing area of research in oncology. Skeletal muscle and adipose tissue can be precisely quantified on cross-sectional imaging,[26] providing a diagnostic tool with multiple clinical applications. Sarcopenia is commonly defined as a low skeletal muscle index (SMI) calculated by measuring muscle surface area at L3 and normalizing for height.[27,42] Sarcopenia has been associated with various poor outcomes in multiple cancer types, including increased risk of chemotherapy toxicity,[50,51] post-operative complications,[52] and decreased overall survival (OS).[53] Low skeletal muscle radiodensity (SMD), also termed myosteatosis, has also been associated with decreased survival in cancer.[30]

A small but growing number of studies are assessing body composition in melanoma. Stage III and IV melanoma patients routinely have computed tomography (CT) scans performed as part of their care,[54] representing a group of patients in whom body composition analysis could be performed at relatively low additional cost. This research is of particular interest given recent changes to melanoma management with emergence of targeted and immunotherapies. While these therapies have dramatically improved outcomes in advanced melanoma,[15–17] predictive biomarkers in these patients are lacking. Sarcopenia has been associated with decreased OS in other cancer types treated with immunotherapy,[55,56] prompting similar investigations of melanoma patients.

Though body composition analysis is a powerful tool, methodological heterogeneity complicates interpretation of results. A multitude of measurements can be taken from a single CT image, including surface area and radiodensity of skeletal muscle, visceral adipose tissue (VAT), and subcutaneous adipose tissue (SAT).[26] A quick survey of the literature reveals a wide variety of methods used to analyze measurements taken from CT; these include stratifying into low versus high based on a cut-off value,[27,42] dividing into quartiles or tertiles,[57] or calculating a ratio of two different tissue compartments.[35] Images taken at varying anatomic levels, or studies focusing on a single muscle versus all skeletal muscle, further contribute to the confusion. Thus, periodic literature reviews are necessary to compare varying methods of body composition analysis and determine best practices for researchers moving forward.

The purpose of this systematic review is to summarize and critically evaluate current literature on body composition analysis in melanoma patients, focusing on associations of baseline body composition with survival outcomes. As a secondary outcome, we collected data on effects of body composition on treatment-related toxicities in patients treated with systemic cancer therapies. For purposes of this review, the terms sarcopenia and myosteatosis will be reserved for parameters determined from total lumbar skeletal muscle; parameters based off a single skeletal muscle such as psoas muscle will be referred to using the muscle name.

2.2 Methods

This systematic review was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[58] MEDLINE and Embase databases were searched for terms relating to melanoma and body composition analysis

(sarcopenia, myosteatosis, skeletal muscle mass, volume, surface area, density, index, and adipose tissue). Date of last search was Oct. 5, 2020. Terms relating to CT scans or survival outcomes were not included as these were found to overly restrict results in preliminary searches. All human-based studies from 2000-2020 with any survival-based outcomes (OS, progression-free survival (PFS), or disease-free survival (DFS)) or cancer treatment toxicities were included.

Only studies that used abdominal cross-sectional imaging to analyze body composition were included given known correlations between abdominal imaging and whole-body skeletal muscle and fat mass.[25,26] Studies including non-melanoma patients had to provide melanoma-specific statistics or have melanoma patients comprise over 50% of the cohort. Conference abstracts, reviews, and commentaries were searched for relevant references but not included. Non-English articles were not included. Study authors were contacted for missing information when necessary.

Two authors (SY, UJ) screened titles and abstracts for inclusion; any studies identified as relevant by either author underwent full text review. Data extraction for each study was independently performed by two authors (SY, UJ) using CHARMS-PF (checklist for critical appraisal and data extraction for systematic reviews of prediction modelling studies and prognostic factors).[59] Information on study participants, outcomes, adjustment for covariates, statistical analyses, and prognostic factors, specifically method of body composition analysis and definition of sarcopenia, were collected from each study. A meta-analysis was not performed due to heterogeneity of parameters identified from body composition analysis and varying statistical methods of analyzing sarcopenia or myosteatosis.

Two authors (SY, UJ) independently assessed risks of bias in each study using the QUIPS (quality in prognostic factor studies) tool, which assigns a low, moderate, or high risk of bias to categories of study participation and attrition, measurement of prognostic factors and outcomes, use of appropriate cut-points for continuous variables, adjustment for important covariates, and statistical analyses and reporting.[59] Any discrepancies in bias ratings were reviewed until an agreement was reached.

2.3 Results

2.3.1 Search outcomes

The initial search yielded 240 results. After removing duplicates and conference abstracts, 171 titles and abstracts were screened for inclusion (Figure 1). Twenty-three studies were selected for full-text review. Of these, four were excluded as they did not perform abdominal cross-sectional imaging (two studies used temporal muscle thickness, one study performed limb imaging only, and one study used serum creatinine as a surrogate for skeletal muscle mass). Six studies were excluded as they did not provide statistics specific to melanoma patients and melanoma patients comprised less than 50% of the entire cohort. A further four studies lacked outcomes of interest, either survival or treatment-related toxicities. No additional studies were identified from review of reference lists.

A total of nine studies involving 914 patients were included in the final systematic review (Table 1).[45–49,60–63] Eight studies were of patients with unresectable or metastatic melanoma treated with systemic therapy,[20-27] while one study evaluated stage III melanoma patients who

underwent completion lymph node dissection.[60] One study did not analyze survival as an outcome but was included for its findings on treatment-related toxicities.[46]

Results of QUIPS assessment are summarized in Table 2. All studies were considered as being at moderate to high risk of participation bias due to the significant number of patients lacking eligible CTs. The percentage of patients with eligible CTs ranged from 36 to 88%.[21-25,27,28] Two studies did not provide this statistic as patients were pre-selected based on the presence of an available CT.[60,62] Timing of baseline CT scans also varied significantly; the longest window of CT inclusion amongst patients on systemic therapy was within six months of starting treatment.[45,49] One study of resected stage III melanoma patients included CT scans performed within one year of diagnosis of stage III disease.[60]

2.3.2 Skeletal muscle and survival

A variety of body composition analysis techniques were observed in this review. Three studies measured psoas muscle only[45,60,62]; this method was considered to be at high risk of bias as the accuracy of using psoas muscle as a surrogate for whole body skeletal muscle mass has been questioned.[64] Furthermore, these studies did not utilize a HU threshold for skeletal muscle but manually outlined or measured psoas muscle. In their study of resected stage III melanoma patients, Sabel *et al.* (2011) found that increased psoas muscle density correlated with prolonged DFS and distant disease-free survival. Another study by Sabel *et al.* (2015) of metastatic melanoma treated with ipilimumab found that increased psoas density was associated with improved OS. Hu *et al.* calculated a psoas muscle index by normalizing psoas area for height and found no associations with OS or PFS.[62]

Remaining studies evaluating skeletal muscle used a threshold of -29 to +150 HU and analyzed all skeletal muscle at the 3rd or 4th lumbar vertebrae, using a variety of SMI and SMD cut-offs to define sarcopenia and myosteatosis, respectively.[46–49,63] While no single cut-point of SMI or SMD has been found to be applicable to all patient populations, studies were considered at low risk of bias if previously established cut-offs were applied or statistical techniques such as optimal stratification were used to identify cut-offs. Prevalence of sarcopenia ranged from 24 to 53.7%.[46,47,49,63]

Three studies used cut-offs established by Martin *et al.*[42] to define sarcopenia.[47–49] Results regarding effects of sarcopenia were conflicting amongst these studies, despite using identical cut-offs. Two studies found no association of sarcopenia with either OS or PFS,[47,49] whereas Chu *et al.* found that sarcopenia negatively impacted both OS and PFS.[48] This association remained significant in multivariable analysis accounting for age, sex, lactate dehydrogenase (LDH) levels, BRAF mutation status, and number of prior lines of treatment.[48] Another study, using SMI cut-offs defined by Fearon *et al*.,[65] found no significant impact of sarcopenia on OS or PFS.[27]

Three studies evaluated myosteatosis[47–49]. Chu *et al.* used optimal stratification to identify BMI-specific cut-offs of SMD that significantly impacted OS and PFS. While their SMD cut-off for patients with BMI <25 was similar to that established by Martin *et al.* (<41 HU vs. <43 HU, respectively), their cut-off in patients with BMI ≥25 was markedly lower (<20 HU vs. <33 HU). Chu *et al.* found that myosteatosis negatively impacted OS and PFS independent of age, sex, LDH levels, line of treatment, and BRAF mutation status. Both Daly *et al.* and Young *et al.* used Martin's[42] SMD cut-offs to define myosteatosis; in contrast to findings by Chu *et al.*, myosteatosis was not significantly associated with OS or PFS. Young *et al.* performed a further

analysis of skeletal muscle using a measurement known as skeletal muscle gauge (SMG), calculated by multiplying SMI by SMD. Low SMG was defined based on a cut-off previously determined in a population of breast cancer patients.[66] SMG alone was not found to impact OS or PFS, though low SMG combined with high total adipose tissue index (TATI), calculated by adding VAT to SAT and normalizing for height, was associated with worse OS and PFS.

2.3.3 Impact of adipose tissue

Three studies evaluated adipose tissue compartments.[45,49,61] Grignol *et al.* measured VAT and SAT surface area at L4 and found that increased visceral-to-subcutaneous fat ratio was associated with worse OS and PFS.[61] Young *et al.* found that increased TATI was also associated with decreased PFS in multivariate analysis.[49]

Sabel *et al.* measured visceral fat distance by averaging the distance from the anterior vertebral aspect to the linea alba from T12-L4; increased visceral fat distance was associated with worse OS.[45] To our knowledge, this measurement has not been used elsewhere in the literature. While some studies have shown a correlation between visceral fat distance and total visceral fat area,[67,68] these studies used different landmarks, measuring from the anterior aspect of the abdominal aorta rather than the vertebrae. This method of quantifying visceral adipose tissue was considered to be at high risk of bias.

2.3.4 Treatment-related toxicities

Seven studies included treatment-related toxicities as an outcome (Table 2). Six studies recorded treatment-related adverse events (AE) according to National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE).[46–49,62,63] One study used their own classification system, grading side effects as mild, intermediate, or severe.[45]

Overall results for associations of sarcopenia with treatment toxicities were inconsistent. Two studies found that sarcopenia did not increase the risk of AE or dose-limiting toxicity,[49,63] while one study found that sarcopenia was associated with increased risk of high-grade (grade III-IV) AE specifically.[47] Heidelberger *et al.* found that a majority of patients (63%) were sarcopenic by Prado's cut-offs and elected to use median SMI by gender to define sarcopenia; in their study, sarcopenia was associated with increased risk of dose-limiting toxicity but only in overweight females.[46]

Two studies evaluated impacts of myosteatosis on treatment toxicity.[47,48] Chu *et al.* found that low SMD was actually associated with decreased risk of transaminitis and dermatitis but not other toxicities.[48] In contrast, Daly *et al.* found that low SMD was associated with increased risk of all high-grade AE.[47] Amongst studies of psoas muscle specifically, neither psoas muscle density nor index were found to predict treatment toxicity.[45,62]

2.4 Discussion

This systematic review highlights significant methodological heterogeneity and a lack of high-quality evidence within existing literature surrounding body composition in melanoma. While some methodological weaknesses are inherent to retrospective study design, such as risk of selection bias due to patients lacking eligible CTs, multiple studies were further limited by small sample size and use of non-validated CT analysis methods.

Standardization of CT analysis is lacking, with three out of nine studies in this review focusing on psoas muscle though single-muscle approaches to diagnosing sarcopenia or myosteatosis are not well-supported in the literature. The psoas muscle in particular is strongly

associated with benign spinal pathology,[64] and measurements of psoas muscle have been found to correlate poorly with both total skeletal muscle surface area and average radiodensity at L3.[43,69] Furthermore, in a study of patients awaiting liver transplant, psoas muscle index was shown to be a poor predictor of mortality compared to SMI.[44] No studies analyzing psoas muscle in this review performed further analyses to determine whether psoas muscle area correlated with total skeletal muscle surface area or lean body mass, or assessed the prognostic utility of psoas muscle against all skeletal muscle at L3. Psoas muscle should not continue to be used as a substitute for total cross-sectional muscle surface area and impacts of skeletal muscle on survival in melanoma patients cannot be determined from studies using psoas muscle exclusively.

Amongst studies that measured total lumbar skeletal muscle surface area, four studies assessed sarcopenia using different sets of SMI cut-offs. Overall results were conflicting, with only one study identifying sarcopenia as a negative prognostic factor.[48] Given the small number of studies assessing sarcopenia using validated CT-based techniques, prognostic effects of sarcopenia in melanoma cannot be clearly determined from this review. It is also unclear whether cut-offs determined in other cohorts of cancer patients should be applied in melanoma studies. The most commonly used cut-offs in the literature, Martin's and Prado's, were established in groups of gastrointestinal and respiratory cancer patients which did not include melanoma patients or patients treated with immunotherapy. In contrast, the majority of studies in this review were of patients with metastatic disease who in some cases received multiple lines of immunotherapy. To better assess impacts of skeletal muscle depletion, future studies may benefit from comparing baseline body composition parameters to those of other studies to determine whether applying previously established cut-offs is appropriate.

The role of myosteatosis in cancer is still emerging and very few studies have assessed impacts of myosteatosis in melanoma. Myosteatosis is a complex, multifactorial process driven in part by increased systemic inflammation and has been associated with various processes including aging, frailty, and comorbidities such as diabetes.[30] The only study in this review to identify a significant prognostic effect of myosteatosis used SMD cut-offs determined within their cohort,[48] whereas other studies utilizing Martin's cut-offs found no impact on survival. Again, these cut-offs may not be applicable to metastatic melanoma patients.

Another measurement of skeletal muscle found in this review was SMG in a study by Young *et al.,* determined by multiplying SMI by SMD. This is a relatively uncommon method of analyzing skeletal muscle in body composition literature. The physiologic basis of multiplying two different measurements of skeletal muscle together is unclear, as sarcopenia and myosteatosis are driven by distinct biological processes and may or may not occur together. Given the small number of studies utilizing SMG, compared to the vast majority of body composition literature that analyzes SMI and SMD separately, further studies should avoid relying on SMG alone as a prognostic marker.

The distribution of adipose tissue has been shown to be prognostic in cancer. Visceral obesity, defined as an excess of intraabdominal fat, correlated with comorbidities and risk of recurrence more accurately than body mass index (BMI) in colorectal cancer.[35,36] Mechanisms behind these associations are not fully understood, but it is apparent that adipose tissue regulates systemic inflammation through production of adipokines such as adiponectin and leptin, which in turn can contribute to cancer progression.[70] Leptin, for example, has been shown to promote melanoma tumor growth in mice.[71] It has also been recognized that visceral vs. subcutaneous fat compartments are biologically distinct and may have disparate effects on cancer prognosis.[36] The

optimal method of measuring and analyzing adipose tissue is unknown. This was demonstrated by the variety of methods used to quantify adipose tissue in this review, which included visceral-to-subcutaneous fat ratios, visceral fat distance, and total adipose tissue index (TATI). Both increased visceral-to-subcutaneous fat ratio and increased TATI were associated with worse PFS. Taken together these findings suggest that adipose tissue may serve as a potential biomarker in melanoma.

