From G551D to F508del: An Inquiry Into The Development Of Targeted Therapeutics For The Treatment Of Cystic Fibrosis

by

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ABSTRACT

Cystic Fibrosis (CF) is a fatal inherited disease caused by mutations in the gene encoding the Cystic Fibrosis Transmembrane Regulator (CFTR) protein. CFTR plays an integral role in salt and water transport across the epithelial membrane of major organs, such as the lungs. CFTR-targeted therapeutic strategies can theoretically reduce the effects of CFTR ion dysfunction through potentiation, correction, or both. Potentiators work by increasing the length of time CFTR channels remain open following activation while correctors work by increasing the cell surface density of CFTR.

In 2012, regulatory approval by the United Stated Food and Drug Administration (FDA) and European Medicines Agency (EMA) was granted to a potentiator compound, Ivacaftor (trade named Kalydeco). In 2015, the FDA and EMA granted regulatory approval to a corrector-potentiator combination, Lumacaftor-Ivacaftor (trade named Orkambi). The regulatory approval of these compounds has been met with both excitement and concern. For the first time since the discovery of CFTR gene in 1989, an agent which works by directly targeting the CFTR channel has been developed, and this in turn has paved the way towards a potential cure. On the other hand, Kalydeco and Orkambi are not curative, and the amount of clinical benefit seen in clinical trials ranges from, at best, an absolute improvement of 12.5% (Kalydeco) and 3.6% (Orkambi) from baseline for one measure of lung function, Percent Predicted Forced Expiratory Volume in 1 Second (FEV1% predicted). Given that they need to be taken throughout a patient's lifetime, there has been some concern regarding the cost-effectiveness of these treatments, which range from $259,000 USD (Orkambi) to over $300,000 USD (Kalydeco) per patient per year

This thesis research involves three investigations pertaining to the development of Kalydeco and Orkambi for the treatment of CF: 1) A review of Phase II/III clinical trials which have lead to the regulatory approval of Kalydeco and Orkambi; 2) An evaluation of clinical trials that have studied Ivacaftor, Lumacaftor, or their combination in patients homozygous for the F508del mutation, which affects nearly half of all CF patients; and 3) A study of gaps that existed in CF treatment when Kalydeco first received regulatory approval and which gaps remain since Orkambi's regulatory approval.

Our first study is important to understand the historical development of Kalydeco and Orkambi, and in particular, to gain a better understanding of the underlying biology of CFTR as well as the clinical endpoints used in clinical trials. The results of our second investigation suggest that, although Lumacaftor-Ivacaftor combination therapy appears to be superior to Lumacaftor monotherapy, studies of longer duration that are adequately powered towards key clinical endpoints, like FEV1% predicted, are needed to distinguish Lumacaftor-Ivacaftor as being superior to Ivacaftor monotherapy. The results of our third study indicate that several gaps in CF treatment through the use of these targeted agents have been fully or partially filled, but there are certain key gaps which remain. In particular, there is still uncertainty pertaining to the clinical benefit of Ivacaftor in certain sub-populations eligible to receive the treatment, the cost effectiveness of Ivacaftor, as well as the usefulness of sweat chloride concentration as a clinical endpoint in clinical trials.

PREFACE

The thesis work contained in this document is original work conducted by Sohaib Mohammad, with supervision from Dr. Yutaka Yasui. Sohaib Mohammad was responsible for the conception, design, and conduct of all three studies contained in the main text of this thesis as well as the single study contained in the appendix, with guidance from Dr. Yutaka Yasui.

DEDICATION

I dedicate this work first and foremost to Tahir Asif, Alberta's youngest ever double-lung transplant recipient who passed away due to Cystic Fibrosis on December 31, 2014. I would like to further dedicate this thesis to my dearly beloved parents and siblings.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

From G551D to F508del: A Review of Phase II/III Trials Leading to the Regulatory Approval of Orkambi for the Treatment of Cystic Fibrosis

1.1 Introduction

On July 2, 2015, the United States Food and Drug Administration (FDA) approved Orkambi [1] for the treatment of Cystic Fibrosis with the European Medicines Agency (EMA) following suit soon after [2]. CF is a fatal inherited genetic condition caused by mutations in the gene encoding the Cystic Fibrosis Transmembrane Regulator (CFTR) protein [3] which plays a vital role in salt and water transport across the epithelial membrane of multiple organs including the lungs [4-6]. To date, over 2,000 mutations [7] in the CFTR gene have been identified; mutations may effectively reduce epithelial CFTR density, hinder CFTR channel gating/conductance, or both [8]. Orkambi, which directly targets CFTR, works through a dual mechanism consisting of both correction and potentiation; correctors serve to increase the cell surface density of CFTR while potentiators prolong the opening of CFTR channels following normal activation.

Until recently, CF therapies were aimed at managing the effects of CFTR dysfunction, such clearing the airways of thick, sticky mucous through chest physiotherapy and bronchodilator use [8]. The development and subsequent journey to approval for Orkambi began with the identification of Ivacaftor [9], a potentiator. Ivacaftor, trade named Kalydeco, was initial marketed as a personalized treatment for CF patients with the G551D mutation (a gating mutation affecting ~4% of CF patients) and was first approved on January 31, 2012 by the United States FDA [10]. Despite subsequent approval for additional CF mutations, clinical trials for Ivacaftor failed to demonstrate clinically meaningful benefit for patients homozygous for the F508del mutation [11], a mutation affecting approximately

half of all CF patients [12-14]. An approach that served to fill this critical gap in the treatment of CF, by targeting the most prevalent mutation, was needed. Orkambi was aimed to fill this gap through the addition of the corrector compound, Lumacaftor, to the potentiator, Ivacaftor.

At least 50 clinical trials on Ivacaftor, Lumacaftor, or their combination have been initiated. A review of these trials, especially Phase II/III studies, is necessary to better understand key developments and how a personalized treatment for a single mutation grew to become approved for a large number of CF mutations. The *CFTR Biology* section aims to provide a basic molecular and genetic overview which is important for understanding the rationale behind the clinical trials. The *Clinical Endpoints* section focuses on key clinical endpoints used in the clinical trials and their relevance to CF in order to better understand the observed study results. Lastly, the *Clinical Trials* section summarizes clinical trial findings with reference to both the underlying biology of CFTR and clinical endpoints.

1.1.1 CFTR Biology

The prevalence of CF varies worldwide and along with it, the presence of different CFTR mutations [12-14]. According to the CF Mutation Database, over 2,000 unique *CFTR* mutations have been identified among CF cases thus far [7]. CF has an autosomal recessive inheritance pattern, where patients with the disease inherit one copy of the mutation from each parent. CFTR mutations influence the degree of functioning CFTR which in turn influence disease severity and phenotype. The F508del mutation is the most common *CFTR* mutation and is present on at least one allele in approximately 86-90% of CF cases [12-14]; approximately half of these patients also have the F508del mutation on their second allele. Following this, the prevalence of other *CFTR* mutations dramatically decreases, with no other mutation cumulatively accounting for more than 5% of observed CF cases worldwide.

Table 1-1 provides relevant demographic information regarding prevalent *CFTR* mutations obtained from national registry reports [12-14].

CFTR mutations have historically been categorized into 5 main classes [15] (Table 1-2). Class I mutations inhibit protein synthesis resulting in little or no CFTR production; CFTR that is produced with these mutations has a truncated structure and is not able to reach the cell surface [16]. Class II mutations, including the F508del mutation [17], result in impaired transport/processing of CFTR. It is believed that class II mutations result in, among other things, misfolded CFTR, preventing it from reaching the cell surface [18]. Class III mutations, which include the G551D mutation, are known as gating mutations. As such, CFTR production and subsequent processing to the cell surface is largely unaffected but the CFTR channel itself opens less frequently [15, 19]. Class IV mutations, much like Class III mutations, also have largely undisturbed production/processing, but the CFTR channel has altered conductance (specifically, CFTR has a reduced ability to pass chloride ions) [20]. Lastly, Class V mutations result in decreased synthesis of the otherwise normal protein [21]. A sixth functional class (Class VI) of mutations is often distinguished from Class V and also results in reduced but normal CFTR at the cell surface; however, the reduction is attributed to increased CFTR removal from the cell surface rather than decreased synthesis [22].

Regarding the different classes of *CFTR* mutations, it is important to consider that the majority of mutations are not mechanistically understood well and some mutations, although largely characterized by a single defect (and thus categorized into a corresponding class), may share some commonalities with other classes. Additionally, disease severity and residual CFTR activity may vary and may further be influenced by both genetic modifiers and environmental factors [8,19]. Lastly, anion reduction is usually greater for Class I, II, and III mutations generally resulting in more severe disease than Class IV or V mutations [8, 23].

1.1.2 In-Vitro Studies

The identification of Ivacaftor (also called VX-770) [9] followed a high-throughput screening, a technique commonly used in pharmaceutical chemistry to automatically assay the biological activities of a large number of drug-like compounds. VX-770 was selected for drug development out of 228,000 compounds that underwent the high-throughput screening. When tested in epithelia isolated from the bronchi of CF patients with the G551D and/or the F508del mutation, VX-770 was shown to prolong the opening of CFTR channels and to increase the transepithelial current (i.e., increase of about 10 fold in chloride ion secretion). Other effects were also noted, such as an increase in surface fluid which prevents dehydration of the epithelium surface.