The predictive potential of body composition in melanoma patients treated with immunotherapy is of particular interest, given significant side effect profiles associated with these therapies. By delineating lean and adipose tissue compartments, body composition analysis may reflect drug distribution volumes and has been suggested as a method of predicting cancer treatment toxicity.[72,73] Immunotherapies, which are dosed by body weight, are typically distributed in blood plasma and extracellular fluid volumes.[39] Increases in adipose tissue, while contributing to body weight, may not lead to corresponding increases in drug distribution volume. As such, patients with low lean body mass relative to adipose tissue (such as those with sarcopenic obesity) may be exposed to higher immunotherapy concentrations and therefore be at higher risk of toxicity. This review did not identify any strong predictors of immunotherapy toxicity, with inconsistent results regarding effects of sarcopenia. However, based on the proposed pharmacokinetics of immunotherapy drugs, measurements of both skeletal muscle and adipose tissue may be necessary to identify those at greater risk of toxicity.

To our knowledge, this systematic review is the first to provide an in-depth examination of the existing literature surrounding body composition analysis in melanoma. Most studies are of patients with metastatic disease and sarcopenia is relatively common in this population. Due to disparate methods of body composition analysis, prognostic effects of sarcopenia,

myosteatosis, and increased adiposity have not been clearly established. There are some limitations to this review. Bias ratings should be interpreted with caution, as there is some disagreement in the literature as to how skeletal muscle and adipose tissue should be analyzed as prognostic factors. Nonetheless, we establish that some methods, such as single-muscle approaches, should be avoided by future researchers in this area.

In summary, current literature regarding body composition in melanoma, while presenting some promising findings, is limited by methodological disparities, small sample sizes, and lack of evidence-based methods of CT analysis. As immunotherapy becomes increasingly prominent and patients with advanced disease enjoy prolonged survival, the need to prognosticate and predict outcomes in these patients will grow. Further research, with an emphasis on standardization of CT analysis techniques, is needed to elucidate the role of body composition in melanoma.

Figure 1. Flow diagram to illustrate study inclusion or exclusion in this review.

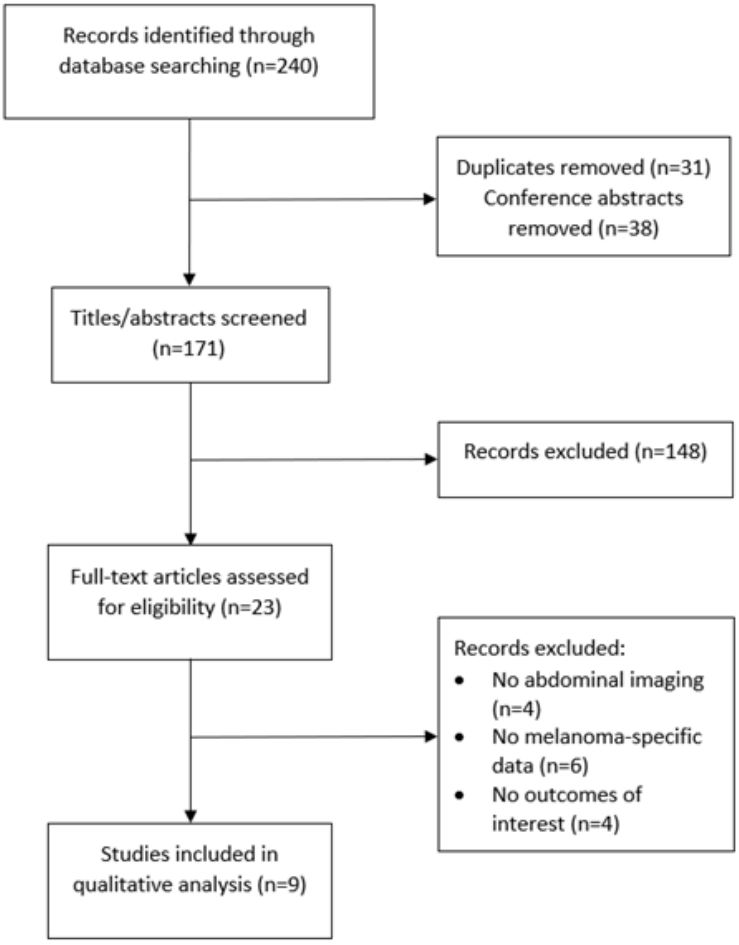


Table 1. Summary of body composition analysis techniques, cut-offs, and impacts of body composition on survival.

Author (year)	**Population, number with CT (percentage)**	**Body composition analysis method (image type, timing, anatomic level, parameter, HU threshold)**	**Body composition parameter and cut-offs**	**Association with survival (95% CI, *p*-value)**	**Adjustment factors in survival analysis**
Sabel *et al.* (2011)[60]	Stage III melanoma undergoing completion lymph node dissection (n=101). Patients pre-selected based on CT availability.	CT Within 1 year of diagnosis of stage III disease L4 Psoas density: muscle outlined and average HU measured.	Psoas density (HU): analyzed as continuous variable Psoas area (cm^2): divided into tertiles.	Psoas density (continuous variable): 1) DFS: HR 0.40 (0.20-0.78, *p*=0.01) 2) DDFS: HR 0.55 (0.35-0.87, *p*=0.01) Psoas area: 1) No significant association with DFS, DDFS	Age, Breslow thickness, ulceration, macroscopic vs. microscopic nodes, number of positive nodes
Sabel *et al.* (2015)[45]	Metastatic melanoma treated with ipilimumab (n=133). 48 patients (36%) with CTs	CT Within 6 months of starting ipilimumab L4, psoas density: muscle outlined and averaged HU measured. T12-L4, VFD: average distance from anterior vertebrae to linea alba	Psoas density (HU): divided into quartiles Psoas area (cm^2): divided into quartiles VFD (cm): divided into quartiles	Psoas density: 1) OS: 1-year survival 71.4% in highest quartile vs. 40.1% in lowest 3 quartiles (*p*=0.04). VFD: 1) OS: 1-year survival 25.0% in highest quartile vs. 56.3% in lowest 3 quartiles (*p*=0.022) Psoas area: 1) No significant association with OS.	None.
Grignol *et al.* (2015)[61]	Metastatic melanoma treated with bevacizumab ±interferon-α (n=62). 42 patients (68%) with CT	CT Within 3 months prior to starting treatment L3-L4 VAT, SAT: -190 to -30 HU	VAT/SAT ratio: divided into increments of 0.5	VAT/SAT ratio (per 0.5 increase): 1) OS: HR 1.60 (1.18-2.19, *p*=0.003) 2) PFS: HR 1.32 (0.99-1.74, *p*=0.056)	LDH, presence of liver metastases. (BMI, age, sex, ECOG PS, treatment type, lung metastases, lymph node metastases, VAT, SAT excluded after

					screening univariate analysis)
Heidelberger *et al.* (2017)[46]	Metastatic melanoma treated with nivolumab or pembrolizumab (n=77). 68 patients (88%) with CT	CT Within 2 months prior to starting treatment L3 Skeletal muscle: -29 to +150 HU	Sarcopenia defined as below median SMI by gender M: <47.68 cm^2/m^2 F: <37.15 cm^2/m^2	N/A	N/A
Daly *et al.* (2017)[47]	Metastatic melanoma treated with ipilimumab (n=96). 84 patients (88%) with CT	CT Pre-treatment L3 Skeletal muscle: -29 to +150 HU	Sarcopenia (Martin's cut-offs): M, BMI <25: <43 cm^2/m^2 M, BMI ≥25: <53 cm^2/m^2 F: <41 cm^2/m^2 Myosteatosis (Martin's cut-offs): BMI <25: <41 HU BMI ≥25: <33 HU	Sarcopenia and myosteatosis not significantly associated with OS.	None.
Chu *et al.* (2020)[48]	Metastatic melanoma treated with ipilimumab (n=121). 97 patients (80%) with CT	CT Within 30 days of starting treatment L3 Skeletal muscle: -29 to +150	Sarcopenia (Martin's cut-offs) M, BMI <25: <43 cm^2/m^2 M, BMI ≥25: <53 cm^2/m^2 F: <41 cm^2/m^2 Myosteatosis (cut-offs determined through optimal stratification): BMI <25: <42 HU BMI ≥25: <20 HU	Sarcopenia: 1) OS: HR 1.85 (1.06-3.22, *p*=0.003) 2) PFS: HR 2.46 (1.35-4.51, *p*=0.004) Myosteatosis (ref: high SMD) 1) OS: HR 2.47 (1.84-6.02, *p*=0.001) 2) PFS: HR 1.77 (1.12-3.31, *p*=0.008)	Age, sex, LDH, line of treatment, BRAF status, ipilimumab dose per MSA (mg/cm^2)

Hu *et al.* (2020)[62]	Metastatic melanoma treated with pembrolizumab (n=156). Patients pre-selected based on CT availability.	CT Within 3 months of starting treatment L3 Psoas area: no HU threshold used.	Psoas muscle index (cm^2/m^2): divided into sex-specific tertiles.	Psoas muscle index: not significantly associated with OS or PFS.	None.
Young *et al.* (2020)[49]	Metastatic melanoma treated with PD1 ± CTLA-4 inhibitors (n=349). 287 patients (82%) with CT	CT Within 6 months of starting treatment L3 Skeletal muscle: -29 to +150 SAT: -190 to -30 VAT: -150 to +50	Sarcopenia (Martin's cut-offs): M, BMI <25: <43 cm^2/m^2 M, BMI ≥25: <53 cm^2/m^2 F: <41 cm^2/m^2 Myosteatosis (Martin's cut-offs): BMI <25: <41 HU BMI ≥25: <33 HU TATI (cm^2/m^2): Divided into tertiles	Sarcopenia: 1) OS: HR 1.28 (0.93-1.77, *p*=0.135) 2) PFS: HR 1.15 (0.87-1.51, *p*=0.33) Myosteatosis: 1) OS: HR 0.76 (0.55-1.04, *p*=0.09) 2) PFS: HR 0.97 (0.74-1.28, *p*=0.85) TATI (highest tertile vs. lowest tertile): 1) OS: HR 1.44 (0.80-2.61, *p*=0.22) 2) PFS: HR 1.71 (1.01-2.87, *p*=0.04)	(Multivariable analysis of TATI) Age, sex, stage, prior treatment, SMG*TATI interaction term
Samanci *et al.* (2020)[63]	Metastatic melanoma treated with BRAF/MEK inhibitors (n=41). 31 patients (76%) with CT	CT L3 Before starting treatment Skeletal muscle: -29 to +150	Sarcopenia (Fearon's cut-offs): M: <55 cm^2/m^2 F: <33 cm^2/m^2	Sarcopenia not significantly associated with OS (*p*=0.326) or PFS (*p*=0.172).	None.

SAT, subcutaneous adipose tissue; IMAT, intermuscular adipose tissue; SFI, subcutaneous fat index; IFI, intermuscular fat index; SMA skeletal muscle area; SMI, skeletal muscle index; SMD, skeletal muscle gauge; SMD, skeletal muscle density; VAT, visceral adipose tissue; VFD, visceral fat distance; TATI, total adipose tissue index; HU, Hounsfield units; OS, overall survival; PFS, progression-free survival; DFS, disease-free survival; DDFS, distant disease-free survival; HR, hazard ratio; 95% CI, 95% confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; AE, adverse event; DLT, dose-limiting toxicity

Table 2. Summary of findings regarding associations of body composition with treatment toxicity. NCI-CTCAE=National Cancer Institute Common Terminology and Criteria for Adverse Events; DLT=dose limiting toxicity.

Study	Toxicity Criteria	Association of body composition with survival
Sabel *et al.* (2015)[45]	Mild, intermediate, severe	Decreased psoas density not associated with increased risk of treatment toxicity.
Daly *et al.* (2017)[47]	NCI-CTCAE version 4.0	Sarcopenia and myosteatosis associated with increased risk of high-grade toxicity.
Heidelberger *et al.* *(*2017)[46]	NCI-CTCAE version 4.0 DLT defined as toxicity leading to temporary or definitive treatment discontinuation	Sarcopenia associated with increased risk of DLT in overweight females.
Chu *et al.* (2020)[48]	NCI-CTCAE version 4.0	Myosteatosis associated with decreased risk of all grades of transaminitis and dermatitis.
Hu *et al.* (2020)[62]	NCI-CTCAE version 5.0	Decreased psoas muscle index not associated with increased risk of treatment toxicity.
Young *et al.* (2020)[49]	NCI-CTCAE version 4.0	Sarcopenia not associated with increased risk of toxicity.
Samanci *et al.* (2020)[63]	NCI-CTCAE version 4.0	Sarcopenia not associated with increased risk of toxicity.

Table 3. Summary of bias ratings of included studies using the Quality in Prognostic Studies (QUIPS) tool

Study	**Study participation**	**Study attrition**	**Prognostic factor measurement**	**Outcome measurement**	**Adjustment for covariates**	**Statistical analysis and reporting**
Sabel *et al.* (2011)[60]	High risk	Low risk	High risk	Low risk	Low risk	Moderate risk
Sabel *et al.* (2015)[45]	High risk	Low risk	High risk	Moderate risk	High risk	Moderate risk
Grignol *et al.* (2015)[61]	Moderate risk	Low risk	Low risk	Low risk	Low risk	Low risk
Daly *et al.* (2017)[47]	Moderate risk	Low risk	Low risk	Low risk	High risk	Low risk
Heidelberger *et al. (*2017)[46]	Moderate risk	Low risk	Moderate risk	Low risk	High risk	Low risk
Chu *et al.* (2020)[48]	Moderate risk	Low risk	Low risk	Low risk	Low risk	Low risk
Hu *et al.* (2020)[62]	High risk	Low risk	High risk	Low risk	High risk	Moderate risk
Young *et al.* (2020)[49]	Moderate	Low risk	Low risk	Low risk	Moderate risk	Low risk
Samanci *et al.* (2020)[63]	Moderate	Low risk	Low risk	Low risk	High risk	Low risk

Chapter 3: Myosteatosis is prognostic in metastatic melanoma treated with nivolumab

3.1 Introduction

Melanoma is one of the most common types of cancer in North America and its incidence is on the rise.[74] Management of advanced melanoma has significantly changed in recent years with the emergence of immunotherapies such as nivolumab, an inhibitor of cell programmed death-1 (PD-1) receptors, and ipilimumab, an antibody targeting anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). These agents have dramatically improved survival in metastatic melanoma (MM).[15,16] Nivolumab, either as monotherapy or in combination with ipilimumab, is now considered one of the standards of care for metastatic melanoma (MM).