The discovery of Lumacaftor, also called VX-809, followed a high-throughput screening of 164,000 compounds [24]. VX-809 was selected for two main reasons: 1) enhanced processing of CFTR; and 2) improved functionality of CFTR upon reaching the cell surface. In bronchial epithelia of CF patients homozygous for the F508del mutation, VX-809 was shown to improve the processing of CFTR, increase secretion of chloride ions, and demonstrate improved functionality that was comparable to CFTR without any defects.

1.2 Clinical Endpoints

Following the evidence from in-vitro studies, clinical trials on CF patients with selected clinical endpoints were conducted. Although more than 50 clinical trials evaluating Ivacaftor, Lumacaftor, or their combination, Orkambi, have been conducted, we focus here on 13 main studies (Table 1-3). The selection of these studies was through a systematic process that is outlined in Figure 1-1 (see the Supplementary Material sections for greater detail). We only included completed Phase II/III studies because they are generally conducted on larger groups of patients and evaluate specific safety and efficacy endpoints on

CF patients. In this section, we briefly describe key clinical endpoints used in these studies which are necessary to better understand clinical trial results described in Section 1.3.

1.2.1 FEV1% Predicted and Other Measures of Lung Function

The primary efficacy endpoint in 8 of the 13 selected clinical trials reviewed here was absolute change from baseline in percent predicted forced expiratory volume in 1 second (FEV1% Predicted). FEV1 is the volume of air following full inspiration which is exhaled during the first second of forced expiration [25], usually measured in milliliters (mL) or litres (L). Based on contextual factors like age, sex, height, and ethnicity, a predictive value is obtained from studies of "normal" or "healthy" subjects [26]. FEV1% predicted is generally regarded as the best prognostic measure for assessing lung disease in CF [27]. However, valid inferences require good quality measurements, selection of appropriate prediction equations which take into account different sources of lung function variability, and a systematic approach to interpretation [28]. Some literature has argued that the heavy reliance on FEV1 values is questionable and has suggested the need for alternatives which could replace or be used in conjunction with FEV1 values [29].

Since respiratory failure is the leading cause of death in CF, improvements in FEV1% predicted would likely signal improved respiratory function in patients. One study on lung function decline found that mean FEV1% predicted decline was -3.89% (± 4.11%) per year [30] and was purported to be similar to the results of other studies. In the 13 clinical trials under review where a sample size was formally calculated before the trials, the lowest power threshold for FEV1% predicted was an absolute improvement from baseline of at least 4.5% in FEV1% predicted [31], although no literature currently exists on what would constitute a minimal clinically important difference (MCID) for this measure [32].

1.2.2 Sweat Chloride Concentration

During an End-of-Phase 1 meeting in 2008, the FDA recommended a more established endpoint, such as FEV1, instead of sweat chloride concentration which had never been used as a clinical endpoint, as the primary efficacy endpoint in clinical trials of Ivacaftor [33]. The significance of sweat chloride is that it is the gold-standard for CF diagnosis [34, 35]. In general, with some exceptions, a sweat chloride concentration greater than 60mmol/L is a positive indication of CF, while a concentration of 30mmol/L or less can generally rule out the disease [34-36]. However, the reliability of reference intervals for sweat chloride tests have been questioned [37].

Additionally, sweat chloride levels have been shown to change significantly in response to changes in CFTR activity [38, 39] with a dose-response relationship [40]. Despite this, change in sweat chloride has not been found to correlate well with changes in lung function in any individual study [41]; a finding in line with initial assessments of clinical trial data of Ivacaftor by both EMA and FDA [42, 43]. As such, positive results seen in clinical trials for this measure should be interpreted cautiously as its relevance as a clinical biomarker has not been fully established.

1.2.3 CFQ-R Respiratory Domain

The Cystic Fibrosis Questionnaire Revised (CFQ-R) is a validated health-related quality of life questionnaire for CF [44]. In the 13 trials under review, the focus was placed on the CFQ-R respiratory domain, as a secondary outcome measure, which focuses on symptoms such as coughing, wheezing, congestion, and sputum production. Respiratory domain scores range from 0-100 with higher scores indicating fewer symptoms and better quality of life. An MCID of 4 points [45] has been determined for this domain. It is important to note that the CFQ-R has not always been found to correlate well with changes in FEV1% predicted [46] and thus is purported to measure different aspects of overall

respiratory health which, taken together with other markers of clinical improvement, could lend support to its use for measuring the benefit of the treatment and associated improvements in the quality of life of CF patients.

1.2.4 Weight

Change in weight since baseline was primarily used as a secondary endpoint in most clinical trials in this review. Weight gain is considered an important outcome in CF because CF patients generally have difficulty gaining or maintaining weight, even though obesity in certain CF subpopulations has been documented [47]. Primarily, this is attributed to malabsorption of nutrients, such fat, caused by pancreatic insufficiency as well as increased metabolic demands [48]. Furthermore, compared to patients who fall within a normal CF weight range, patients in lower weight percentiles have been shown to have reduced lung function [49] as well as increased morbidity/mortality [50]. Owing to this, weight increases of even of a few kilograms per year may be considered clinically meaningful. Other related measures used in clinical trials and in approval body evaluations included absolute change since baseline in body mass index (BMI) and weight/height ratio.

1.2.5 Pulmonary Exacerbations

Although considered to be an important predictor of quality of life [51, 52] and short-term mortality [53, 54], there is no single universally agreed upon definition for pulmonary exacerbations [55]. The Cystic Fibrosis Foundation Clinical Practice Guidelines [56] define a pulmonary exacerbation as a change or new finding in at least 3 out of 11 possible symptoms (cough, sputum production, fever, weight loss, school or work absenteeism, respiratory rate, chest examination, exercise tolerance, spirometry, oximetry, and chest radiograph) compared to the previous visit. The 13 clinical trials in this review used a modified version of Fuchs criteria [57] which define a pulmonary exacerbation to

have occurred when a patient is treated with parenteral antiobiotics for any 4 of 12 possible symptoms: change in sputum; new or increase hemoptysis; increased cough; increased dyspnea; malaise, fatigue, or lethargy; temperature above 38°C; anorexia or weight loss; sinus pain or tenderness; change in sinus discharge; change in physical examination of the chest; decrease in pulmonary function by 10% or more from a previously recorded value; or radiographic changes which are indicative of pulmonary infection.

The 13 clinical trials in question considered pulmonary exacerbations to be serious adverse events (SAEs) and some studies utilized several measures including rate of pulmonary exacerbations, time to first pulmonary exacerbation, number/frequency of pulmonary exacerbations, and pulmonary exacerbations leading to hospitalization, in order to assess the safety/efficacy of the intervention. In addition to pulmonary exacerbations, dozens of adverse events (AEs) and SAEs were also measured during these clinical trials (see appendices of studies in Table 1-3 for a complete list). AE severity in the clinical trials was determined by the study investigator using a grading scale that can be found in the study protocols of the clinical trials in this review.

1.3 Key Clinical Trials

The emphasis of this section is on key clinical trial findings (with a focus on clinical endpoints described in the previous section) of studies listed in Table 1-3.

1.3.1 Clinical Trials of Ivacaftor (Kalydeco)

1.3.1.1 Establishing the 150mg dose (Study 101)

The first Phase II trial of Ivacaftor [58] was conducted on adult CF patients with the G551D mutation on at least one *CFTR* allele and aimed to evaluate the safety profile of Ivacaftor. The overall frequency of AEs was similar between Ivacaftor and placebo-treated groups. Ivacaftor was also shown to confer benefits for secondary efficacy endpoints such as

FEV1% predicted and sweat chloride concentration. Based on pharmacokinetic/pharmacodynamics data, the 150mg dose was deemed optimal because it provided the greatest benefit for FEV1% predicted improvement of all study doses. Overall, this trial lent support to the safety of Ivacaftor for use in adult patients with a G551D mutation and led to establishing 150mg as the optimal dose for further testing and development.

1.3.1.2 Evidence for Ivacaftor's Efficacy (STRIVE/ENVISION)

STRIVE [59] and ENVISION [60] were Phase III studies of Ivacaftor on CF patients with the G551D mutation with an identical study designs except for the age of study subjects: STRIVE patients were 12 years of age and older; ENVISION patients were 6-11 years of age. The primary efficacy endpoint was absolute change from baseline in FEV1% predicted through week 24. An absolute mean improvement from baseline for Ivacaftor-treated patients in FEV1% predicted of 10.4% and 12.6% were seen in STRIVE and ENVISION, respectively, which translated to a treatment difference of 12.6% and 12.5% in STRIVE and ENVISION, respectively, which were also statistically significant ($p<0.001$); these effects were largely maintained through the 48-week study period. Absolute improvements in weight gain (2.7kg in STRIVE and 1.9kg in ENVISION) and sweat chloride concentration (-47.9mmol/L in STRIVE and -54.3mmol/L in ENVISION) were also noted for the treatment group as compared to the placebo group, all of which were statistically significant ($p<0.001$). In both studies, the incidence of AEs was similar between placebo and treatment arms; however, a 55% reduction in pulmonary exacerbation risk was seen in STRIVE's treatment group, while the pulmonary exacerbation rate did not differ by arm in ENVISION.

The efficacy and safety results from STRIVE and ENVISION were heavily relied on by approval bodies [42, 61, 62] in evaluating Ivacaftor. The benefits seen in these clinical trials were integral in approval bodies' decisions to approve Kalydeco for the treatment of patients with the G551D mutation who were over the age of 6. The results from these studies are consistent with Ivacaftor's potentiator function which prolongs the opening of CFTR channels and the G551D mutation, a Class III mutation whereby CFTR production/processing is largely unaltered, but CFTR channels fail to open normally following activation.

1.3.1.3 PERSIST – Long-term safety/efficacy of Ivacaftor

PERSIST [63] was a phase III, 96-week rollover study of Ivacaftor on CF patients who had successfully completed 48 weeks of STRIVE or ENVISION. At baseline, FEV1% predicted, weight, and BMI, were higher among subjects who had received Ivacaftor in either STRIVE/ENVISION, compared to placebo; these effects were largely sustained for patients who continued to receive Ivacaftor for 96-weeks. Meanwhile, patterns of improvement similar to what was observed for treatments arms in STRIVE/ENVISION were seen in FEV1% predicted, weight gain, BMI, and CFQ-R respiratory domain scores throughout 96-weeks for patients who switched from placebo to Ivacaftor.

For patients who had received Ivacaftor in STRIVE or ENVISION, there existed a total of 144-weeks of continuous data on the outcomes under treatment by Ivacaftor. PERSIST was the first long-term study to demonstrate that treatment with Ivacaftor for CF patients with the G551D mutation who were over the age of 6 was both safe and led to sustained clinical benefit.

1.3.1.4 DISCOVER - Lack of efficacy for the F508del mutation

During an FDA End-of-Phase I meeting [33] for Ivacaftor, a key discussion point was the requirement to conduct a large-scale clinical trial in CF patients over the age of 12 homozygous for the F508del mutation. F508del is a Class II mutation, which results in impaired processing/transport of CFTR, effectively reducing its density at the cell surface; however, CFTR that does reach the cell surface may further display defective gating [64]. This lends support for potential benefit to patients with this mutation through the action of a potentiator compound. The rationale for selecting a homozygous F508del population was to isolate any potentiation effects of the F508del mutation without confounding from another mutation.

DISCOVER [11] was a phase II study of Ivacaftor for patients over the age of 12 homozygous for the F508del mutation; part A was a parallel design with participants given Ivacaftor every 12 hours or placebo for 16 weeks and part B was a 96-week open-label extension period. The overall proportion of subjects with AEs was similar between the two groups and pulmonary exacerbations did not differ significantly during part A. Additionally, no statistically significant treatment effects were noted for FEV1% predicted, sweat chloride concentration, CFQ-R respiratory domain score, or weight (and BMI) between the study arms in part A. The open-label extension period did not add any meaningful findings regarding safety or key clinical endpoints. Benefits observed for patients treated with Ivacaftor were limited and not considered clinically meaningful; an observation that aligns with the view of Class II mutations, such as F508del, as predominantly resulting in reduced CFTR density at the cell surface that would likely not be improved by a potentiator alone.

1.3.1.5 KONDUCT, KONNECTION, and KIWI

KONDUCT [65], KONNECTION [66], and KIWI [67] were Phase III studies of Ivacaftor on largely new CF subpopulations than previous studies. KONDUCT specifically

looked at the safety and efficacy of Ivacaftor in CF patients with an R117H mutation which affects approximately 3% of all CF patients [12]. KONNECTION was the first study to evaluate the efficacy and safety of Ivacaftor in patients with any of the following Class III non-G55ID gating mutations which collectively account for approximately 1% of CF cases [68]; G178R, G551S, S549N, S549R, G970R, G1244E, S1251N, S1255P, or G1349D. KIWI was the first study of Ivacaftor in pediatric patients 2 to 5 years of age with a CFTR-gating mutation.

The R117H mutation is a Class IV mutation where CFTR displays impaired channel conductance and to a lesser extent, reduced gating [69]. KONDUCT used a 24-week long parallel study design (Ivacaftor every 12 hours or placebo) with eligible subjects able to roll-over into an open-label extension study, KONTINUE. Following 24 weeks of treatment, an FEV1% predicted treatment difference of 2.1% ($p=0.20$) was noted between the Ivacaftor and placebo arm. Additionally, treatment with Ivacaftor did not result in any increase in BMI from baseline. Time to first pulmonary exacerbation was also not significantly different between the treatment and placebo groups. However, significant improvements for Ivacaftor-treated patients in CFQ-R respiratory domain score (8.4 points, $p=0.009$) and in sweat chloride concentration reduction (-24.0mmol/L, $p<0.0001$) compared to placebo at 24 weeks were observed. Although FEV1% predicted improvements did not reach the levels from previous G551D studies, sub-group analyses showed clinically meaningful and statistically significant improvements in FEV1% predicted in adult patients. One explanation for this is that lung disease generally manifests itself at an older age in patients with the R117H mutation [70], supporting the benefit of Ivacaftor for treating patients with the R117H mutation that are older and have more established lung disease.

KONNECTION was a two-part study on CF patients. At the end of Part 1, absolute change since baseline for FEV1% predicted for Ivacaftor-treated patients was 7.5% (corresponding to a treatment effect of 10.7%, $p<0.0001$). Significant improvements for BMI, sweat chloride, and CFQ-R respiratory domain scores were also seen for Ivacaftor-treated patients compared to the placebo. Improvements seen in Part 1 were sustained during the Part 2 open-label period. AEs and pulmonary exacerbations were largely comparable between the arms during Part 1. Although the trial showed evidence to support some benefit of Ivacaftor in non-G551D gating mutations, there was a lack of clinically meaningful improvement in some subgroups of patients, such as those with the G970R mutation [71], suggesting the need for further investigation in this CF sub-population.

KIWI was a two-part, open-label trial undertaken to study the safety, pharmacokinetics, pharmacodynamics and to explore the efficacy of Ivacaftor in patients aged 2-5 years old with a *CFTR* gating mutation on at least one allele. Doses of 50mg or 75mg were administered depending on the weight of patients. Part A, which was 4 days in duration, was undertaken to assess short-term safety and pharmacokinetics of Ivacaftor, while Part B, 24-weeks in duration, aimed to assess longer-term safety of Ivacaftor. Detailed safety results are available in the clinical trial publication for KIWI [67]. Overall, the pharmacokinetic data as well as the safety profile were found to be similar to that of previous phase II/III adult studies of Ivacaftor [31, 60]. Preliminary efficacy results showed improvements in sweat chloride concentration and in nutritional parameters; at the end of Part B, mean absolute change from baseline in sweat chloride concentration was -46.9mmol/L ($p<0.0001$), weight Z score increase of 0.3 ($p<0.0001$), and BMI Z increase score by 0.4 ($p<0.0001$).

The results from KONDUCT, KONNECTION, and KIWI have been evaluated by approval bodies, with Kalydeco now approved at 50mg/75mg doses for 2-5 year old CF patients as well as at doses of 150mg for patients over the age of 6 with any of the following mutations: G551D, G1244E, G1349D, G178R, G551S, S1251N, S1255P, S549N, S549R or R117H [72]. Given that Ivacaftor prolongs CFTR opening, the results observed in KONNECTION, which evaluated non-G551D Class III gating mutations, are logically consistent. The results from KONDUCT further support improved CFTR function as evidenced by sweat chloride reductions while KIWI results added support to the benefit of Ivacaftor in a younger population.

1.3.1.6 Ivacaftor for mutations with residual CFTR function

The final completed study of Ivacaftor alone was conducted on CF patients who had either phenotypic or molecular evidence to support residual (or partial) CFTR function [73]. The study is completed but results with statistical analyses have not been formally published. Among other inclusion/exclusion criteria, study subjects were 12 years and older with one of at least 38 *CFTR* mutations associated with residual CFTR function.

1.3.2 Clinical Trials of Lumacaftor

1.3.2.1 Lumacaftor in F508del subjects

The only completed trial of Lumacaftor monotherapy [74] was a Phase II study which aimed to evaluate the safety and tolerability of Lumacaftor, a CFTR corrector, at varying doses. The study found similar AE incidence across dosing groups and no difference in pulmonary exacerbations between Lumacaftor-treated and placebo patients (17% (Lumacaftor) vs. 12% (placebo), p=0.62). Absolute reductions in sweat chloride after 28 days of treatment were +0.10mmol/L, -4.61mmol/L, -6.13mmol/L, and -8.21mmol/L in 25mg, 50mg, 100mg, and 200mg dosing groups, respectively, and were statistically significant

in the 100mg and 200mg groups as compared to placebo. No improvements in FEV1% predicted or CFQ-R scores were observed.

Given the primary objective of the study, the results lent support to the safety and tolerability of Lumacaftor at all doses tested. Given that Lumacaftor is a CFTR corrector, it should theoretically lead to improvements in other efficacy measures for patients homozygous for the F508del mutation, but such effects were not seen. Given the lack of statistically significant and clinically meaningful effects, the study pointed to the need for additional trials, perhaps of a longer duration, to evaluate Lumacaftor in F508del homozygous patients.