However, few predictive biomarkers in patients treated with immunotherapy have been identified. Body composition analysis is increasingly being studied as a method of prognosticating MM treated with immunotherapy. Imaging analysis software can quantify surface area and radiodensity of skeletal muscle on computed tomography (CT) scans. Muscle surface area (MSA) measured at the L3 vertebrae correlates with total lean body mass and skeletal muscle,[25,26] while the mean attenuation of skeletal muscle in Hounsfield units (HU) is a surrogate of muscle density and the degree of fatty infiltration.[29] Low skeletal muscle density (SMD), also termed *myosteatosis*, has been associated with worse OS in multiple cancer types.[30,75]

As patients with advanced melanoma typically have PET-CT or CT scans performed as part of their work-up, myosteatosis can be easily identified from existing data and is promising as a low-cost method of prognosticating MM. The impact of myosteatosis in melanoma is unclear. Chu *et al.* identified myosteatosis as a negative prognostic factor in MM patients treated

with ipilimumab,[48] while a recent study by Young *et al.* of MM patients treated with anti-PD-1 or anti-CTLA-4 immunotherapy found no impact of myosteatosis on survival.[49]

Body composition analysis has also been studied as a predictor of immunotherapy-related toxicities. Treatment-related toxicities are common, with over 50% of patients experiencing a high-grade adverse event (AE) in a pooled analysis of MM treated with nivolumab plus ipilimumab.[20] Several studies have examined associations of myosteatosis with immunotherapy toxicity in melanoma with conflicting results: Chu *et al.* found that low SMD patients treated with ipilimumab actually had fewer immune-related AE,[48] while Daly *et al.* found that low SMD was associated with a higher incidence of ipilimumab toxicity.[47]

MSA, as a surrogate of lean body mass, has been suggested as a method of predicting cancer treatment toxicity. Immunotherapy drugs are typically distributed in blood plasma and extracellular fluid volumes.[39] Currently immunotherapies are dosed by body weight, which can fluctuate widely depending on levels of adipose tissue and therefore may not correlate with lean body mass. By distinguishing lean and adipose tissue compartments, imaging-based body composition analysis may reflect drug distribution volumes more accurately than body weight. Studies of cytotoxic chemotherapy, which are similarly distributed in the lean compartment, have found that patients who received a higher dose per kilogram of lean body mass (derived from measurements of MSA) were more likely to experience toxicity.[73,76] Quantifying skeletal muscle may have a similar role in patients on immunotherapy.

The primary aim of this study was to assess whether SMD was prognostic of OS in melanoma patients treated with nivolumab. We also aimed to determine whether calculating nivolumab dose based on MSA could predict OS and treatment-related toxicities.

3.2 Methods

Ethics approval was obtained from the Health Research Ethics Board of Alberta. A retrospective analysis was performed of all MM patients who were treated with nivolumab at a single Northern Alberta cancer institute from 2015 to 2017. All patients who had CT scans performed within 30 days prior to starting treatment were included in the study. Demographic information including age, sex, BRAF status, lactate dehydrogenase (LDH) levels, stage, and Eastern Cooperative Oncology Group Performance Status (ECOG PS) were collected. Weight, height, body mass index (BMI), nivolumab dose received per cycle, and number of previous lines of therapy were collected. Primary outcome was OS, which was determined from the date of starting treatment. Secondary outcomes were incidence of hospitalizations and treatment-related toxicities, including gastrointestinal toxicities (nausea, vomiting, or diarrhea), transaminitis, dermatitis, and endocrinopathy.

Baseline CT scans were analyzed using Sliceomatic™ software by a trained user to determine MSA in cm^2 and SMD in HU from a single image at the L3 vertebrae (version 5.0, TomoVision, Magog, Quebec, Canada). The threshold used for skeletal muscle tissue was -29 to +150 HU. Average nivolumab dosing by muscle area was calculated by dividing nivolumab dose in milligrams received per cycle by MSA (mg/cm^2).

Optimal stratification was used to determine cut-offs of low versus high SMD and nivolumab dosing that most significantly impacted OS. This method is used to stratify continuous variables hypothesized to have a threshold value above which outcomes are significantly different.[77] Relatively few studies have determined myosteatosis cut-offs in MM or in patients on immunotherapy. The most commonly used cut-offs in the literature, Martin's and Prado's, were established in cohorts of gastrointestinal and lung cancer patients.[17,18] A study by

Chu *et al.* determined SMD cut-offs in 97 MM patients treated with ipilimumab[9]; these cut-offs were tested in our study in addition to determining our own cut-offs due to smaller sample size. Baseline patient characteristics were compared using Mann-Whitney, t-test, or Chi-square analysis. Kaplan-Meier curves and log rank tests were used to compare survival between groups. Univariate analysis was performed to screen for variables that significantly impacted OS ($\alpha \leq 0.10$); these variables were included in subsequent multivariate Cox regression analyses. All statistical analyses were performed using Stata (version 16, StataCorp, College Station, Texas).

3.3 Results

3.3.1 Patients

Fifty patients from 2015-2017 received nivolumab for MM. Forty-four patients (88%) had pre-treatment CT scans eligible for analysis. Twelve patients (27.3%) received nivolumab only; 32 patients (72.7%) received three induction cycles of ipilimumab at the start of treatment before proceeding to nivolumab maintenance therapy. A significant proportion of patients received prior systemic treatment for melanoma, with 13 patients (29.5%) having received 2 or more prior lines of therapy. Median age was 57 and 25 patients (56.8%) were male. Twenty-two (50%) harbored a BRAF mutation, while 6 patients (13.6%) had an elevated LDH at start of treatment. The most common treatment-related toxicity was gastrointestinal toxicity, with an incidence of 50%. Twenty-one patients (47.8%) died by the end of the study period and one patient was lost to follow up; all deaths were attributable to disease progression. Baseline patient characteristics are shown in Table 1.

In univariate analysis, sex, number of prior lines of therapy, and ECOG PS significantly impacted OS ($\alpha \leq 0.10$, Table 2). These variables were included in subsequent multivariate Cox

regressions. Advanced age, BRAF status, LDH levels, and BMI category did not significantly impact survival.

3.3.2 Skeletal Muscle Density

Median SMD was 31.67 HU (range 10.06-54.85 HU). Optimal stratification yielded a cut-off of 25.65 HU for low versus high SMD that significantly impacted OS. With this cut-off, 12 patients (27.3%) had low SMD and 32 patients (72.7%) had high SMD. Low SMD patients had significantly shorter OS compared to high SMD patients (median 12.03 vs. 34.96 months, p=0.001 by log rank test, Figure 1). Using cut-off values determined by Chu *et al*. that were able to be stratified by BMI due to the larger numbers in that study (<42 HU in BMI <25 kg/m^2, <20 HU in BMI ≥25 kg/m^2),[48] median OS was 12.05 vs. 34.73 months, p=0.007 by log-rank test, in the low SMD and high SMD groups.

When comparing baseline characteristics between low vs. high SMD patients by our cut-offs, median age was found to be significantly different (64 vs. 55 years respectively, p=0.001, Table 1). In multivariate Cox regression analysis performed with age, sex, ECOG PS, and number of prior lines of therapy as covariates, low SMD still significantly impacted OS (HR 4.40, 95% CI 1.44-13.42, p=0.009, Table 3). Hazard ratios of multivariate analysis are shown in Table 3. The incidence of hospitalizations and treatment-related toxicities was not significantly different between low vs. high SMD patients (Table 1).

3.3.3 Nivolumab Dosing by Muscle Surface Area

Patients on combination immunotherapy received nivolumab at a standard dose of 1 mg/kg per cycle during induction before proceeding to a maintenance dose of 3 mg/kg. Those on nivolumab alone received 3 mg/kg throughout treatment. Calculations were based on the initial dose of nivolumab received, as only 11 patients in the combination treatment group (34.4%)

completed induction and went on to receive maintenance therapy. The median dose of nivolumab by MSA was 0.68 mg/cm^2 (range 0.41-2.67 mg/cm^2). A significant cut-point for low versus high nivolumab dose was identified at 0.62 mg/cm^2. Those who received a total dose below this cut-off had significantly improved OS (median 42.9 vs. 12.3 months, p<0.001, see Figure 2).

Age, number of prior lines of therapy, BMI, MSA, and ECOG PS were significantly different between patients who received low vs. high doses of nivolumab by MSA (Table 1). Patients who received a lower dose per MSA were younger, received fewer prior therapies, were more likely to be overweight (BMI ≥25), and had higher ECOG PS. When accounting for age, sex, ECOG PS, and number of prior lines of therapy, lower nivolumab dosing by MSA remained significantly associated with improved OS (HR 0.05, 95% CI 0.01-0.30, *p*=0.001, Table 3). Additional covariates were not included in multivariable analysis due to small sample size. The incidence of transaminitis was significantly higher in patients who received a lower dose of nivolumab (55.0 vs 25.0%, p=0.04). Rates of other treatment-related toxicities and hospitalizations were not significantly different between low vs. high nivolumab dose.

Given that nivolumab dose varied by treatment group (combination vs. monotherapy), survival analysis was repeated amongst the combination treatment group to eliminate this potential confounding effect. In this cohort of 32 patients, lower nivolumab dose by MSA remained significantly associated with prolonged OS (median survival 43.4 vs. 7.4 months, p<0.001, Figure 2B).

3.4 Discussion

Myosteatosis is thought to prognosticate cancer patients in multiple ways. Whereas measurements of MSA reflect total body skeletal muscle mass and can be used to identify

sarcopenic patients, SMD is thought to reflect intrinsic muscle quality.[29] Decreased SMD has been associated with aging,[78] frailty,[79] and various comorbidities such as obesity and diabetes. In this sense, myosteatosis may be a marker of poor functional status and as such identify those with a poor prognosis. Low SMD is also associated with increased systemic inflammation,[80] which in itself has been acknowledged as an important driver of cancer progression.[81] In melanoma, elevated markers of systemic inflammation have been identified as a poor prognostic factor, with several studies demonstrating that increased neutrophil-to-lymphocyte ratio were associated with worse OS and PFS in MM treated with immunotherapy.[82,83] In our study, myosteatosis was associated with worse OS even when accounting for known prognostic factors such as age, sex, performance status, and number of prior lines of therapy. Due to a lack of readily available data, we did not perform further analyses to determine whether inflammatory markers such as an increased neutrophil-to-lymphocyte ratio were associated with myosteatosis or survival.

Our findings stand in contrast to those of several studies which found no impact of SMD on survival in similar groups of metastatic melanoma patients treated with immunotherapy.[47,84] Of note, our study used the technique of optimal stratification to identify SMD cut-offs within our cohort, whereas studies by Daly *et al.* and Young *et al.* used SMD cut-offs established by Martin *et al.* in a cohort of gastrointestinal and lung cancer patients (<41 HU in BMI <25 kg/m^2 and <33 HU in BMI ≥25 kg/m^2) .[42,47,49] Martin's cohort did not include any melanoma patients or patients treated with immunotherapy. While these are among the most common SMD cut-offs used in body composition literature, it is unclear whether they are applicable to all cancer populations. Disparate definitions of myosteatosis may explain some of the variation between studies. Interestingly, the SMD cut-off determined in our study (<25.65 HU) is similar to that

determined by Chu *et al.* in patients with BMI ≥25 kg/m^2 (<20 HU).[48] A study by Bhullar *et al.* that took repeated measures of SMD from CT scans within a 5 cm range found a coefficient of variation of 5 HU[85]; given this, our cut-off is consistent with that of Chu *et al.* and suggests that the threshold value for SMD in MM lies somewhere within this range.

Body composition analysis has been studied as a way of tailoring immunotherapy dosing to both improve outcomes and avoid toxicities. In this study, we calculated nivolumab dosing based on muscle surface area, as total body lean body mass is linearly correlated with muscle area on cross-sectional imaging.[26] Based on this calculation, those who received lower doses of nivolumab (<0.62 mg/cm^2) actually had improved OS, even when controlling for age, sex, and performance status. While mechanisms underlying this association are unclear, these results are similar to those of Chu *et al.*, who found that lower doses of ipilimumab based on MSA were associated with improved PFS and OS, independent of age, sex, number of prior treatments, LDH, BRAF status, and presence of sarcopenia.[48] While our analyses were limited by small sample size, they contribute to a growing body of evidence that suggests body composition may be used as a method of cancer therapy dosing to improve survival.

Our study did not identify any strong predictors of immunotherapy toxicity. Rates of treatment toxicity were relatively high and in line with other studies of MM treated with nivolumab ± ipilimumab. While myosteatosis has been identified as a poor prognostic factor in cancer, its relation to cancer treatment toxicity is unclear. In our study, there were no significant differences in the incidence of hospitalizations and treatment-related toxicities between low vs. high SMD patients. Thus, while SMD may impact OS, it may not predict which patients are more likely to experience adverse events. As well, patients who received a higher concentration of nivolumab based on MSA were not more likely to experience toxicities. We based our dosing

on MSA measured at L3 to avoid introducing further error by calculating total lean body mass through linear regression formulas established in other studies of cancer patients. Our findings suggest that this method of calculating nivolumab dose may be of limited value in predicting toxicity.

The main limitations of this study are its retrospective nature and small sample size. This limited the number of potential confounders we could account for in multivariate regression, though we attempted to evaluate all variables through a screening univariate analysis. Given our small sample size and specific patient population, SMD cut-offs identified here should be regarded as hypothesis-generating and may not be generalizable to other patient cohorts. Furthermore, there is some evidence that SMD cut-offs may vary with BMI category, though underlying associations between myosteatosis and BMI are unclear.[17,18] Our small sample size precluded us from further stratifying our population to determine BMI-specific SMD cut-offs, though of note we did apply BMI-specific cut-offs established in another study and found similar effects on survival. This study also looked at all patients receiving nivolumab as a whole, though some patients also received several cycles of ipilimumab at the start of treatment. As the two therapies have different side effect profiles,[20] this may have impacted our ability to identify predictors of treatment toxicity.

Despite these limitations, this study contributes to a growing body of evidence on the prognostic and predictive value of SMD in melanoma. In contrast to several other studies, we identify myosteatosis as a negative prognostic factor in MM patients treated with nivolumab. As immunotherapies become increasingly prevalent, further research will be needed to identify prognostic and predictive markers in MM.

Table 1. Baseline patient characteristics and toxicities based on SMD and nivolumab dosing.

Patient characteristic	**All (n=44)**	**Low SMD (n=12)**	**High SMD (n=32)**	***p*-value (low vs. high SMD)**	**Nivolumab dose <0.62 mg/cm^2 (n=20)**	**Nivolumab dose ≥0.62 mg/cm^2 (n=24)**	***p*-value (low vs. high dose)**
Median age, years (range)	57 (29-79)	64 (55-79)	55 (29-75)	0.001	55 (29-74)	62 (34-79)	0.005
Sex, n				0.90			0.03
Male	25 (56.8%)	7 (58.3%)	18 (56.3%)		15 (75%)	10 (41.7%)	
Female	19 (43.2%)	5 (41.7%)	14 (43.7%)		5 (25%)	14 (58.3%)	
Stage, n				0.83			0.74
III	10 (22.7%)	3 (25%)	7 (21.9%)		5 (25%)	5 (20.8%)	
IV	34 (77.4%)	9 (75%)	25 (78.1%)		15 (75%)	19 (79.2%)	
Prior lines of therapy, n				0.069			0.009
<2	31 (70.5%)	6 (50%)	25 (78.1%)		18 (90%)	13 (54.2%)	
≥2	13 (29.5%)	6 (50%)	7 (21.9%)		2 (10%)	11 (45.8%)	
BMI, n							
<25 kg/m^2	11 (25%)	5 (41.7%)	6 (18.8%)	0.118	2 (10%)	9 (37.5%)	0.036
≥25 kg/m^2	33 (75%)	7 (58.3%)	26 (81.3%)		18 (90%)	15 (62.5%)	
BRAF mutation, n				0.50			0.99
Negative	22 (50%)	7 (58.3%)	15 (46.9%)		10 (50%)	12 (50%)	
Positive	22 (50%)	5 (41.7%)	17 (53.1%)		10 (50%)	12 (50%)	
LDH, n				0.72			0.52
Normal	38 (86.3%)	10 (83.3%)	28 (87.5%)		18 (90%)	20 (83.3%)	
Elevated	6 (13.6%)	2 (16.7%)	4 (12.5%)		2 (10%)	4 (16.7%)	
ECOG PS, n				0.69			0.03
0	29 (65.9%)	7 (58.3%)	22 (68.8%)		17 (85%)	12 (50%)	
1	13 (29.6%)	4 (33.3%)	9 (28.1%)		2 (10%)	11 (45.8%)	
2	2 (4.5%)	1 (8.3%)	1 (3.1%)		1 (5%)	1 (4.2%)	
3	0	0	0				
Mean MSA, cm^2 (SD)	137.8 (±40.5)	142.3 (±42.8)	135.9 (±40.0)	0.634	160.2 (±36.8)	119.1 (±33.8)	<0.001
Hospitalization, n	11 (25%)	4 (33.3%)	7 (21.9%)	0.43	5 (25%)	6 (25%)	0.99
Gastrointestinal toxicity, n	22 (50%)	4 (33.3%)	18 (56.3%)	0.18	12 (60%)	10 (41.7%)	0.23
Transaminitis, n	17 (38.6%)	5 (41.7%)	12 (37.5%)	0.80	11 (55%)	6 (25%)	0.04
Dermatitis, n	18 (40.9%)	4 (33.3%)	14 (43.8%)	0.53	10 (50%)	8 (33.3%)	0.26
Endocrinopathy, n	4 (9.1%)	1 (8.3%)	3 (9.4%)	0.92	2 (10%)	2 (8.3%)	0.85

BMI=body mass index; LDH=lactate dehydrogenase; ECOG PS=Eastern Cooperative Oncology Group performance status; SMD=skeletal muscle density; MSA=muscle surface area; SD=standard deviation.