1.3.3 Clinical Trials of Lumacaftor-Ivacaftor (Orkambi)

1.3.3.1 A Phase II evaluation of Lumacaftor-Ivacaftor

The rationale to combine Ivacaftor and Lumacaftor was driven, in part, by a lack of clinically meaningful improvement, particularly in lung function, among CF patients homozygous for the F508del mutation in previous trials as well as in-vitro evidence [24] showing nearly twice as much CFTR chloride transport following combination of the two drugs. A Phase II study [75] of Lumacaftor-Ivacaftor was conducted with 3 consecutive cohorts. Study patients in the study were homozygous for the F508del mutation (except in Cohort 2, where a group of heterozygous patients were allowed in order to assess gene dosing effects comparing homozygous and heterozygous patients). Patients in each of the cohorts were randomized to receive either Lumacaftor at a certain dose or placebo followed by Ivacaftor after a period of time or placebo (Table 1-3). Change in sweat chloride concentration was the primary efficacy endpoint for this study.

Although statistical significance does not always result in clinically meaningful benefit, we focus mainly on key statistically significant findings for this study. For Cohort 1,

200mg of Lumacaftor combined with 250mg of Ivacaftor led to significant reductions in sweat chloride compared to baseline (-12.6mmol/L, $p<0.001$) and relative to placebo (-10.9mmol/L, $p=0.002$). Additionally, in Cohort 1, mean FEV1% predicted in the Lumacaftor 200mg and Ivacaftor 150mg group showed a significant improvement compared to baseline (3.1%, $p=0.047$) but not compared to placebo ($p=0.18$). For both Cohorts 2 and 3, among patients homozygous for the F508del mutation, monotherapy with Lumacaftor at all doses resulted in significant reductions in sweat chloride compared to baseline and relative to placebo. However, combination with Ivacaftor did not confer any additional statistically significant benefits in sweat chloride reduction compared to just Lumacaftor. Among patients heterozygous for the F508del mutation, reductions in sweat chloride with the combined therapy were only statistically significant compared to baseline and relative to placebo.

At the end of the trial, the only group which showed statistically significant improvements compared to baseline and placebo in absolute FEV1% predicted change was a group of patients homozygous for the F508del mutation given a 600mg dose of Lumacaftor and a 250mg dose of Ivacaftor (mean absolute change from baseline of 3.6% ($p=0.027$) and a treatment difference of 5.6% ($p=0.017$). Lumacaftor-Ivacaftor combination therapy resulted in statistically significant changes compared to monotherapy (Lumacaftor) in absolute FEV1% predicted for both the Lumacaftor 400mg (6.2%, $p<0.001$) and 600mg (6.1%, $p=0.004$) groups. In all three study cohorts, the AE proportion was similar across all treatment and placebo arms. Overall, this study lent some support for the safety of the combination therapy in certain dosing groups. The results also supported improved CFTR channel function given sweat chloride concentration reductions which were greater than previous studies of Ivacaftor or Lumacaftor administered alone on the F508del population.

1.3.3.2 TRAFFIC/TRANSPORT

TRAFFIC and TRANSPORT [76] were Phase III studies on F508del homozygous patients who were randomized in a 1:1:1 ratio to receive 600mg Lumacaftor and 250mg Ivacaftor, 400mg Lumacaftor and 250mg Ivacaftor, or a matched placebo for a 24-week period. The primary endpoint was absolute change from baseline in FEV1% predicted. Interestingly, sweat chloride was not included as an outcome measure in these clinical trials. The only difference between the two trials was the inclusion of ambulatory electrocardiography for TRAFFIC only and adolescent pharmacokinetic assessment for TRANSPORT only.

Statistically significant ($p<0.001$) improvements in mean absolute change from baseline in FEV1% were in both TRAFFIC and TRANSPORT treatment groups, but these changes ranged on average from 2.2% to 3.6% (a relative treatment difference of 2.6% to 4.0%, $p<0.001$). CFQ-R respiratory domain scores of 5 points or more, which were clinically and statistically significantly better compared to the within-group baseline, were seen in 3 out of 4 treatment groups. Rate ratios for pulmonary exacerbations were found to be statistically significantly lower in all treatment groups compared to placebo, ranging from 0.57 to 0.72. The overall AE proportion was found to be similar across treatment arms and the placebo group in both studies.

The TRAFFIC/TRANSPORT studies were the largest of the clinical trials in this review with over 1100 patients studied at 187 centers globally. Although the mean absolute improvement from baseline in FEV1% predicted did not reach levels seen in studies like STRIVE/ENVISION, relative improvements from baseline in FEV1% of 5% or 10% were noted in approximately twice as many subjects as compared to placebo in both studies. Additionally, pulmonary exacerbations occurred less frequently in treatment groups than in

placebo groups. Coupled with sweat chloride reductions from the previous combined Phase II trial, benefits of the combined therapy in improving certain clinical outcomes for CF patients homozygous for F508del were shown. The results from TRAFFIC, TRANSPORT, as well as the Phase II study of the combination therapy were used by the FDA and EMA [77, 78] to approve Orkambi for the treatment of CF in patients over the age of 12 homozygous for the F508del mutation.

1.4 Conclusion

The past decade has witnessed tremendous strides, from the initial discovery of two compounds, Ivacaftor and Lumacaftor, to a therapeutic agent, Orkambi, which is now available for the most common CFTR mutation, the F508del homozygous mutation. This review paper has traced the development of Orkambi from its initial in-vitro discovery to Phase II/III clinical trials, in the context of the underlying CFTR biology and clinical endpoints measures used. Currently, a second corrector compound, VX-661 [79], has shown initial promise in improving clinical outcomes for patients homozygous for the F508del mutation when combined with Ivacaftor, and may be next in a line of treatments aimed at improving the well-being of CF patients by directly targeting its underlying cause.

Table 1-1 –Demographic Information of Prevalent CFTR Mutations							
Country	**Mutation (n(%))**						
	F508del		**G551D**	**R117H**	**G542X**	**621+1G->T**	**A455E**
	Total	**Homozygous/ Heterozygous**					
United Kingdom	7,990 (90.8%)	4,551 (51.3%)/ 3,479 (39.5%)	514 (5.8%)	398 (4.5%)	318 (3.6%)	186 (2.1%)	--
United States	23,478 (86.4%)	12, 636 (46.5%)/ 10,842 (39.9%)	1,182 (4.4%)	767 (2.8%)	1,252 (4.6%)	437 (1.6%)	142 (0.5%)
Canada	3,563 (89.7%)	1,986 (50%)/ 1,577 (39.7%)	122 (3.1%)	--	138 (3.5%)	241 (6.1%)	102 (2.6%)

Table 1-2 – CFTR Mutation Classes and Their Functional Consequences		
Class	**Mutations (Examples)**	**Functional Consequences**
I	G542X, 621+1G->T	- Defective protein synthesis - CFTR not expressed
II	F508del	- Misfolded CFTR - Lack of CFTR transport to cell surface
III	*G551D, G178R, G551S, S549N, S549R, G970R, G1244E, S1251N, S1255P, G1349D	- CFTR reaches cell surface - Defective channel opening following activation
IV	R117H	- CFTR reaches cell surface - Defective conductance (restriction of chloride ion movement)
V	A455E	- CFTR synthesis reduced - CFTR produced is normal
* These Class III mutations, except G551D, were studied in clinical trials KONNECTION and KIWI		

Table 1-3 - Clinical Trial Overview with Study Population and Trial Design		
Trial Name (ID)	**Study Population**	**Trial Design**
Effect of VX-770 in Persons with Cystic Fibrosis and the G551D Mutation (NCT00457821)	- G551D on at least one allele - 18 years or older	Two-part, randomized, double-blind, placebo-controlled study: - Part 1: 25mg, 75mg, 150mg Ivacaftor or Placebo for 14 days, followed by washout and crossover to different dose/placebo for 14 days (modified crossover design) - Part 2: 150mg, 250mg or Placebo for 28 days
A CFTR Potentiator in Patients with Cystic Fibrosis and the G551D Mutation (STRIVE) (NCT00909532)	- G551D on at least one allele - 12 years or older	Randomized, double-blind, placebo-controlled, parallel arm study: - 48 weeks with 150mg Ivacaftor every 12 hours or placebo
Efficacy and Safety of Ivacaftor in Patients Aged 6 to 11 Years with Cystic Fibrosis with a G551D Mutation (ENVISION) (NCT00909727)	- G551D on at least one allele - 6 to 11 years old	Randomized, double-blind, placebo-controlled, parallel arm study: - 48 weeks with 150mg Ivacaftor every 12 hours or placebo
Long-term safety and efficacy of ivacaftor in patients with cystic fibrosis who have the Gly551Asp-CFTR mutation: a phase 3, open-label extension study (PERSIST) (NCT01117012)	- G551D on at least one allele - Completed 48 weeks of STRIVE or ENVISION	Open-label extension study: - All subjects received 150mg Ivacaftor every 12 hours for 96 weeks
Ivacaftor in Subjects With Cystic Fibrosis Who are Homozygous for the F508del-CFTR Mutation (DISCOVER) (NCT00953706)	- F508del/ F508del - 12 years or older	Two-part study: - Part A: randomized, double-blind, placebo-controlled of 150 mg Ivacaftor every 12 hours or placebo for 16 weeks - Part B: open-label extension period of 150mg Ivacaftor every 12 hours for 96 weeks
Efficacy and safety of ivacaftor in patients with cystic fibrosis who have an Arg117His-CFTR mutation: a double-blind, randomised controlled trial (KONDUCT) (NCT01614457)	- R117H on at least one allele - 6 years or older	Randomized, double-blind, placebo-controlled, parallel arm study: - 150mg Ivacaftor every 12 hours or placebo for 24 weeks
Efficacy and safety of ivacaftor in patients with cystic fibrosis and a non-G551D gating mutation (KONNECTION) (NCT01614470)	- G178R, S549N, S549R, G551S, G907R, G1244E, S1251N, S1255P, or G1349D on at least one allele - 6 years or older	Two-part design: - Part 1: Double-blind crossover design of 150mg Ivacaftor every 12 hours or placebo for 8 weeks followed by crossover - Part 2: 16-week open-label extension of Ivacaftor every 12 hours
Study of Ivacaftor in Cystic Fibrosis Subjects 2 Through 5 Years of Age With a CFTR Gating Mutation (KIWI) (NCT01705145)	- CFTR-gating mutation on at least one allele* - 2 to 5 years old	Two-part, open-label design: - Part A: 50mg or 75mg (weight-dependent) every 12 hours for 4 days - Part B: 50mg or 75mg (weight-dependent) every 12 hours for 24 weeks
Pilot Study Testing the Effect of Ivacaftor on Lung Function in Subjects with Cystic Fibrosis and Residual CFTR Function	- CFTR mutations associated with residual function* - 12 years or older	Crossover design with open-label extension: - Cycle 1 (1-29 days), Washout (4-weeks), Cycle 2 (1-29 days), Washout (4-weeks), Open-label (1- 57 days).