Table 2. Univariate analysis of characteristics associated with overall survival (OS).

Variable	Hazard ratio	95% CI	*p*-value
Age (≥60)	1.71	0.72 - 4.03	0.223
Female sex	2.13	0.90 - 5.08	0.087
Stage IV	1.14	0.38 - 3.39	0.816
≥ 2 prior lines of therapy	3.72	1.56 - 8.90	0.003
BMI ≥25 kg/m^2	0.56	0.22-1.38	0.208
Positive BRAF mutation	1.29	0.55 - 3.05	0.558
Elevated LDH	1.19	0.35 - 4.03	0.784
ECOG PS			0.213
1	2.24	0.92 - 5.42	0.075
2	1.79	0.23 - 13.87	0.579
Low SMD	3.81	1.60 - 9.08	0.003
Low nivolumab dose by MSA	0.06	0.01 - 0.28	<0.001

CI=confidence interval; LDH=lactate dehydrogenase; ECOG PS=Eastern Cooperative Oncology Group performance status; SMD=skeletal muscle density; MSA=muscle surface area.

Table 3. Multivariate analyses of variables assessing for impact on overall survival (OS) based on SMD and Nivolumab dosing by MSA.

	Variable	Hazard ratio	95% CI	*p*-value
SMD	Low SMD (<25.65 HU)	4.40	1.44-13.42	0.009
	Female sex	2.98	1.07-8.31	0.037
	Age (≥60 years)	0.85	0.25-2.86	0.791
	≥2 prior lines of therapy	3.21	1.22-8.47	0.018
	ECOG PS			
	1	1.56	0.59-4.08	0.367
	2	1.58	0.18-14.01	0.681
Nivolumab dose by MSA	Low dose (<0.62 mg/cm^2)	0.05	0.01-0.30	0.001
	Female sex	1.00	0.38-2.68	0.993
	Age (≥60 years)	0.61	0.20-1.87	0.388
	≥2 prior lines of therapy	2.32	0.85-6.36	0.101
	ECOG PS			
	1	0.88	0.34-2.27	0.787
	2	4.54	0.47-43.63	0.191

CI=confidence interval; SMD=skeletal muscle density; ECOG PS=Eastern Cooperative Oncology Group performance status; Nivo=nivolumab; MSA=muscle surface area.

Figure 1. Kaplan-Meier curve of overall survival (OS) based on low versus high skeletal muscle density (SMD).

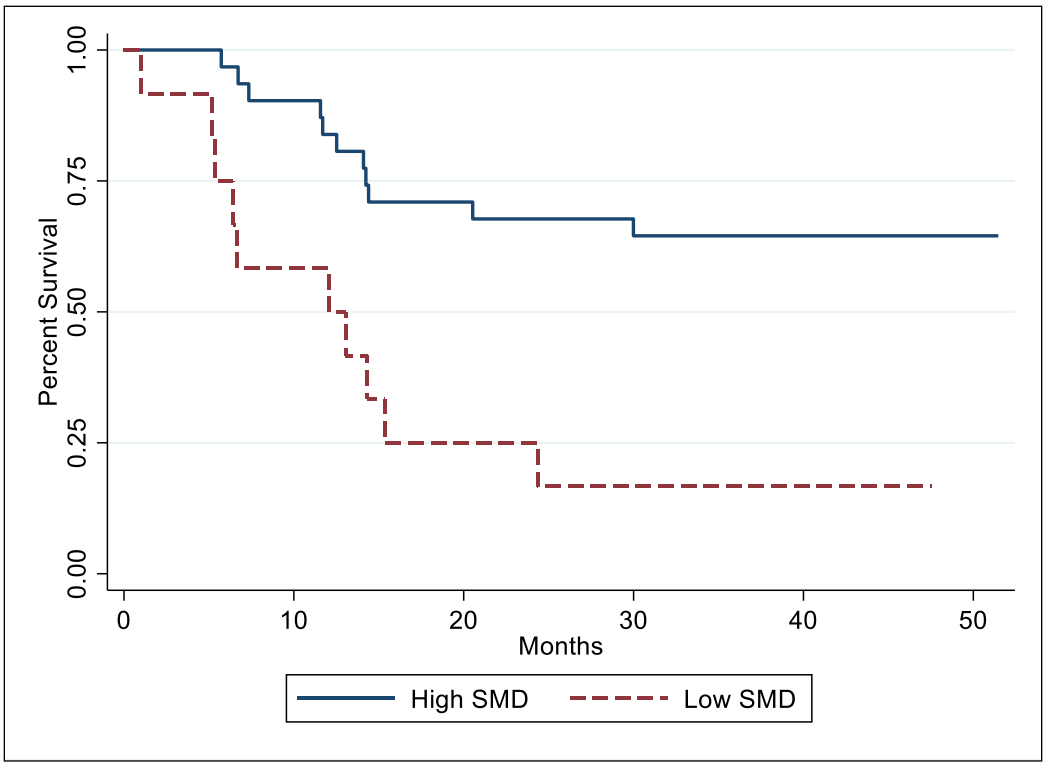
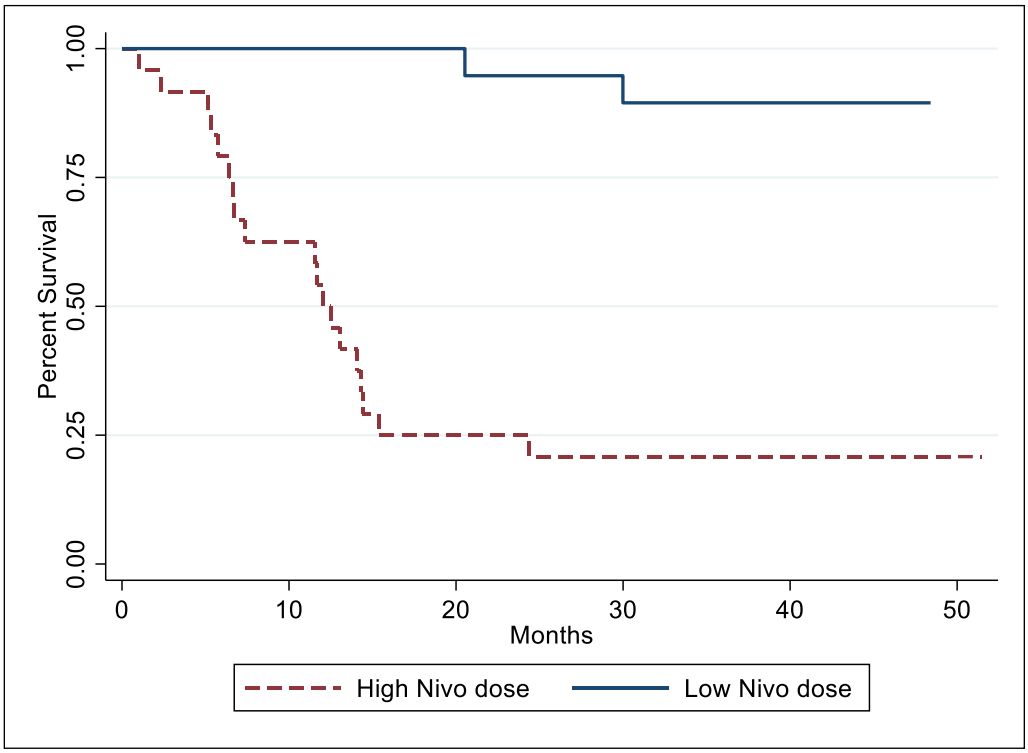
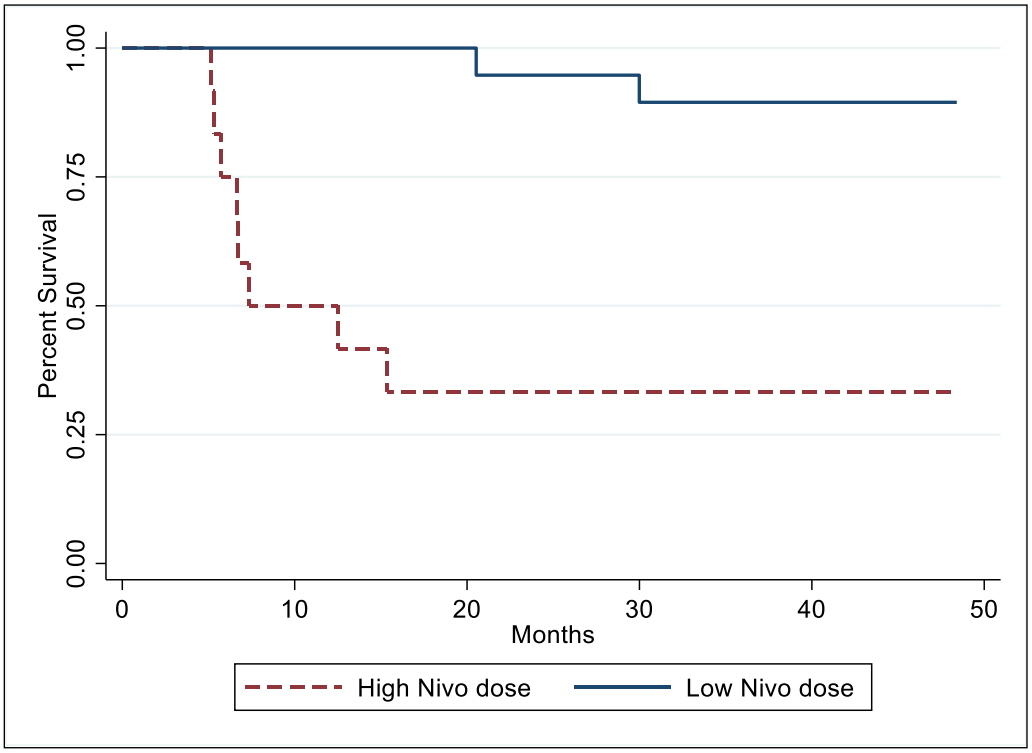


Figure 2. Kaplan-Meier curves of overall survival (OS) based on low versus high nivolumab dosing received per muscle surface (cm^2); (A) all patients (B) patients in cohort treated with combination ipilimumab plus nivolumab.

A.



B.



Chapter 4: Skeletal muscle is prognostic in resected stage III malignant melanoma

4.1 Background

Body composition analysis is a source of valuable prognostic and predictive information in cancer. Hallmarks of malnutrition such as sarcopenia, or skeletal muscle depletion, may not be evident on physical exam, leading to a growing reliance on imaging-based assessments of body composition. Sarcopenia is commonly defined as a low skeletal muscle index (SMI), determined by measuring muscle surface area (MSA) on cross-sectional imaging and normalizing for height.[26,27,42] Multiple studies have associated sarcopenia with decreased survival in a variety of cancer patients.[27,42] Another measurement of skeletal muscle is skeletal muscle density (SMD), which can be assessed on computed tomography (CT) scans by measuring average muscle attenuation in Hounsfield units (HU).[29] Decreased SMD, also known as myosteatosis, is distinct from sarcopenia but also carries negative prognostic implications.[30] A landmark study by Martin *et al.* assessing body composition in a cohort of stage I-IV gastrointestinal and respiratory cancer patients established SMI and SMD cut-offs that are widely used in Western literature as definitions of sarcopenia and myosteatosis, respectively.[42]

A small, but growing number of studies, are focusing on impacts of body composition in melanoma.[45,47–49,60] Most studies are of patients with metastatic disease, given the wide availability of diagnostic imaging in these patients. However, patients with stage III disease also have CT or PET-CT scans routinely performed and represent an understudied group in the body composition field. A previous study of body composition in stage III patients identified decreased psoas muscle density as a negative prognostic factor,[60] though single muscle approaches to body composition analysis have been questioned for their reliability.[43,64]

Management of stage III melanoma has undergone significant changes in recent years, with results of the Multicenter Selective Lymphadenectomy Trial II (MSLT-II) altering indications for completion lymph node dissection[11] and the recent approval of immunotherapy agents and BRAF-inhibitors as adjuvant treatment for resected stage III disease.[19,86] Prevalence of sarcopenia and long-term impacts of baseline body composition on survival in these patients are unknown.

Our study's objectives were to assess impacts of body composition on survival in resected stage III melanoma, alongside other known prognostic factors and anthropometric measures such as body mass index (BMI).

4.2 Methods

Patients

This was a retrospective study of all patients in Alberta, Canada referred to one of two cancer care centers (Cross Cancer Institute and Tom Baker Cancer Center) with resected stage III melanoma, as determined from a prospectively collected cancer database. Stage III patients were defined as those with pathologically positive lymph nodes, satellite lesions, or in-transit metastases; all patients had pathologic lymph node sampling. Patients with multiple primary lesions were excluded. Patients were included if an abdominal CT scan was performed within 60 days of primary surgery (wide local excision). In the case of patients presenting with lymphadenopathy and unknown site of primary, date of surgery was defined as date of regional node dissection. Height, weight, and Eastern Cooperative Oncology Group performance status (ECOG PS) were collected from medical records closest to date of CT. Type of surgery

performed (sentinel lymph node biopsy only vs. regional node dissection) and any adjuvant therapies given were recorded. Stage subgroup was determined retrospectively based on pathologic and clinical data using AJCC (American Joint Committee on Cancer) 8th edition criteria.

CT analysis technique

A single image at L3 was analyzed in SliceOMatic (version 5.0, TomoVision, Magog, Quebec, Canada) body composition software by a trained user. Hounsfield unit (HU) thresholds used were -20 to +150 HU for skeletal muscle, -190 to -30 HU for subcutaneous adipose tissue, and -150 to -50 HU for visceral adipose tissue. SMI was calculated by normalizing SMA for height (cm^2/m^2). Average SMD at L3 was measured in HU. Total adipose tissue surface area was calculated as the sum of visceral and subcutaneous fat areas. Visceral, subcutaneous, and total adipose tissue indices were calculated by dividing surface area by height in m^2.

Statistical approach

Primary endpoints were overall survival (OS), melanoma-specific survival (MSS), and recurrence-free survival (RFS). Survival was calculated from date of surgery and patients were followed until death, loss to follow-up, or date of last chart review (Dec 7, 2020). Recurrence was defined as date of either biopsy- or imaging-proven recurrence, whichever occurred first. Baseline characteristics were compared using t-tests or Chi-square tests for continuous and categorical variables, respectively. Cut-offs established by Martin *et al.* were used to define sarcopenia and myosteatosis.[42] Martin's SMI cut-offs for sarcopenia are stratified by sex and BMI (males with BMI<25, <43 cm^2/m^2; males with BMI $\geq$25, <53 cm^2/m^2; females, <41

cm^2/m^2) and SMD cut-offs for myosteatosis are stratified by BMI (BMI <25, <41 HU; BMI ≥25, <33 HU).

As Martin's cohort did not include melanoma patients and it is unknown whether body composition varies significantly between these populations, cohort-specific cut-offs were also determined using optimal stratification. SMI and SMD cut-offs that optimally predicted OS based on log-rank statistics were identified using a minimal p-value approach.[77] Cut-points were selected from the inner 80% of values in order to avoid small numbers in subgroups and loss of statistical power.[87,88] To further assess joint effects of myosteatosis and sarcopenia, patients were also classified into phenotypes based on presence or absence of these factors using cohort-specific cut-offs.

As prognostic cut-offs of adipose tissue have not been well-established in the literature, an exploratory analysis was performed of whether adipose tissue indices predicted OS in our study. Adipose tissue indices were divided into quartiles and survival was compared between the highest and lowest three quartiles. If survival significantly varied between these groups, minimal p-value analysis was applied to identify cut-offs that optimally predicted OS.[77] These cut-offs were then tested in multivariate analysis.

Log-rank tests and Kaplan-Meier curves were used to compare survival between groups. Univariate and multivariate survival analyses were conducted using Cox proportional hazards models. Multivariate survival models included the following variables selected *a priori:* age, sex, stage subgroup, ECOG PS, and tumor location. Variables that were considered for inclusion in univariate analysis were BMI, surgery (regional node dissection vs. no dissection), and adjuvant treatment, using an alpha threshold of 0.1. Testing of Schonfield residuals demonstrated no

violation of assumptions of proportional hazards models. All statistical analyses were performed in Stata (version 16, StataCorp, College Station, Texas).

4.3 Results

4.3.1 Patients

Of 589 stage III patients initially identified from the database, 44 patients (7.5%) were excluded due to having multiple primary tumors (Figure 1). 367 (67.3%) of these patients had a baseline CT within 60 days of surgery. Twenty-five scans could not be analyzed due to image quality and a further 12 patients had incomplete data, leaving 330 patients included in the final analysis. The mean age was 56.4 years (Table 1), the majority of patients were male (62.4%), and 72.4% of patients were overweight or obese with a BMI ≥25. Most patients (97%) had a baseline ECOG of 0-1. A majority (80.2%) of patients underwent regional node dissection and 43.6% received a form of adjuvant treatment, with radiation and interferon being the most common therapies. Only 6 patients (1.8%) received adjuvant immunotherapy and 1 patient (0.3%) received an adjuvant BRAF-inhibitor.

4.3.2 Survival

Median OS was 56.4 months (range 1.84-165.1) and 150 patients (45.6%) had died at time of censoring. The majority of deaths were attributable to melanoma recurrence (110/150, 73.3%). In total, 182 patients (55.2%) experienced disease recurrence. Median MSS was 55.8 months (range 1.84-165.1). Based on log-rank statistics, BMI category (<25 vs. >25) did not significantly predict OS (p=0.159) or MSS (p=0.394). Receiving regional node dissection did not significantly predict OS (p=0.218) or MSS (p=0.770). Finally, type of adjuvant treatment

received did not predict OS (p=0.654) or MSS (p=0.612). These variables were therefore not included in subsequent multivariate survival models.

4.3.3 Sarcopenia

Mean SMI was 54.5 cm^2/m^2 in men and 40.8 cm^2/m^2 in women (Table 2). For comparison, mean SMI in Martin's study was 51.5 cm^2/m^2 in men and 41.3 cm^2/m^2 in women. Based on SMI cut-offs determined by Martin *et al.*,[42] 46.7% of patients were sarcopenic. Sarcopenia as defined by these cut-offs was associated with significantly decreased OS in both univariate analysis (HR 1.44, 95% CI 1.04-1.98, p=0.026) and multivariate analysis accounting for age, sex, ECOG status, tumor location, and stage (HR 1.50, 95% CI 1.08-2.09, p=0.016).

Sex- and BMI-specific cut-offs generated using optimal stratification are shown in Table 3. These cut-offs were lower than those established by Martin *et al.*[42] Prevalence of sarcopenia based on cohort-specific cut-offs was 20%. Sarcopenic patients were significantly older, more likely to have higher ECOG PS, and less likely to undergo regional node dissection (Table 1). Sarcopenic patients had significantly decreased OS based on log-rank statistics (p<0.0001, Figure 2) and in univariate Cox regression analysis (HR 2.43, 95% CI 1.73-3.43, p<0.001). This association remained significant in multivariate analysis accounting for age, sex, stage subgroup, ECOG PS, and tumor location (HR 2.38, 95% CI 1.64-3.45, p<0.001, Table 4). Sarcopenia was associated with worse MSS in multivariate analysis (HR 1.85, 95% CI 1.16-2.96, p=0.009) but did not significantly impact RFS (HR 1.43, 95% CI 0.98-2.08, p=0.062).

4.3.4 Myosteatosis

Mean SMD did not vary significantly by sex, with an average of 36.0 HU in men and 34.9 HU in women (p=0.365, Table 2). These were similar to Martin's average baseline values

of 35.5 HU in men and 34.5 HU in women. Using Martin's SMD cut-offs, 46.4% of patients had myosteatosis. These patients had significantly decreased OS in univariate (HR 1.56, 95% CI 1.13-2.5, p=0.006) and multivariate analysis (HR 1.57, 95% CI 1.10-2.24, p=0.013, Table 4). Martin's cut-offs of myosteatosis also significantly impacted MSS in multivariate analysis (HR 1.55, 95% CI 1.03-2.34, p=0.037).

Mean SMD was significantly lower in patients with BMI $\geq$25 (Table 2). BMI-specific SMD cut-offs determined using optimal stratification were lower than Martin's cut-offs (Table 3).[42] Myosteatosis prevalence based on cohort-specific cut-offs was 18.2%. Patients with myosteatosis were significantly older, more likely to be female, had worse ECOG PS, and were more likely to have a BMI <25 (Table 1). Patients with myosteatosis had significantly decreased OS based on log-rank statistics (p<0.0001, Figure 3) and in univariate Cox regression analysis (HR 2.20, 95% CI 1.53-3.16, p<0.001, Table 4). This association remained significant in multivariate analysis (HR 2.30, 95% CI 1.56-3.37, p<0.001, Table 4). Myosteatosis was prognostic of both decreased MSS (HR 2.33, 95% CI 1.48-3.69, p<0.001) and RFS (HR 1.50, 95% CI 1.03-2.19, p=0.035) in multivariate analysis.

4.3.5 Combined phenotypes based on skeletal muscle

To assess joint effects of sarcopenia and myosteatosis on survival, patients were divided into four phenotypes based on presence or absence of these factors as defined using cohort-specific cut-offs. 25 patients (7.6%) had both sarcopenia and myosteatosis, 41 patients (12.4%) had sarcopenia only, and 35 patients (10.6%) had myosteatosis only. Patients with both sarcopenia and myosteatosis had significantly decreased OS compared to other phenotypes (p<0.0001, Figure 3). In multivariate analysis, patients who had combined sarcopenia and myosteatosis had significantly decreased OS (HR 4.07, 95% CI 2.42-6.85, p<0.001, Table 4)

compared to those without either factor. Combined sarcopenia and myosteatosis had a similar association with MSS (HR 3.00, 95% CI 1.52-5.94, p=0.002, Table 4) and RFS (HR 1.85, 95% CI 1.04-3.29, p=0.035) in multivariate analysis.

4.3.6 Adipose tissue

An exploratory analysis was performed of whether amounts of visceral, subcutaneous, and total adipose tissue impacted survival. Patients in the highest quartile of visceral fat index (VFI) had significantly decreased median OS (45.7 months) compared to those in the lowest three quartiles (58.5 months, p=0.0330). Though mean VFI was significantly higher in men (p<0.001, Table 2), no cut-point that significantly predicted OS was identified in women. A cut-point of VFI that optimally predicted OS for all patients was identified at 38.0 cm^2/m^2, with patients above this cut-off experiencing decreased OS (p=0.0009, Table 3). 213 patients (64.6%) fell above this cut-off. However, in multivariate analysis, increased VFI did not significantly impact OS, MSS, or RFS (Table 4). Subcutaneous and total adipose tissue indices, when divided into quartiles, did not significantly impact OS (p=0.1504 and 0.3538, respectively), and further optimal stratification analyses were not undertaken of these variables.

4.4 Discussion

This is the largest study to date assessing prognostic effects of body composition in resected stage III melanoma. This disease has not been previously associated with high rates of sarcopenia or myosteatosis; we demonstrate here that both are prevalent amongst resectable stage III patients based on a set of cut-offs widely used in cancer literature. As these cut-offs were established in a cohort that did not include melanoma patients,[42] we also determined cohort-

specific sarcopenia and myosteatosis cut-offs that were strongly predictive of both OS and MSS. These associations were significant independent of other known prognostic factors in melanoma, including age, sex, performance status, and stage subgroup. Associations with RFS were somewhat mixed, as myosteatosis but not sarcopenia was associated with decreased RFS. This inconsistency may be a consequence of variable follow-up and surveillance in resected melanoma patients, which would affect time to diagnosis of recurrent disease. Average SMI and SMD values for men and women were somewhat similar between our population and that of Martin *et al.*'s study, suggesting that our cohort of stage III melanoma patients has similar baseline body composition to their population of stage I-IV mixed gastrointestinal and respiratory cancer patients. While our cut-offs were generally lower than Martin's, this is likely a reflection of the nature of optimal stratification, which selects a threshold that most strongly prognosticates survival within a specific population.

Sarcopenia is increasingly being studied in cancer as both a prognostic marker and as a method of identifying patients who may benefit from nutritional intervention and/or rehabilitation. Sarcopenic patients may not be easily identifiable based on body habitus, especially with increasing rates of overweight and obese patients. Indeed, a majority of patients (72.3%) in this study had a BMI greater than or equal to 25. While malignant melanoma lacks certain drivers of malnutrition that may be present in other cancers, such as bowel symptoms associated with gastrointestinal malignancies, these patients may still be prone to significant skeletal muscle loss.

Myosteatosis, or decreased skeletal muscle density, is less well characterized than sarcopenia but has also been recognized as a negative prognostic factor in a variety of cancers. We have previously reported on negative prognostic effects of low SMD in metastatic melanoma

treated with immunotherapy[48,89]; here we demonstrate that myosteatosis, as defined by two different sets of SMD cut-offs, is also predictive of decreased OS and MSS in resectable stage III patients. Furthermore, patients with both sarcopenia and myosteatosis had decreased OS and MSS compared to those with either factor alone, demonstrating that these measures of skeletal muscle have distinct, additive effects on prognosis.

While adipose tissue is increasingly recognized as a major endocrine organ that may influence cancer progression,[90,91] the optimal method of analyzing adipose tissue in body composition research is unknown. Several studies have demonstrated decreased survival in patients with visceral obesity,[36,37,92] which has been variously defined as increased VAT surface area, increased VFI, and increased visceral-to-subcutaneous fat ratios. We undertook a simple exploratory analysis by dividing visceral, subcutaneous, and total adipose tissue indices into quartiles and selectively applying optimal stratification to identify significant thresholds. While a threshold of VFI that significantly impacted OS was identified, this association was not significant in multivariate analysis. Such measurements may be of limited value in prognosticating stage III melanoma, and further studies may be needed to elucidate impacts of adipose tissue compartments in melanoma.

Previous studies have described an obesity paradox in metastatic melanoma whereby patients with greater BMI have improved survival,[21,22,24,93] though results have been somewhat inconsistent and most studies have been of patients with metastatic disease. In our study of resectable stage III patients, BMI category did not significantly impact OS or MSS. Our study demonstrates that skeletal muscle, rather than BMI, seems most strongly associated with prognosis. Underlying variation in skeletal muscle amongst patients with similar BMI may explain varying results in previous studies regarding associations of BMI with survival.

Several limitations should be noted to our study. As a retrospective study there may be possible confounders impacting our findings, though we attempted to account for a variety of potential prognostic factors in melanoma. Certain pathologic and genetic factors, such as maximal thickness of sentinel lymph node tumor deposits and tumor BRAF mutation status, are known to impact survival in stage III melanoma;[94,95] however, this data was not consistently available in our database. In addition, the recent emergence of adjuvant immunotherapy and BRAF-inhibitors has significantly improved outcomes in high-risk resected melanoma,[19,86] which was not reflected in our study as only a fraction of patients received these during the study period. Interactions of body composition with immuno- and targeted therapies are an area of ongoing study.

Our study may also be at risk of selection bias, as a significant number of patients (32.7%) were excluded due to a lack of available imaging. Nonetheless, our sample size was large enough to perform multivariable analyses and demonstrate impacts of body composition variables alongside a number of other known prognostic factors in melanoma. A strength of this study is its detailed review of clinical progress notes and pathology reports, which allowed us to retrospectively stage patients according to the most recent edition of AJCC criteria. The incorporation of stage subgroup into multivariable analyses strengthens our findings of skeletal muscle as an important prognostic factor in melanoma.

In summary, our study is one of the first to evaluate body composition in melanoma patients with advanced but resectable disease. We demonstrate a high prevalence of sarcopenia and myosteatosis in this population at baseline and their negative impacts on survival. These findings suggest a need for further body composition research in patients with stage III melanoma.

Table 1. Baseline characteristics of patients included in the study, stratified by presence of sarcopenia and myosteatosis defined using cohort-specific cut-offs. Values shown are n(%) unless stated otherwise. BMI=body mass index, ECOG PS=Eastern Cooperative Oncology Group performance status, IO=immunotherapy.