(NCT01685801)		- Participants received 150mg Ivacaftor every 12 hours for 2 weeks and placebo for 2 weeks during each cycle in a random order. All subjects received 150mg every 12 hours during open-label period.
Results of a phase IIa study of VX-809, an investigational CFTR corrector compound, in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation (NCT00865904)	- F508del/ F508del - 18 years or older	Two-part, randomized, double-blind, placebo-controlled study: - Group A: 25 or 50mg Lumacaftor or placebo daily for 28 days. - Group B 100 or 200mg Lumacaftor or placebo daily for 28 days.
A CFTR corrector (lumacaftor) and a CFTR potentiator (ivacaftor) for treatment of patients with cystic fibrosis who have a phe508del CFTR mutation: a phase 2 randomised controlled trial (NCT01225211)	- F508del/ F508del or F508del on at one allele (different study group) - 18 years or older	Randomized, double-blind, placebo-controlled study with three consecutive cohorts: - Cohort 1: 200mg Lumacaftor daily for 21 days combined with either 150mg or 250mg Ivacaftor every 12 hours from day 15-21 or placebo for 21 days. - Cohort 2**: 200, 400, or 600mg Lumacaftor daily for 56 days combined with 250mg Ivacaftor every 12 hours from days 29-56 or placebo. - Cohort 3: Lumacaftor 400mg every 12 hours for 56 days combined with Ivacaftor 250mg every 12 hours from days 29-56 or placebo.
A Study of Lumacaftor in Combination With Ivacaftor in Cystic Fibrosis Subjects Aged 12 Years and Older Who Are Homozygous for the F508del-CFTR Mutation (TRAFFIC) (NCT01807923)	- F508del/ F508del - 12 years or older	Randomized, double-blind, placebo-controlled, parallel arm study: - 600mg Lumacaftor daily with 250mg Ivacaftor every 12 hours or 400mg Lumacaftor daily with 250mg Ivacaftor every 12 hours or matched placebo every 12 hours
A Study of Lumacaftor in Combination With Ivacaftor in Cystic Fibrosis Subjects Aged 12 Years and Older Who Are Homozygous for the F508del-CFTR Mutation (TRANSPORT) (NCT01807949)	- F508del/ F508del - 12 years or older	Randomized, double-blind, placebo-controlled, parallel arm study: - 600mg Lumacaftor daily with 250mg Ivacaftor every 12 hours or 400mg Lumacaftor daily with 250mg Ivacaftor every 12 hours or matched placebo every 12 hours
* A full-list of mutations included in this study can be found on the trial registration page		
** Cohort 2 consisted of one heterozygous F508del group receiving 400mg Lumacaftor daily (56 days) and 250mg Ivacaftor (Days 29-56) every 12 hours, or matched placebo for 56 days.		

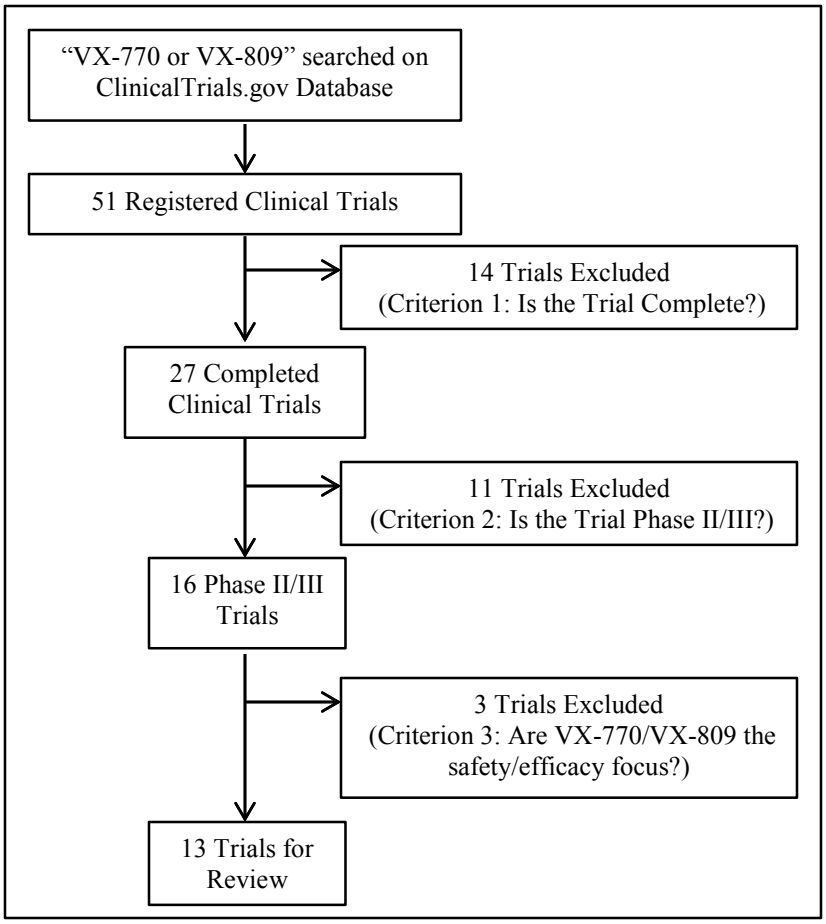

Figure 1-1- Selection Process for Trials Included in Review

Supplementary Material:

Methods:

The selection of relevant clinical trials was done through a systematic review process. We used the clinical trial registration page, clinicaltrials.gov, which contains information about clinical trials around the world, as our search database. We used one key search term "VX-770 OR VX-809" to search for relevant clinical trials. VX-770 and VX-809 are identifiers for Ivacaftor and Lumacaftor, respectively.

Following results from the search, we used three inclusion/exclusion criteria to determine which clinical trials were relevant for our study purpose. Firstly, the study needed to be listed as "completed". As such, studies which were still underway were excluded. Secondly, the study needed to be either a Phase II or a Phase III clinical trial. This led to the exclusion of Animal Studies, Phase I studies, and other studies which did not fit this criterion. Lastly, the study needed to focus its evaluation on the safety/efficacy of Ivacaftor or Lumacaftor or both in a given population, but not in combination with a third compound. Studies that met these criteria were included in our review paper, in the section titled "Key Clinical Trials" and relevant information on the clinical trial registration page, such as publications from the clinical trial, were also accessed.

Results:

The systematic search was conducted on July 2, 2015 which is the day that Orkambi received regulatory approval by the United States Food and Drug Administration (FDA). After searching the clinicaltrials.gov database using "VX-770 or VX-809", 51 studies/results were registered.

After applying inclusion/exclusion criterion 1 (the study needed to be complete), 27 studies remained (14 were excluded) that were listed as "complete". Secondly, of the 27 studies, only 16 were Phase II/III, resulting in an additional 11 studies being excluded. Thirdly, after criterion 3 was applied, 13 studies remained (1 study was excluded because it focused on another compound VX-661, and 2 studies were excluded because they were testing novel measures of lung function). A flow diagram is available in the manuscript Figure 1-1.

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CHAPTER 2

An Evaluation of Clinical Trials of Ivacaftor (Kalydeco), Lumacaftor, and Lumacaftor-Ivacaftor (Orkambi) on Cystic Fibrosis Patients Homozygous for the F508del Mutation

2.1 Introduction

The F508del mutation is the most common cause of Cystic Fibrosis (CF), with patients homozygous for the F508del mutation accounting for nearly half of all observed cases [1-2]. Categorized as a Class II mutation [3], the F508del mutation results in altered transport and processing of the Cystic Fibrosis Transmembrane Regulator (CFTR) ion channel [4]. Primarily, this prevents CFTR from reaching the cell surface, where it plays an important role in salt and water transport across the epithelia of organs such as the lungs [5-7]. CFTR that successfully reaches the cell surface may further display defective gating [8]. Therapeutic strategies to combat these defects may occur through the action of correctors and/or potentiators. Corrector compounds, such as Lumacaftor [9], serve to increase the cell surface density of CFTR while potentiators, such as Ivacaftor [10], prolong the opening of CFTR channels at the cell surface. In theory, correctors and potentiators, alone or in combination, could help improve CFTR function in patients with the F508del mutation.

Kalydeco (Ivacaftor) and Orkambi (Lumacaftor-Ivacaftor) are recently approved therapeutic agents for the treatment of CF which have been hailed as breakthroughs in personalized medicine [11-12]. Kalydeco was first approved in 2012 [13] for the treatment of CF with the G551D mutation, which affects approximately 4% of all CF patients [1]. In 2015, Orkambi received regulatory approval for the treatment of patients homozygous for the F508del mutation [14].

Although the approval of Kalydeco was met with some skepticism [15-17], clinical

trials for patients receiving Ivacaftor for the G551D mutation, on average, showed a 10.0-12.5% absolute improvement from baseline in Percent Predicted Forced Expiratory Volume in 1 second (FEV1% predicted) [18-19]. Meanwhile, benefits seen in clinical trials of Orkambi (Lumacaftor-Ivacaftor) showed, at best, an average improvement of 3.6% in FEV1 % predicted [20-21]. Similarly, improvements for other measures, such as sweat chloride concentration, a biomarker of CFTR activity, also showed noticeably greater benefit for the G551D population administered Ivacaftor, compared to CF patients homozygous for the F508del mutation who were administered Lumacaftor-Ivacaftor.

Additionally, Kalydeco was priced at approximately $300,000 USD per patient per year [22], and this was meant to reflect the cost of developing a treatment for a small subgroup of patients from a disease already given "orphan" designation. However, Orkambi, which is priced $259,000 USD per patient per year [23], is available to a much larger patient group, with a relatively smaller proven clinical benefit compared to Kalydeco. According to some estimates, if all eligible patients were to receive Orkambi, this would amount to an annual revenue of $2 billion dollars for the manufacturer, Vertex pharmaceuticals, in the United States alone [24].

The motivation to combine Lumacaftor and Ivacaftor was purported to be due to a lack of clinical benefit seen from each compound when administered alone to patients homozygous for the F508del mutation. One legitimate concern which arises is whether the degree of clinical benefit seen for F508del homozygous patients administered Lumacaftor-Ivacaftor is discernible from the benefit provided by Ivacaftor or Lumacaftor alone. For this paper, we reviewed, appraised and compared clinical trials of Ivacaftor, Lumacaftor, or Lumacaftor-Ivacaftor that were conducted on the patients homozygous for the F508del mutation. We had one overarching question during the course of our evaluation; does

Lumacaftor-Ivacaftor combination therapy appear to improve outcomes for CF patients homozygous for the F508del mutation compared to Ivacaftor or Lumacaftor alone? Answering this question could help inform whether Orkambi as a newly approved therapeutic agent can be differentiated from Kalydeco in terms of its clinical benefit to patients who are eligible to receive the treatment, and could further shed some light on issues surrounding the clinical benefit and the cost-effectiveness of Orkambi compared to Kalydeco.

2.2 Methods

2.2.1 Identification of Relevant Clinical Trials

We used an online clinical trials registry, clinicaltrials.gov, to identify relevant trials. We searched the database using the key search terms "Ivacaftor OR Lumacaftor" to identify all studies evaluating either Ivacaftor or Lumacaftor. Next, all relevant studies were compiled and two selection criteria were applied. Firstly, the study needed to be completed with results available. Secondly, the clinical trial needed to be focused primarily on evaluating Ivacaftor or Lumacaftor, or both, on patients homozygous for the F508del mutation. If other patient populations were studies, such as patients with one F508del allele, but were not the primary focus of the trial, we still considered the trial relevant for our study purposes.

2.2.2 Analysis of Clinical Trials

All possible content pertaining to each selected clinical trial was compiled, including but not limited to, the clinical trial registration information, relevant publications, supplementary materials of publications, and study protocols. The lead investigator, SM, was responsible for reading, summarizing, and appraising all content. Clinical trials were initially appraised using CONSORT (Consolidated Standards of Reporting Trials) [25], which aims to improve the quality of reporting of randomized controlled trials.

Next, the comparison and appraisal of clinical trials were performed along three specific dimensions: 1) clinical trial study design; 2) sample size and power considerations; and 3) important clinical endpoints (FEV1% predicted, sweat chloride concentration, Cystic Fibrosis Questionnaire-Revisited (CFQ-R) respiratory domain score).

Upon completion of the analysis of clinical trials, findings were scrutinized and refined through the help of Dr. Yutaka Yasui, a biostatistician. Subsequently, a manuscript with study findings was prepared and evaluated and edited by Dr. Winnie Leung, a respirologist with expertise in CF. The results section comprises a summary of the study designs, sample sizes and power, and endpoint results. The discussion section focuses on trial comparisons and appraisals.

2.3 Results

2.3.1 F508del Clinical Trials

Fifty nine clinical trials were identified using "Ivacaftor OR Lumacaftor" in the clinicaltrials.gov database. Criterion 1, requiring that clinical trials be completed with results, led to the exclusion of 44 clinical trials. Criterion 2, requiring that clinical trials be focused primarily on evaluating Lumacaftor, Ivacaftor, or both, on patients homozygous for the F508del mutation, was then applied to the 15 remaining clinical trials, of which 10 were excluded; this left 5 remaining studies. The selection process is summarized in Figure 2-1.

2.3.2 Clinical Trial Study Designs

Table 1-1 contains basic information regarding the 5 selected clinical trials. DISCOVER [26] was a Phase II study evaluating Ivacaftor monotherapy on F508del homozygous patients. Subjects in the study were randomized to receive Ivacaftor 150mg or placebo for 16 weeks (Figure 2-2). Patients were eligible to enroll in a 96-week extension (Part B) if they had a relative change from baseline in FEV1% predicted of 10% or greater at

any time during the study or a sweat chloride reduction of greater than 15mmol/L from baseline on both the day 15 and week 8 visit.

The Phase II study of Lumacaftor monotherapy [27] consisted of two cohorts of homozygous F508del patients. Group A subjects were randomized to receive Lumacaftor 25mg or 50mg or a placebo for 28 days. Following a safety review of Group A results, Group B patients were randomized to receive 100mg or 200mg of Lumacaftor or a placebo for 28 days (Figure 2-3).

The Phase II study of Lumacaftor-Ivacaftor [20] combined therapy consisted of three consecutive cohorts, with results from previous cohorts informing dosing for the subsequent cohort. All subjects enrolled in the study were homozygous for the F508del mutation (except for one F508del heterozygous group in Cohort 2). Except for Cohort 3, where Lumacaftor was administered every 12 hours, Ivacaftor was assigned every 12 hours and Lumacaftor was assigned once daily. Each cohort began with a Lumacaftor monotherapy period (or matched placebo) for a period of 14-28 days followed by a combination therapy period for 7-28 days (see Figure 2-4).

TRAFFIC and TRANSPORT [21] were both Phase III studies with parallel designs evaluating different combinations of Lumacaftor-Ivacaftor therapy. Patients in these studies were given either, 600mg Lumacaftor and 250mg Ivacaftor, 400mg Lumacaftor and 250mg Ivacaftor, or a placebo for 24 weeks (Figure 2-5).

2.3.3 Sample Size

There was no formal sample size calculation conducted for DISCOVER which the authors attributed to the fact that the primary objective was to evaluate safety. The authors estimated that 120 subjects would be adequate to provide safety data for the given

population based on unspecified "clinical considerations" [26]. In DISCOVER, 112 patients were randomized to Ivacaftor and 28 were randomized to placebo.

With respect to the Phase II study of Lumacaftor, a sample size of 90 subjects was planned and was reported to give more than 97% power to detect a 20mmol/L reduction in sweat chloride and a probability of 99% to observe at least 1 adverse event. Overall, 89 subjects were randomized to a treatment arm (72 to different doses of Lumacaftor and 17 to placebo).

The Phase II study of Lumacaftor-Ivacaftor provided a formal sample size calculation for each study cohort. Cohort 1 had at least 81% power to detect decrease of 10-16mmol/L in sweat chloride as well as to detect a 5% increase in FEV1% predicted. Cohort 2 had at least 86% power to detect sweat chloride reductions of 10-16mmol/L and 81% power to detect increases in FEV1% predicted by 5%. Cohort 3 had 74% power to detect decreases of 10-16mmol/L in sweat chloride concentration and 50% power to detect a 6-8% increase in FEV1% predicted. 64, 111, and 15 subjects were randomized to cohorts 1, 2, and 3, respectively.

TRAFFIC and TRANSPORT used data from the Phase II study of Lumacaftor-Ivacaftor to estimate that a sample of 501 patients (167 per treatment group) would result in 99% power to detect a 5% absolute change from baseline in FEV1% predicted treatment difference between each of the treatments arms and the placebo group. In the trials, 559 TRAFFIC subjects and 563 TRANSPORT subjects were randomized into one of the study arms.

2.3.4 Clinical Endpoints

A full list of all available FEV1% predicted, sweat chloride concentration, and CFQ-R respiratory domain score results from the clinical trials described above can be found in

Table 2-2, Table 2-3, and Table 2-4, respectively, and are elaborated on in the upcoming sections.