Variable	n (%)	Sarcopenic (n=66)	Not sarcopenic (n=264)	p-value	Myosteatosis (n=60)	No myosteatosis (n=270)	p-value
Sex				0.140			0.057
Male	206 (62.4%)	36 (54.5%)	170 (64.4%)		31 (51.7%)	175 (64.8%)	
Female	124 (37.6%)	30 (45.5%)	94 (35.6%)		29 (48.3%)	95 (35.2%)	
Age (mean, SD)	56.4 (15)	66.9 (12.7)	53.8 (14.4)	<0.001	66.8 (11.6)	54.1 (14.7)	<0.0001
BMI category				0.056			<0.0001
<25	91 (27.6%)	12 (18.2%)	79 (29.9%)		29 (48.3%)	62 (23.0%)	
≥25	239 (72.4%)	54 (81.8%)	185 (70.1%)		31 (51.7%)	208 (77.0%)	
Stage*				0.932			0.915
IIIa	48 (14.6%)	8 (12.1%)	40 (15.2%)		7 (11.7%)	41 (15.2%)	
IIIb	77 (23.3%)	16 (24.2%)	61 (23.1%)		14 (23.3%)	63 (23.3%)	
IIIc	173 (52.4%)	35 (53.0%)	138 (52.3%)		33 (55.0%)	140 (51.9%)	
IIId	32 (9.7%)	7 (10.6%)	25 (9.5%)		6 (10.0%)	26 (9.6%)	
ECOG PS				<0.001			0.001
0	267 (80.9%)	43 (65.2%)	224 (84.5%)		39 (65.0%)	228 (84.4%)	
1	53 (16.1%)	17 (25.8%)	36 (13.6%)		16 (26.7%)	37 (13.7%)	
2	10 (3.0%)	6 (9.0%)	4 (1.5%)		5 (8.3%)	5 (1.9%)	
Tumor location				0.010			0.407
Arm/leg	124 (37.6%)	36 (54.5%)	88 (33.3%)		22 (36.7%)	102 (37.8%)	
Head/neck	44 (13.3%)	9 (13.6%)	35 (13.3%)		6 (10.0%)	38 (14.1%)	
Trunk	126 (38.2%)	17 (25.8%)	109 (41.3%)		22 (36.7%)	104 (38.5%)	
Unknown	36 (10.9%)	4 (6.1%)	32 (12.1%)		10 (16.7%)	26 (9.6%)	
Regional node dissection				0.002			0.032
Yes	264 (80.0%)	22 (33.3%)	44 (16.7%)		18 (30.0%)	48 (17.8%)	
No	66 (20.0%)	44 (66.7%)	220 (83.3%)		42 (70.0%)	222 (82.2%)	
Adjuvant therapy				0.669			0.157
None	186 (56.4%)	38 (57.6%)	148 (56.1%)		39 (65.0%)	147 (54.4%)	
IO	6 (1.8%)	2 (3.0%)	4 (1.5%)		2 (3.3%)	4 (1.5%)	
Other	138 (41.8%)	26 (39.4%)	112 (42.4%)		19 (31.7%)	119 (44.1%)	

Table 2. Baseline body composition variables stratified by sex and BMI category. HU=Hounsfield units.

Variable	Males	Females	p-value	BMI <25	BMI ≥25	p-value
Skeletal muscle index (cm^2/m^2)	54.54 ±9.63	40.75 ±7.22	<0.001	43.03 ±9.32	51.77 ±10.71	<0.001
Skeletal muscle density (HU)	36.03 ±10.78	34.93 ±10.51	0.365	40.19 ±10.34	33.88 ±10.30	<0.001
Visceral fat index (cm^2/m^2)	67.29 ±39.12	37.18 ±30.25	<0.001	23.75 ±21.16	68.24 ±36.99	<0.001
Subcutaneous fat index (cm^2/m^2)	65.19 ±32.77	85.11 ±50.40	<0.001	43.21 ±22.57	83.89 ±41.42	<0.001
Total adipose tissue index (cm^2/m^2)	132.48 ±62.33	122.29 ±73.23	0.179	66.96 ±35.69	152.14 ±60.50	<0.001

Table 3. Cohort-specific skeletal muscle index (SMI), skeletal muscle density (SMD), and visceral fat index (VFI) cut-offs associated with decreased overall survival.

	SMI (cm^2/m^2)		**SMD** (HU)		**VFI** (cm^2/m^2)	
BMI category ($kg/m^{2)}$	Males	Females	Males	Females	Males	Females
<25	<40.7	<33.2	<36.8	<36.8	<38.0	<38.0
≥25	<47.3	<39.7	<22.0	<22.0	<38.0	<38.0

Table 4. Univariate and multivariate analyses assessing impacts of body composition on survival based on A) cut-offs established by Martin *et al*. B) cohort-specific cut-offs. VFI=visceral fat index. Phenotype 1=myosteatosis only, 2=sarcopenia only, 3=sarcopenia and myosteatosis.

All multivariate models adjusted for age, sex, ECOG PS, stage, and tumor location.

*Sarcopenia and myosteatosis excluded as covariates.

A)

Overall survival				
	Univariate		*Multivariate*	
	HR (95% CI)	*p-value*	*HR (95% CI)*	*p-value*
Sarcopenia	1.44 (1.04-1.98)	0.026	1.50 (1.08-2.09)	0.016
Myosteatosis	1.56 (1.13-2.15)	0.006	1.57 (1.10-2.24)	0.013
Melanoma-specific survival				
	Univariate		*Multivariate*	
	HR (95% CI)	*p-value*	*HR (95% CI)*	*p-value*
Sarcopenia	1.26 (0.87-1.83)	0.217	1.42 (0.97-2.09)	0.072
Myosteatosis	1.36 (0.94-1.97)	0.107	1.55 (1.03-2.34)	0.037
Recurrence-free survival				
	Univariate		*Multivariate*	
	HR (95% CI)	*p-value*	*HR (95% CI)*	*p-value*
Sarcopenia	0.96 (0.72-1.29)	0.803	1.01 (0.75-1.37)	0.930
Myosteatosis	1.18 (0.88-1.58)	0.265	1.25 (0.92-1.71)	0.156

B)

Overall survival				
	Univariate		*Multivariate*	
	HR (95% CI)	*p-value*	*HR (95% CI)*	*p-value*
Sarcopenia	2.43 (1.73-3.43)	<0.001	2.38 (1.64-3.45)	<0.001
Myosteatosis	2.20 (1.53-3.16)	<0.001	2.30 (1.56-3.37)	<0.001
Phenotype*				
1	1.92 (1.16-3.16)	0.011	2.00 (1.19-3.38)	0.009
2	2.23 (1.45-3.43)	<0.001	2.14 (1.36-3.36)	0.001
3	3.67 (2.27-5.94)	<0.001	4.07 (2.42-6.85)	<0.001
Increased VFI	1.81 (1.27-2.59)	0.001	1.49 (0.99-2.26)	0.057
Melanoma-specific survival				
	Univariate		*Multivariate*	
	HR (95% CI)	*p-value*	*HR (95% CI)*	*p-value*
Sarcopenia	1.69 (1.10-2.60)	0.016	1.85 (1.16-2.95)	0.009
Myosteatosis	2.01 (1.31-3.10)	0.001	2.33 (1.48-3.69)	<0.001
Phenotype*				
1	2.11 (1.24-3.61)	0.006	2.45 (1.39-4.32)	0.002
2	1.69 (0.99-2.89)	0.053	1.83 (1.05-3.21)	0.034
3	2.35 (1.24-4.47)	0.009	3.00 (1.52-5.94)	0.002
Increased VFI	1.71 (1.12-2.58)	0.011	1.51 (0.93-2.44)	0.094
Recurrence-free survival				
	Univariate		*Multivariate*	
	HR (95% CI)	*p-value*	*HR (95% CI)*	*p-value*
Sarcopenia	1.36 (0.95-1.93)	0.089	1.43 (0.98-2.08)	0.062
Myosteatosis	1.40 (0.97-2.02)	0.070	1.50 (1.03-2.19)	0.035
Phenotype*				
1	1.44 (0.91-2.27)	0.116	1.46 (0.91-2.35)	0.118
2	1.37 (0.90-2.10)	0.143	1.38 (0.88-2.15)	0.161
3	1.53 (0.88-2.67)	0.132	1.85 (1.04-3.29)	0.035
Increased VFI	1.17 (0.84-1.64)	0.351	1.00 (0.70-1.43)	0.998

Figure 1. Flow diagram to illustrate inclusion and exclusion criteria.

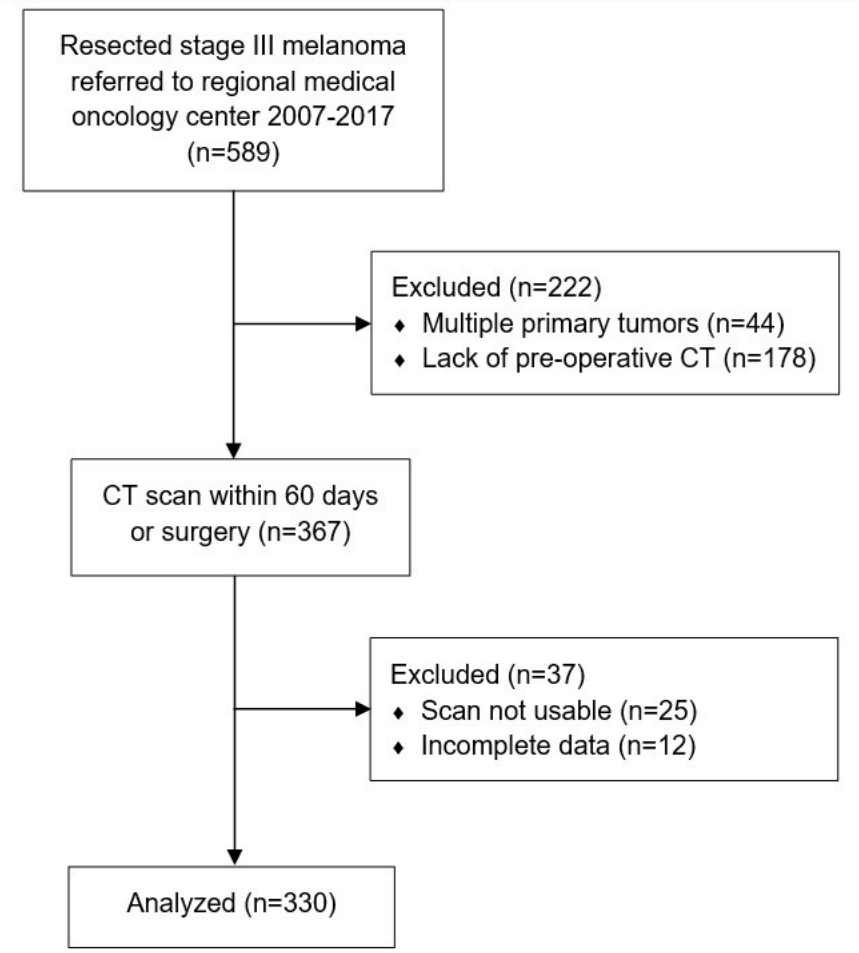


Figure 2. Associations of sarcopenia with survival based on cohort-specific cut-offs.

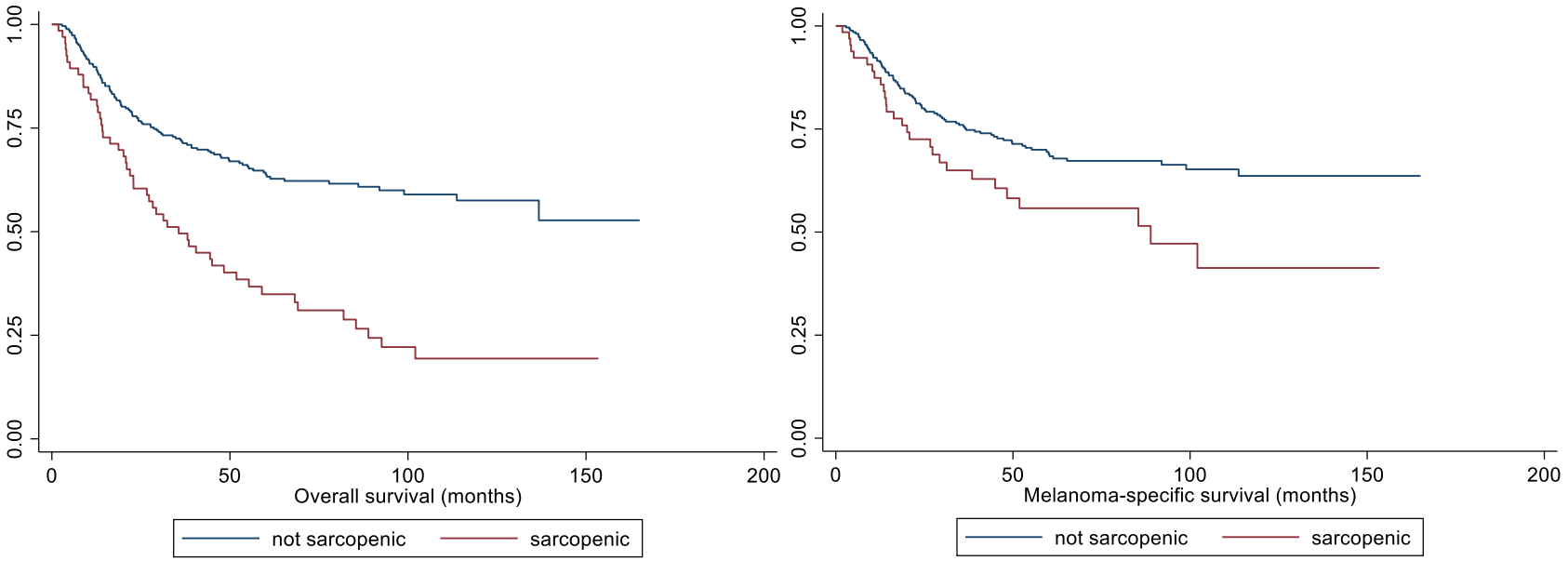


Figure 3. Associations of myosteatosis with survival based on cohort-specific cut-offs.

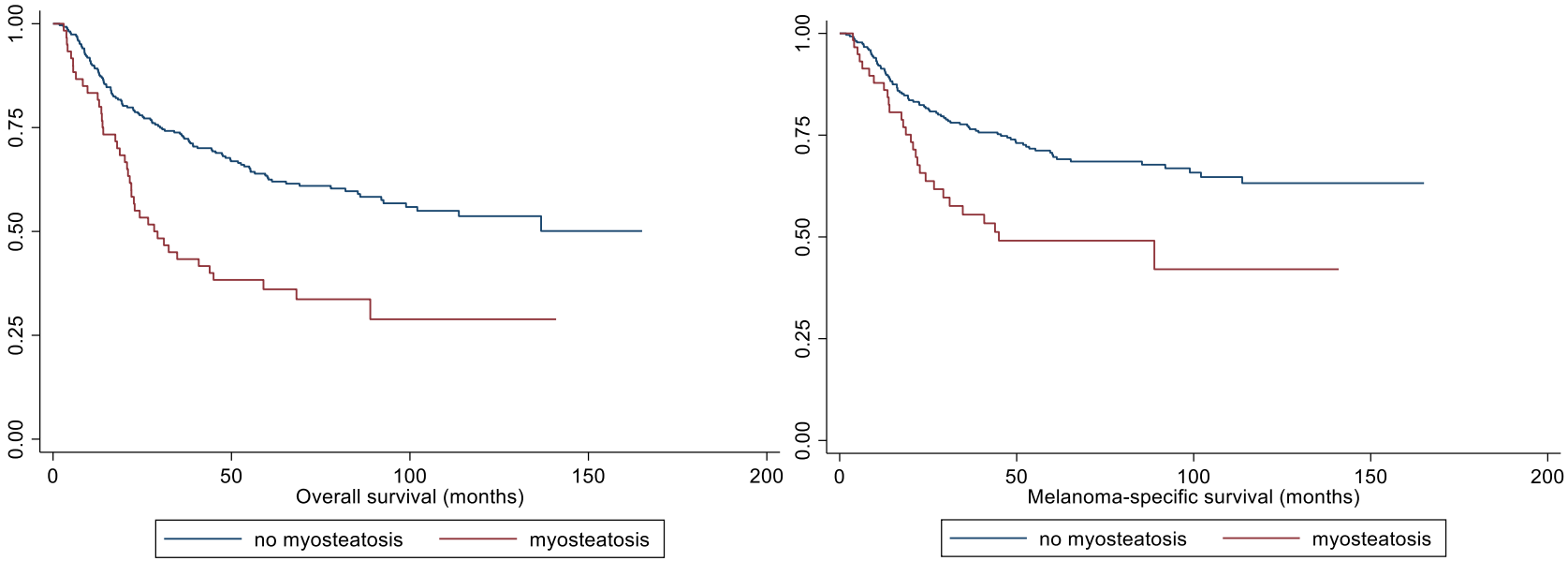
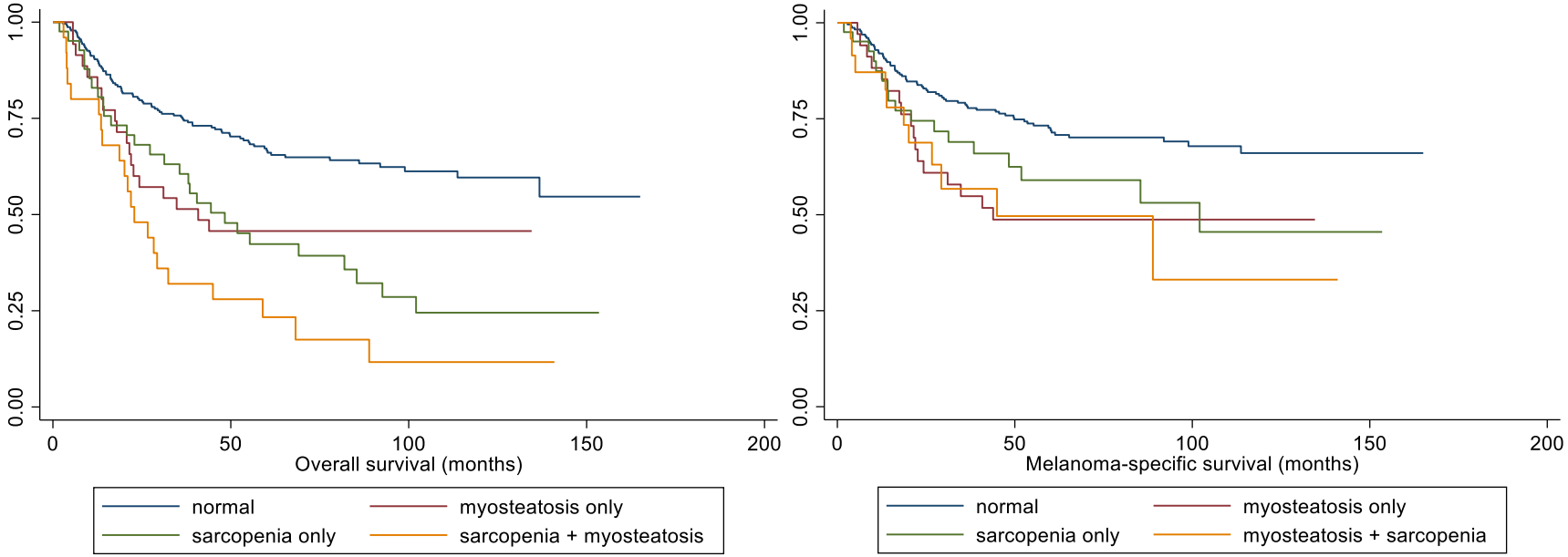


Figure 4. Impacts of skeletal muscle phenotype on survival using cohort-specific cut-offs.



Chapter 5: Conclusion

This thesis presents a comprehensive assessment of current body composition research in melanoma and makes several new additions to the literature. Though melanoma is a common malignancy, relatively few studies have been performed assessing the clinical utility of body composition analysis in these patients. Though several of these studies presented interesting findings, especially regarding skeletal muscle as a prognostic factor in metastatic melanoma patients receiving immunotherapy, many are limited by methodological weaknesses including use of non-validated CT analysis techniques. To further clarify roles of body composition in metastatic melanoma, we performed a retrospective study evaluating skeletal muscle in stage IV melanoma patients treated with nivolumab and found that myosteatosis was prognostic of decreased survival. Our study was limited by a small sample size, given the small number of patients who have been treated with these recently approved drugs. As such, there is a need for further, higher-powered studies with an emphasis on using body composition analysis techniques previously validated in the literature.

To our knowledge, our study of resected stage III melanoma patients is the largest study to date assessing body composition in this population. Baseline characteristics of skeletal muscle, including average skeletal muscle index and radiodensity, are similar to those identified in studies of other cancer patients, notably in Martin's landmark study of stage I-IV gastrointestinal and respiratory cancer patients. Though patients with surgically resectable melanoma have not traditionally been thought of as being at high risk for malnutrition, especially given the high prevalence of overweight and obese patients in this population, nearly half the patients in our study had sarcopenia or myosteatosis based on Martin's cut-offs. Furthermore, using several sets of cut-offs to define sarcopenia and myosteatosis, we demonstrated strong negative impacts of

these factors on overall, melanoma-specific, and recurrence-free survival, independent of other variables including age, sex, stage, and performance status. As an isolated study, cut-offs identified within our cohort should not be accepted as universal values for clinically significant thresholds of skeletal muscle loss. Nonetheless, these findings point towards skeletal muscle as an important prognostic factor in resected stage III melanoma and suggest a need for greater awareness of risks associated with sarcopenia and myosteatosis in this population.

This study also evaluated factors such as BMI and adipose tissue as potential prognostic factors. There were no significant differences in survival between BMI categories. Previous studies have suggested an obesity paradox in melanoma; our findings suggest that variation in skeletal muscle, rather than BMI, may be more responsible for differences in survival. We performed an exploratory analysis of adipose tissue, as there are no widely accepted cut-offs of increased or decreased adipose tissue in the literature. While adipose tissue loss is associated with cancer cachexia and has been observed in patients with palliative cancer,[96] the significance of increased adiposity in patients with less advanced disease is unclear. Our analysis demonstrated a trend towards reduced overall survival in patients with increased visceral fat index, an association that was only borderline significant in multivariate analysis. This suggests some potential of visceral obesity to prognosticate patients; however, measurements of skeletal muscle remain a stronger and more consistent predictor of survival.

In summary, this thesis is the culmination of several new studies confirming the prognostic value of body composition analysis, particularly measurements of skeletal muscle, in advanced melanoma. Our findings represent a substantial new addition to a relatively understudied field and justify further research into applications of body composition in melanoma.

References

1. Erdmann F, Lortet-Tieulent J, Schüz J, et al. International trends in the incidence of malignant melanoma 1953-2008-are recent generations at higher or lower risk? *Int J Cancer*. 2013;132(2):385-400. doi:10.1002/ijc.27616

2. Canadian Cancer Society's Advisory Committee on Cancer Statistics. *Can Cancer Stat 2014*. 2014.

3. Brenner DR, Weir HK, Demers AA, et al. Projected estimates of cancer in Canada in 2020. *CMAJ*. 2020;192(9):E199-E205. doi:10.1503/cmaj.191292

4. Howlader N, Noone A, Krapcho M, et al. *SEER Cancer Statistics Review, 1975-2009 (Vintage 2009 Populations), National Cancer Institute*. Bethesda, MD; 2012. https://seer.cancer.gov/archive/csr/1975_2009_pops09/. Accessed March 4, 2021.

5. Edge SB, Byrd DR, Fritz AG, et al. *AJCC Cancer Staging Manual*. 8th ed. (Amin M, Edge S, Greene F, eds.). Springer; 2017. http://springer.com/978-0-387-88440-0. Accessed March 4, 2021.

6. Joosse A, De Vries E, Eckel R, et al. Gender differences in melanoma survival: Female patients have a decreased risk of metastasis. *J Invest Dermatol*. 2011;131(3):719-726. doi:10.1038/jid.2010.354

7. Joosse A, Collette S, Suciu S, et al. Superior outcome of women with stage I/II cutaneous melanoma: Pooled analysis of four European organisation for research and treatment of cancer phase III trials. *J Clin Oncol*. 2012;30(18):2240-2247. doi:10.1200/JCO.2011.38.0584

8. Pollack LA, Li J, Berkowitz Z, et al. Melanoma survival in the United States, 1992 to 2005. *J Am Acad Dermatol*. 2011;65(5 SUPPL. 1):S78.e1-S78.e10. doi:10.1016/j.jaad.2011.05.030

9. Morton DL, Thompson JF, Cochran AJ, et al. Sentinel-Node Biopsy or Nodal Observation in Melanoma. *N Engl J Med*. 2006;355(13):1307-1317. doi:10.1056/NEJMoa060992

10. Morton DL, Thompson JF, Cochran AJ, et al. Final Trial Report of Sentinel-Node Biopsy

versus Nodal Observation in Melanoma. *N Engl J Med*. 2014;370(7):599-609. doi:10.1056/NEJMoa1310460

11. Faries MB, Thompson JF, Cochran AJ, et al. Completion Dissection or Observation for Sentinel-Node Metastasis in Melanoma. *N Engl J Med*. 2017;376(23):2211-2222. doi:10.1056/NEJMoa1613210

12. Leiter U, Stadler R, Mauch C, et al. Complete lymph node dissection versus no dissection in patients with sentinel lymph node biopsy positive melanoma (DeCOG-SLT): a multicentre, randomised, phase 3 trial. *Lancet Oncol*. 2016;17(6):757-767. doi:10.1016/S1470-2045(16)00141-8

13. Melero I, Hervas-Stubbs S, Glennie M, Pardoll DM, Chen L. Immunostimulatory monoclonal antibodies for cancer therapy. *Nat Rev Cancer*. 2007;7(2):95-106. doi:10.1038/nrc2051

14. Hodi FS, O'Day SJ, McDermott DF, et al. Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. *N Engl J Med*. 2010;8:711-734. doi:10.1056/NEJMoa1003466

15. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *N Engl J Med*. 2015;373(1):23-34. doi:10.1056/NEJMoa1504030

16. Wolchok JD, Chiarion-Sileni V, Gonzalez R, et al. Overall Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma. *N Engl J Med*. 2017;377(14):1345-1356. doi:10.1056/NEJMoa1709684

17. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Five-Year Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma. *N Engl J Med*. 2019;381(16):1535-1546. doi:10.1056/NEJMoa1910836

18. Eggermont AMM, Dummer R. The 2017 complete overhaul of adjuvant therapies for high-risk melanoma and its consequences for staging and management of melanoma patients. *Eur J Cancer*. 2017;86:101-105. doi:10.1016/j.ejca.2017.09.014

19. Weber J, Mandala M, Del Vecchio M, et al. Adjuvant Nivolumab versus Ipilimumab in

Resected Stage III or IV Melanoma. *N Engl J Med*. 2017;377(19):1824-1835. doi:10.1056/NEJMoa1709030

20. Ferrucci PF, Sznol M, Hogg D, et al. Pooled Analysis Safety Profile of Nivolumab and Ipilimumab Combination Therapy in Patients With Advanced Melanoma. *ournal Clin Oncol*. 2017;35:3815-3822. doi:10.1200/JCO.2016.72.1167

21. McQuade JL, Daniel CR, Hess KR, et al. Association of body-mass index and outcomes in patients with metastatic melanoma treated with targeted therapy, immunotherapy, or chemotherapy: a retrospective, multicohort analysis. *Lancet Oncol*. 2018;19(3):310-322. doi:10.1016/S1470-2045(18)30078-0

22. Donnelly D, Bajaj S, Yu J, et al. The complex relationship between body mass index and response to immune checkpoint inhibition in metastatic melanoma patients. *J Immunother Cancer*. 2019;7(1):222. doi:10.1186/s40425-019-0699-5

23. Naik GS, Waikar SS, Johnson AEWW, et al. Complex inter-relationship of body mass index, gender and serum creatinine on survival: Exploring the obesity paradox in melanoma patients treated with checkpoint inhibition. *J Immunother Cancer*. 2019;7(1):89. doi:http://dx.doi.org/10.1186/s40425-019-0512-5

24. Cortellini A, Bersanelli M, Buti S, et al. A multicenter study of body mass index in cancer patients treated with anti-PD-1/PD-L1 immune checkpoint inhibitors: when overweight becomes favorable. *J Immunother Cancer*. 2019;7(1):57. doi:10.1186/s40425-019-0527-y

25. Shen W, Punyanitya M, Wang ZM, et al. Total body skeletal muscle and adipose tissue volumes: Estimation from a single abdominal cross-sectional image. *J Appl Physiol*. 2004;97(6):2333-2338. doi:10.1152/japplphysiol.00744.2004

26. Mourtzakis M, Prado CMM, Lieffers JR, Reiman T, McCargar LJ, Baracos VE. A practical and precise approach to quantification of body composition in cancer patients using computed tomography images acquired during routine care. *Appl Physiol Nutr Metab*. 2008;33(5):997-1006. doi:10.1139/H08-075

27. Prado CMM, Lieff JR, Mccargar LJ, et al. Prevalence and clinical implications of

sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: a population-based study. *Lancet Oncol*. 2008;9:629-635. doi:10.1016/S1470

28. Xia L, Zhao R, Wan Q, et al. Sarcopenia and adverse health-related outcomes: An umbrella review of meta-analyses of observational studies. *Cancer Med*. 2020;9(21):7964-7978. doi:10.1002/cam4.3428

29. Goodpaster BH, Kelley DE, Thaete FL, He J, Ross R. Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. *J Appl Physiol*. 2000;89(1):104-110. doi:10.1152/jappl.2000.89.1.104

30. Aleixo GFP, Shachar SS, Nyrop KA, Muss HB, Malpica L, Williams GR. Myosteatosis and prognosis in cancer: Systematic review and meta-analysis. *Crit Rev Oncol Hematol*. 2020;145. doi:10.1016/j.critrevonc.2019.102839

31. Malietzis G, Johns N, Al-Hassi HO, et al. Low muscularity and myosteatosis is related to the host systemic inflammator y response in patients undergoing surger y for colorectal cancer. *Ann Surg*. 2016;263(2):320-325. doi:10.1097/SLA.0000000000001113

32. Okugawa Y, Toiyama Y, Yamamoto A, et al. Close Relationship Between Immunological/Inflammatory Markers and Myopenia and Myosteatosis in Patients With Colorectal Cancer: A Propensity Score Matching Analysis. *J Parenter Enter Nutr*. 2019;43(4):508-515. doi:10.1002/jpen.1459

33. Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. *Mol Cell Endocrinol*. 2010;316(2):129-139. doi:10.1016/j.mce.2009.08.018

34. Doyle SL, Donohoe CL, Lysaght J, Reynolds J V. Visceral obesity, metabolic syndrome, insulin resistance and cancer. In: *Proceedings of the Nutrition Society*. Vol 71. Cambridge University Press; 2012:181-189. doi:10.1017/S002966511100320X

35. Moon HG, Ju YT, Jeong CY, et al. Visceral obesity may affect oncologic outcome in patients with colorectal cancer. *Ann Surg Oncol*. 2008;15(7):1918-1922. doi:10.1245/s10434-008-9891-4

36. Lee CS, Murphy DJ, Mcmahon C, et al. Visceral Adiposity is a Risk Factor for Poor

Prognosis in Colorectal Cancer Patients Receiving Adjuvant Chemotherapy. *J Gastrointest Canc*. 2015;46:243-250. doi:10.1007/s12029-015-9709-0

37. Hopkins JJ, Reif RL, Bigam DL, Baracos VE, Eurich DT, Sawyer MB. The Impact of Muscle and Adipose Tissue on Long-term Survival in Patients With Stage I to III Colorectal Cancer. *Dis Colon Rectum*. 2019;62(5):549-560. doi:10.1097/DCR.0000000000001352

38. Sawyer M, Ratain MJ. Body surface area as a determinant of pharmacokinetics and drug dosing. *Invest New Drugs*. 2001;19(2):171-177. doi:10.1023/A:1010639201787