2.4 Discussion

2.4.1 Study Design Considerations

All three Phase II investigations on the F508del homozygous population were conducted with different study designs. DISCOVER used a two-part study design with 16 weeks of blinded treatment followed by a 96-week rollover extension. Despite being specified a priori, no justification was provided as to why patients needed to show a relative change in FEV1 % predicted of 10% from baseline at any time during Part A or sweat chloride reduction greater than 15mmol/L from baseline on both day 15 and week 8 visits for eligibility into Part B. The Phase II study of Lumacaftor consisted of two cohorts, each 28 days in duration. The Phase II study of Lumacaftor-Ivacaftor consisted of 3 consecutive cohorts receiving Lumacaftor monotherapy and subsequently combined therapy over a period of 21 or 56 days, depending on the cohort. Furthermore, patients in DISCOVER could be as young as 12 years of age while the other Phase II studies were comprised of adults.

Arguably, the first Phase II investigation of Ivacaftor on patients with the G551D mutation had already shown that is to be safe by August 2008 (when primary data were first registered on clinicaltrials.gov). As such, this would justify, in the case of DISCOVER which began in September 2009, the longer study duration, age of study patients, and the study design employed. Lumacaftor and Lumacaftor-Ivacaftor on the other hand, needed an essential investigation of dosing and their safety profiles before a study of longer duration could be justified.

While Phase II studies are not focused on efficacy, the issue of different study designs is nonetheless important because it is difficult to compare 16 weeks of data available on Ivacaftor with, at most, 28 days of Lumacaftor monotherapy or 28 days of combined Lumacaftor-Ivacaftor therapy.

2.4.2 Sample Size Considerations

Sufficient sample size in clinical trials assumes appropriate power in statistical inferences of a trial [28]. Sample size issues are especially a concern in the conduct of trials for rare diseases, such as CF [29], where a sufficient sample of patients that meet a set of eligibility criteria may not be available to adequately power the study. Small and underpowered studies would yield imprecise results/estimates and provide a greater chance for random error to conflate results [30, 31].

In general, Phase II trials are conducted to evaluate safety and adverse events of the treatment, determine optimal dosing, and to evaluate efficacy. If a Phase II study shows that a therapeutic agent has an acceptable safety profile and is potentially efficacious, a Phase III study is conducted, usually on a larger population, to test the effectiveness of the intervention [31].

DISCOVER and the Phase II trial of Lumacaftor monotherapy did not indicate efficacy of Ivacaftor and Lumacaftor monotherapy on the patients homozygous for the F508del mutation, particularly in terms of FEV1% predicted. The Phase II study objectives were nonetheless satisfied as there were no major outstanding safety concerns associated with the treatment. Conclusions about the lack of efficacy with respect to FEV1% predicted are questionable from Phase II studies because the studies were never powered to detect such changes. As such, a point can be made that given that the primary aim of the study was fulfilled, it may have justified, larger studies with more patients, and in the case of

Lumacaftor monotherapy, of a longer duration, which were adequately powered towards efficacy endpoints, such as FEV1% predicted. If results from adequately powered studies of Ivacaftor or Lumacaftor monotherapy showed a similar trend towards a lack of benefit for patients homozygous for the F508del mutation, there would exist much stronger support for greater benefit of Lumacaftor-Ivacaftor combination therapy.

The Phase II/III studies of the combined therapy were the only studies powered to detect changes in FEV1% predicted. Interpretation of results from these studies, although in generally support of greater relative benefit than Ivacaftor or Lumacaftor alone, cannot be directly compared to results from the monotherapy studies given that the latter studies were not powered to detect FEV1% predicted changes.

DISCOVER was not powered to detect any changes in sweat chloride concentration while the remainder of the Phase II clinical trials were. Additionally, a uniform power threshold for sweat chloride concentration reduction was not used consistently; the Phase II study of Lumacaftor was powered to detect reductions of 20mmol/L while the combined Lumacaftor-Ivacaftor Phase II trial was powered to detect reductions between 10-16mmol/L. If a study is powered to detect a smaller change, a larger sample size is required, but statistically significant reductions of at least 10mmol/L in sweat chloride concentration may not be clinically meaningful.

2.4.3 FEV1% Predicted and CFQ-R Respiratory Domain Scores

FEV1% predicted and CFQ-R respiratory domains are both measures of respiratory function, but they have not been found to correlate with one another [32]. The CFQ-R is a validated questionnaire with respiratory domain scores providing key patient-reported quality of life information. A minimal clinically important difference of 4 points has been

established for this domain [33]. There is currently no universally agreed upon minimal improvement in FEV1% predicted that would be considered clinically meaningful [34].

Initial studies of Ivacaftor on patients with the G551D mutation powered their studies to detect a 4.5% improvement in absolute FEV1% predicted change from baseline [18]. None of the studies on the F508del population were able to reach this level of clinical benefit in any of the treatment groups. The largest change from baseline in mean absolute FEV1% predicted was 3.6%. This improvement was seen in the Phase II trial of Lumacaftor-Ivacaftor and in TRANSPORT, a Phase III trial of Lumacaftor-Ivacaftor, both in a group receiving 600mg of Lumacaftor and 250mg of Ivacaftor. There is some uncertainty regarding the clinical relevance of an absolute improvement of 3.6% from baseline in FEV1% predicted.

Improvements for FEV1% predicted seen in DISCOVER for all measures were neither clinically nor statistically significant, although the study was not powered to detect such changes. Additionally, in the Phase II study of Lumacaftor-Ivacaftor, which was powered to detect improvements in FEV1% predicted, the monotherapy period resulted in a decrease in FEV1% predicted in 6 out of 7 groups (2 of which were statistically significant) given Lumacaftor at varying doses (the only improvement was an absolute FEV1% predicted increase of 0.2% in Cohort 2 group given Lumacaftor 200mg and was not statistically significant). Interestingly, in one study group (Cohort 2, Lumacaftor 400mg), absolute FEV1% predicted change from baseline worsened statistically significantly and arguably, clinically meaningfully (-4.5%, $p=0.032$). Although data from the Phase II trial of Lumacaftor on mean absolute changes in FEV1% predicted was not provided, the mean relative change from baseline decreased in 2 out of 4 treatment groups.

CFQ-R respiratory domain changes in DISCOVER and in the Phase II study of Lumacaftor monotherapy did not provide any support for improved quality of life through the treatment with Ivacaftor or Lumacaftor alone. Additionally, all groups during the monotherapy period for the Lumacaftor-Ivacaftor Phase II study showed reductions in CFQ-R respiratory domain scores except one. Moreover, in the Lumacaftor monotherapy period, CFQ-R respiratory domain reductions that were seen in half of the groups were greater than 4 points, signaling clinically meaningfully worse outcomes compared to baseline. In contrast, all study arms showed a statistically significant improvement in CFQ-R respiratory domain score in TRAFFIC and TRANSPORT and 3 out of 4 groups showed improvements that would be considered clinically meaningful. This lends support to improved quality of life as measured by this domain through the combined action of Lumacaftor-Ivacaftor.

Based on the available data from the Phase II studies of Lumacaftor monotherapy and Lumacaftor-Ivacaftor therapy, Lumacaftor monotherapy does not only appear to not improve lung function as measured by FEV1% predicted and CFQ-R respiratory domain results, but rather it appears to adversely affect it. Additionally, the study of Lumacaftor monotherapy was not powered towards these ends points and in both of these studies, the treatment administration period for Lumacaftor was only between 14-28 days in duration; longer treatment with Lumacaftor on more patients may rule out the possibility that these results are artifacts.

In contrast, the combination of both Lumacaftor and Ivacaftor appears to show small yet sustained overall benefits for patients with the F508del homozygous patients compared to Lumacaftor alone. Upon administration of combination therapy in the Phase II Lumacaftor-Ivacaftor study, improvements in all groups of patients with the F508del

homozygous mutation were seen for measures of FEV1% predicted (three of which were also statistically significant). Furthermore, improvements of at least 5% in absolute FEV1% predicted change from baseline were seen in 11 out of 20 (55%) subjects receiving Lumacaftor 600mg and Ivacaftor 250mg in Cohort 2, in 5 out of 10 (50%) subjects receiving Lumacaftor 400mg and Ivacaftor 250mg in Cohort 3, compared to only 3 of 24 (13%) of subjects receiving placebo.

Similarly, in TRAFFIC and TRANSPORT, a larger proportion of patients receiving Lumacaftor-Ivacaftor showed a relative improvement in FEV1% predicted of at least 5% compared to placebo. In TRAFFIC, 22 out of 184 (11.9%), 46 out of 183 (25.1%), and 37 out of 182 (20.3%) in the placebo, Lumacaftor 600mg and Ivacaftor 250mg group, and Lumacaftor 400mg and Ivacaftor 250mg group, showed a relative improvement of at least 5% in FEV1% predicted, respectively. In TRANSPORT, 23 out of 186 (12.3%), 46 out of 186 (24.7%), and 41 out of 187 (21.9%) showed at least a 5% relative improvement from baseline in FEV1% in the placebo, Lumacaftor 600mg and Ivacaftor 250mg group, and Lumacaftor 400mg and Ivacaftor 250mg group, respectively.