39. Hendrikx JJMA, Haanen JBAG, Voest EE, Schellens JHM, Huitema ADR, Beijnen JH. Fixed Dosing of Monoclonal Antibodies in Oncology. *Oncologist*. 2017;22(10):1212-1221. doi:10.1634/theoncologist.2017-0167

40. Antoun S, Borget I, Lanoy E. Impact of sarcopenia on the prognosis and treatment toxicities in patients diagnosed with cancer. *Curr Opin Support Palliat Care*. 2013;7(4):383-389. doi:10.1097/SPC.0000000000000011

41. Cruz-Jentoft AJ, Bahat G, Bauer J, et al. Sarcopenia: revised European consensus on definition and diagnosis European Working Group on Sarcopenia in Older People 2 (EWGSOP2), and the extended group for EWGSOP2. *Age Ageing*. 2019;48:16-31. doi:10.1093/ageing/afy169

42. Martin L, Birdsell L, MacDonald N, et al. Cancer cachexia in the age of obesity: Skeletal muscle depletion is a powerful prognostic factor, independent of body mass index. *J Clin Oncol*. 2013;31(12):1539-1547. doi:10.1200/JCO.2012.45.2722

43. Rutten IJG, Ubachs J, Kruitwagen RFPM, Beets-Tan RGH, Olde Damink SWM, Van Gorp T. Psoas muscle area is not representative of total skeletal muscle area in the assessment of sarcopenia in ovarian cancer. *J Cachexia Sarcopenia Muscle*. 2017;8(4):630-638. doi:10.1002/jcsm.12180

44. Ebadi M, Wang CW, Lai JC, et al. Poor performance of psoas muscle index for identification of patients with higher waitlist mortality risk in cirrhosis. *J Cachexia*

Sarcopenia Muscle. 2018;9(6):1053-1062. doi:10.1002/jcsm.12349

45. Sabel MS, Lee J, Wang A, Lao C, Holcombe S, Wang S. Morphomics predicts response to ipilimumab in patients with stage IV melanoma. *J Surg Oncol*. 2015;112(4):333-337. doi:10.1002/jso.24003

46. Heidelberger V, Goldwasser FFF, Kramkimel N, et al. Sarcopenic overweight is associated with early acute limiting toxicity of anti-PD1 checkpoint inhibitors in melanoma patients. *Invest New Drugs*. 2017;35(4):537. doi:https://dx.doi.org/10.1007/s10637-017-0475-7

47. Daly LE, Power DG, O'Reilly Á, et al. The impact of body composition parameters on ipilimumab toxicity and survival in patients with metastatic melanoma. *Br J Cancer*. 2017;116(3):310-317. doi:10.1038/bjc.2016.431

48. Chu MP, Li Y, Ghosh S, et al. Body composition is prognostic and predictive of ipilimumab activity in metastatic melanoma. *J Cachexia Sarcopenia Muscle*. 2020;11(3):748-755. doi:10.1002/jcsm.12538

49. Young AC, Quach HT, Song H, et al. Impact of body composition on outcomes from anti-PD1 +/-anti-CTLA-4 treatment in melanoma. *J Immunother Cancer*. 2020;8(2):821. doi:10.1136/jitc-2020-000821

50. Prado CMM, Baracos VE, McCargar LJ, et al. Sarcopenia as a determinant of chemotherapy toxicity and time to tumor progression in metastatic breast cancer patients receiving capecitabine treatment. *Clin Cancer Res*. 2009;15(8):2920-2926. doi:10.1158/1078-0432.CCR-08-2242

51. Barret M, Antoun S, Dalban C, et al. Sarcopenia Is Linked to Treatment Toxicity in Patients With Metastatic Colorectal Cancer. *Nutr Cancer*. 2014;66(4):583-589. doi:10.1080/01635581.2014.894103

52. Jones K, Gordon-Weeks A, Coleman C, Silva M. Radiologically Determined Sarcopenia Predicts Morbidity and Mortality Following Abdominal Surgery: A Systematic Review and Meta-Analysis. *World J Surg*. 2017;41(9):2266-2279. doi:10.1007/s00268-017-3999-

2

53. Joglekar S, Nau PN, Mezhir JJ. The impact of sarcopenia on survival and complications in surgical oncology: A review of the current literature. *J Surg Oncol*. 2015;112(5):503-509. doi:10.1002/jso.24025

54. Michielin O, Van Akkooi ACJ, Ascierto PA, Dummer R, Keilholz & U, Committee G. Cutaneous melanoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up †. *Cancer-related Cogn Impair*. 2019;30:1884-1901. doi:10.1093/annonc/mdz411

55. Bilen MA, Martini DJ, Liu Y, et al. Combined Effect of Sarcopenia and Systemic Inflammation on Survival in Patients with Advanced Stage Cancer Treated with Immunotherapy. *Oncologist*. 2020;25(3):e528-e535. doi:http://dx.doi.org/10.1634/theoncologist.2019-0751

56. Wang J, Cao L, Xu S. Sarcopenia affects clinical efficacy of immune checkpoint inhibitors in non-small cell lung cancer patients: A systematic review and meta-analysis. *Int Immunopharmacol*. 2020;88:106907. doi:10.1016/j.intimp.2020.106907

57. Choi MH, Yoon SB, Lee K, et al. Preoperative sarcopenia and post-operative accelerated muscle loss negatively impact survival after resection of pancreatic cancer. *J Cachexia Sarcopenia Muscle*. 2018;9(2):326-334. doi:10.1002/jcsm.12274

58. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Med*. 2009;6(7):e1000097. doi:10.1371/journal.pmed.1000097

59. Riley RD, Moons KGM, Snell KIE, et al. A guide to systematic review and meta-analysis of prognostic factor studies. *BMJ*. 2019;364. doi:10.1136/bmj.k4597

60. Sabel MS, Lee J, Cai S, et al. Sarcopenia as a prognostic factor among patients with stage III melanoma. *Ann Surg Oncol*. 2011;18(13):3579-3585. doi:http://dx.doi.org/10.1245/s10434-011-1976-9

61. Grignol VP, Smith AD, Shlapak D, Zhang X, Del Campo SM, Carson WE. Increased

visceral to subcutaneous fat ratio is associated with decreased overall survival in patients with metastatic melanoma receiving anti-angiogenic therapy. *Surg Oncol*. 2015;24(4):353-358. doi:10.1016/j.suronc.2015.09.002

62. Hu JB, Ravichandran S, Rushing C, et al. Higher BMI, but Not Sarcopenia, Is Associated with Pembrolizumab-related Toxicity in Patients with Advanced Melanoma. *Anticancer Res*. 2020;40(9):5245-5254. doi:10.21873/anticanres.14528

63. Sengul Samanci N, Çelik E, Bagcilar O, et al. Loss of skeletal muscle area and fat-free mass during dabrafenib/trametinib and vemurafenib/cobimetinib treatments in patients with BRAF-mutant metastatic malignant melanoma. *Melanoma Res*. 2020;30(5):477-483. doi:http://dx.doi.org/10.1097/CMR.0000000000000678

64. Baracos VE. *Psoas* as a sentinel muscle for sarcopenia: a flawed premise. *J Cachexia Sarcopenia Muscle*. 2017;8(4):527-528. doi:10.1002/jcsm.12221

65. Fearon K, Strasser F, Anker SD, et al. Definition and classification of cancer cachexia: An international consensus. *Lancet Oncol*. 2011;12(5):489-495. doi:10.1016/S1470-2045(10)70218-7

66. Shachar SS, Deal AM, Weinberg M, et al. Skeletal muscle measures as predictors of toxicity, hospitalization, and survival in patients with metastatic breast cancer receiving taxane-based chemotherapy. *Clin Cancer Res*. 2017;23(3):658-665. doi:10.1158/1078-0432.CCR-16-0940

67. Ribeiro-Filho FF, Faria AN, Kohlmann O, et al. Ultrasonography for the evaluation of visceral fat and cardiovascular risk. *Hypertension*. 2001;38(3 Pt 2):713-717. doi:10.1161/01.hyp.38.3.713

68. Ribeiro-Filho FF, Faria AN, Azjen S, Zanella MT, Ferreira SRG. Methods of estimation of visceral fat: Advantages of ultrasonography. *Obes Res*. 2003;11(12):1488-1494. doi:10.1038/oby.2003.199

69. Rollins KE, Gopinath A, Awwad A, Macdonald IA, Lobo DN. Computed tomography-based psoas skeletal muscle area and radiodensity are poor sentinels for whole L3 skeletal

muscle values. *Clin Nutr*. 2020;39(7):2227-2232. doi:10.1016/j.clnu.2019.10.003

70. Tilg H, Moschen AR. Adipocytokines: Mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol*. 2006;6(10):772-783. doi:10.1038/nri1937

71. Amjadi F, Javanmard SH, Zarkesh-Esfahani H, Khazaei M, Narimani M. Leptin promotes melanoma tumor growth in mice related to increasing circulating endothelial progenitor cells numbers and plasma NO production. *J Exp Clin Cancer Res*. 2011;30(1):21. doi:10.1186/1756-9966-30-21

72. Prado CMM, Baracos VE, McCargar LJ, et al. Body composition as an independent determinant of 5-fluorouracil-based chemotherapy toxicity. *Clin Cancer Res*. 2007;13(11):3264-3268. doi:10.1158/1078-0432.CCR-06-3067

73. Ali R, Baracos VE, Sawyer MB, et al. Lean body mass as an independent determinant of dose-limiting toxicity and neuropathy in patients with colon cancer treated with FOLFOX regimens. *Cancer Med*. 2016;5(4):607-616. doi:10.1002/cam4.621

74. Berwick M, Buller DB, Cust A, et al. Melanoma epidemiology and prevention. In: *Cancer Treatment and Research*. Vol 167. Kluwer Academic Publishers; 2016:17-49. doi:10.1007/978-3-319-22539-5_2

75. Lee CM, Kang J. Prognostic impact of myosteatosis in patients with colorectal cancer: a systematic review and meta-analysis. *J Cachexia Sarcopenia Muscle*. June 2020:1270-1282. doi:10.1002/jcsm.12575

76. Sjøblom B, Grønberg BH, Benth JŠ, et al. Low muscle mass is associated with chemotherapy-induced haematological toxicity in advanced non-small cell lung cancer. *Lung Cancer*. 2015;90(1):85-91. doi:10.1016/j.lungcan.2015.07.001

77. Williams BA, Mandrekar JN, Mandrekar SJ, et al. *Finding Optimal Cutpoints for Continuous Covariates with Binary and Time-to-Event Outcomes*.; 2006.

78. Miljkovic I, Zmuda JM. Epidemiology of myosteatosis. *Curr Opin Clin Nutr Metab Care*. 2010;13(3):260-264. doi:10.1097/MCO.0b013e328337d826

79. Souza NC, Gonzalez MC, Martucci RB, et al. Frailty is associated with myosteatosis in obese patients with colorectal cancer. *Clin Nutr*. 2020;39(2):484-491. doi:10.1016/j.clnu.2019.02.026

80. Stephens NA, Skipworth RJE, MacDonald AJ, Greig CA, Ross JA, Fearon KCH. Intramyocellular lipid droplets increase with progression of cachexia in cancer patients. *J Cachexia Sarcopenia Muscle*. 2011;2(2):111-117. doi:10.1007/s13539-011-0030-x

81. Diakos CI, Charles KA, McMillan DC, Clarke SJ. Cancer-related inflammation and treatment effectiveness. *Lancet Oncol*. 2014;15(11):e493-e503. doi:10.1016/S1470-2045(14)70263-3

82. Ferrucci PF, Gandini S, Battaglia A, et al. Baseline neutrophil-to-lymphocyte ratio is associated with outcome of ipilimumab-treated metastatic melanoma patients. *Br J Cancer*. 2015;112(12):1904-1910. doi:10.1038/bjc.2015.180

83. Capone M, Giannarelli D, Mallardo D, et al. Baseline neutrophil-to-lymphocyte ratio (NLR) and derived NLR could predict overall survival in patients with advanced melanoma treated with nivolumab. *J Immunother Cancer*. 2018;6(1):1-7. doi:10.1186/s40425-018-0383-1

84. Young A, Quach HT, Davis EJ, Moslehi J, Williams GR, Johnson DB. Impact of body composition on outcomes from anti-programmed death-1 (PD-1) treatment. *J Clin Oncol*. 2019;37(15_suppl):9516-9516. doi:10.1200/jco.2019.37.15_suppl.9516

85. Bhullar AS, Anoveros-Barrera A, Dunichand-Hoedl A, et al. Lipid is heterogeneously distributed in muscle and associates with low radiodensity in cancer patients. *J Cachexia Sarcopenia Muscle*. 2020;11(3):735-747. doi:10.1002/jcsm.12533

86. Long G V., Hauschild A, Santinami M, et al. Adjuvant Dabrafenib plus Trametinib in Stage III BRAF-Mutated Melanoma. *N Engl J Med*. 2017;377(19):1813-1823. doi:10.1056/NEJMoa1708539

87. Lausen B, Schumacher M. Maximally Selected Rank Statistics. *Biometrics*. 1992;48(1):73. doi:10.2307/2532740

88. Mazumdar M, Glassman JR. Categorizing a prognostic variable: Review of methods, code for easy implementation and applications to decision-making about cancer treatments. *Stat Med*. 2000;19(1):113-132. doi:10.1002/(SICI)1097-0258(20000115)19:1<113::AID-SIM245>3.0.CO;2-O

89. Youn S, Reif R, Chu MP, et al. Myosteatosis is prognostic in metastatic melanoma treated with nivolumab. *Clin Nutr ESPEN*. January 2021. doi:10.1016/j.clnesp.2021.01.009

90. Renehan AG, Zwahlen M, Egger M. Adiposity and cancer risk: New mechanistic insights from epidemiology. *Nat Rev Cancer*. 2015;15(8):484-498. doi:10.1038/nrc3967

91. Font-Burgada J, Sun B, Karin M. Obesity and Cancer: The Oil that Feeds the Flame. *Cell Metab*. 2016;23(1):48-62. doi:10.1016/j.cmet.2015.12.015

92. Grignol VP, Smith AD, Shlapak D, et al. Increased visceral to subcutaneous fat ratio is associated with decreased overall survival in patients with metastatic melanoma receiving anti-angiogenic therapy. *Surg Oncol*. 2015;24(4):353-358. doi:http://dx.doi.org/10.1016/j.suronc.2015.09.002

93. Naik GS, Waikar SS, Johnson AEW, et al. Complex inter-relationship of body mass index, gender and serum creatinine on survival: Exploring the obesity paradox in melanoma patients treated with checkpoint inhibition. *J Immunother Cancer*. 2019;7(1):89. doi:http://dx.doi.org/10.1186/s40425-019-0512-5

94. Cadili A, Scolyer RA, Brown PT, Dabbs K, Thompson JF. Total sentinel lymph node tumor size predicts nonsentinel node metastasis and survival in patients with melanoma. *Ann Surg Oncol*. 2010;17(11):3015-3020. doi:10.1245/s10434-010-1145-6

95. Thomas NE, Edmiston SN, Alexander A, et al. Association between NRAS and BRAF mutational status and melanoma-specific survival among patients with higher-risk primary melanoma. *JAMA Oncol*. 2015;1(3):359-368. doi:10.1001/jamaoncol.2015.0493

96. Fouladiun M, Körner U, Bosaeus I, et al. Body composition and time course changes in regional distribution of fat and lean tissue in unselected cancer patients on palliative care - Correlations with food intake, metabolism, exercise capacity, and hormones. *Cancer*.

2005;103(10):2189-2198. doi:10.1002/cncr.21013