However, while an absolute improvement in FEV1% predicted of 5% is arguably likely to be clinically meaningful, a relative FEV1% predicted improvement of 5% is a much smaller improvement unlikely to constitute a clinically meaningful improvement. Additionally, despite more patients reaching a 5% improvement in relative FEV1% predicted in treatment arms compared to placebo, the vast majority of patients in these studies did not show this improvement.

The only comparable data from the Phase II studies of Ivacaftor or Lumacaftor monotherapy are from DISCOVER where 28 (63.6%) Ivacaftor-treated patients showed at least a 10% improvement in relative FEV1% predicted as well as 5 out of 6 (83.3%) placebo

subjects who subsequently rolled over to the extension study. However, these improvements in FEV1% predicted could have been seen at any point during the 16-week period while data from Lumacaftor-Ivacaftor trials were measured along fixed endpoints.

2.4.4 Sweat Chloride Concentration

The clinical significance of a reduction of sweat chloride concentration is not fully established. Sweat chloride is a clinical endpoint that has not been found to correlate well with measures of respiratory function such as FEV1% predicted or any other measure of lung function [35]. Nonetheless, there is support for utilizing sweat chloride as a surrogate marker for CFTR channel function [36].

DISCOVER showed a statistically significant treatment effect in Part A of -2.9mmol/L and an increase of 2.2mmol/L in the open-label period, Part B. Reductions in sweat chloride concentration were seen in all Lumacaftor groups in the Phase II Lumacaftor monotherapy clinical trial; the largest of these was a -6.6mmol/L change from baseline that was statistically significant. Meanwhile, the largest reduction for the Phase II study of Lumacaftor-Ivacaftor was in a Cohort 1 group receiving 200mg of Lumacaftor and 250mg of Ivacaftor (-12.6mmol/L, $p<0.001$).

Interestingly, sweat chloride concentration, a measure used in every clinical trial in this review, was not studied as a clinical endpoint in these TRAFFIC and TRANSPORT and was not listed as a clinical endpoint in the study protocol. Recall that the Phase III clinical trials were most heavily considered by regulatory bodies in their decision to approve Orkambi. Furthermore, there is no information that we are aware of as to why sweat chloride was not evaluated in these final and critical studies. As such, inferences on sweat chloride concentration reduction through the action of Lumacaftor-Ivacaftor are limited to one Phase II investigation.

2.4.5 Other Considerations

Throughout our evaluation of clinical trials, we came across several issues which, although not the focus of our analysis, are still relevant.

Firstly, it is important to mention that DISCOVER evaluated Ivacaftor at the 150mg dose while Phase II and Phase III combination therapy studies always used a 250mg dose. It is therefore important to consider that, although 16-weeks of blinded study in DISCOVER did not show any benefit on the F508del homozygous population in terms of clinical efficacy, the results of a 250mg Ivacaftor monotherapy period have never been evaluated. It is important to note that the dose increase when combining Lumacaftor with Ivacaftor may be due to interactions between the two drugs [37], since the effects of monotherapy at one dose might not correlate with the effects of combined therapy at the same dose.

Secondly, during the Phase II study of Lumacaftor-Ivacaftor therapy, CFQ-R respiratory scores during the 28-day monotherapy period showed within-group improvements of 2.9 points in placebo. However, during the combination therapy period, for days 28-56, these scores dropped to -8.6 points in the placebo arm. It is unclear why such a large reduction in patient-reported respiratory scores may have occurred in a relatively short time.

Lastly, it should be noted that the improvements seen in key efficacy endpoints for Orkambi compared to Kalydeco are vastly different. Phase III clinical trials of Kalydeco on patients with the G551D mutation [18-19] showed improvement within-group for absolute FEV1% predicted, sweat chloride concentration, and CFQ-R respiratory domain of, at minimum, 10.4%, -48.7mmol/L, and 5.9 points, respectively. By these trial investigators' accounts, these improvements, especially with respective to FEV1% predicted and sweat chloride concentration, are likely to be considered clinically meaningful. However, the

clinical significance of the much smaller reported improvements seen in these outcomes for patients with the F508del homozygous mutation treated with Lumacaftor-Ivacaftor is not fully clear.

2.5 Conclusion

Following analysis of the clinical trials evaluating Lumacaftor, Ivacaftor, or their combination, we are unable to fully answer the initial question posed at the start of our research; does Lumacaftor in combination with Ivacaftor, appear to meaningfully improve clinical outcomes for CF patients homozygous for the F508del mutation compared to Ivacaftor alone? Based on the results, Lumacaftor-Ivacaftor therapy appears to be superior to Lumacaftor monotherapy. However, based on considerations discussed above, more definitive results across trials that are comparable and adequately powered will be needed to fully distinguish Orkambi from Kalydeco in terms of their clinical benefits for this patient population.

Table 2-1 – F508del Clinical Trial Information		
Trial Name (ID)	**Study Population**	**Outcomes**
Ivacaftor in Subjects With Cystic Fibrosis Who are Homozygous for the F508del-CFTR Mutation (DISCOVER) (NCT00953706)	- F508del/ F508del - 12 years or older	1° - Safety and FEV1% predicted results 2° - Sweat chloride concentration, CFQ-R respiratory domain scores, change in weight 3° - Time to first Pulmonary exacerbation
Results of a phase IIa study of VX-809, an investigational CFTR corrector compound, in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation (NCT00865904)	- F508del/ F508del - 18 years or older	1° - Safety and Tolerability 2° - CFTR function measures (sweat chloride concentration and nasal potential difference), FEV1% predicted results, CFQ-R scores
A CFTR corrector (lumacaftor) and a CFTR potentiator (ivacaftor) for treatment of patients with cystic fibrosis who have a phe508del CFTR mutation: a phase 2 randomised controlled trial (NCT01225211)	- F508del/ F508del - One group of F508del heterozygotes (Cohort 2) - 18 years or older	<u>Cohorts 1, 2, and 3:</u> 1° - Sweat chloride concentration (combination period) and safety 2° - FEV1% predicted results, sweat chloride concentration (monotherapy), Pharmacokinetic analyses <u>Cohort 2, and 3:</u> 2° - CFQ-R Respiratory domain scores
A Study of Lumacaftor in Combination With Ivacaftor in Cystic Fibrosis Subjects Aged 12 Years and Older Who Are Homozygous for the F508del-CFTR Mutation (TRAFFIC) (NCT01807923)	- F508del/ F508del - 12 years or older	1° - FEV1% predicted results (absolute) 2° - FEV1% predicted results (relative), CFQ-R Respiratory domain scores, body-mass index change, percentage of patients with a relative FEV1% improvement of 5%, pulmonary exacerbations
A Study of Lumacaftor in Combination With Ivacaftor in Cystic Fibrosis Subjects Aged 12 Years and Older Who Are Homozygous for the F508del-CFTR Mutation (TRANSPORT) (NCT01807949)	- F508del/ F508del - 12 years or older	1° - FEV1% predicted results (absolute) 2° - FEV1% predicted results (relative), CFQ-R Respiratory domain scores, body-mass index change, percentage of patients with a relative FEV1% improvement of 5%, pulmonary exacerbations

Table 2-2 – FEV1% Predicted Results from F508del Trials				
	Mean Absolute Change From Baseline		Mean Relative Change From Baseline	
Study	**Within-Group (95% CI)**	**Treatment Effect (95% CI)**	**Within-Group (95% CI)**	**Treatment Effect (95% CI)**
DISCOVER				
Part A				
IVA (150mg)	1.5%	1.7% (-0.6, 4.1) (p=0.15)	2.4% (-0.9, 5.8) (p=0.16)	
Placebo	-0.2%	--		--
Part B				
IVA (150mg)	-3.5% (SD: 11.7)	--		--
Phase II Lumacaftor				
LUM (25mg)			0.07%	
LUM (50mg)			-2.46%	
LUM (100mg)			-2.15%	
LUM (200mg)			0.32%	
Placebo		--	0.47%	--
Phase II Lumacaftor-Ivacaftor				
Cohort 1				
Day 1-14				
LUM (200mg)	-0.3 (-2.4, 1.7) (p=0.74)	-2.1 (-4.8, 1.7) (p=0.010)		
LUM (200mg)	-0.1 (-2.1, 2.0) (=0.96)	-2.2 (-4.7, 1.1) (p=0.12)		
Placebo	1.7 (-0.2, 3.6) (p=0.076)	--		--
Day 14-21				
LUM (200mg) + IVA (150mg)	3.5 (0.9, 6.1) (p=0.010)	4.9 (1.4, 8.4) (p=0.007)		
LUM (200mg) + IVA (250mg)	0.6 (-2.2, 3.5) (p=0.66)	2.1 (-1.8, 5.9) (p=0.28)		
Placebo	-1.4 (-3.9, 1.1) (p=0.24)	--		--
Day 1-21				
LUM (200mg) + IVA (150mg)	3.1 (0.1, 6.1) (p=0.047)	2.8 (-1.3, 7.0) (p=0.18)		
LUM (200mg) + IVA (250mg)	0.5 (-2.8, 3.8) (p=0.76)	0.3 (-4.2, 4.7) (p=0.91)		
Placebo	0.3 (-2.6, 3.1) (p=0.86)	--		--
Cohorts 2 & 3				
Day 1-28				
LUM (200mg)	0.2 (-2.8, 3.2) (p=0.89)	0.2 (-3.7, 4.2) (p=0.91)	0.2 (-4.3, 4.8) (p=0.92)	
LUM (400mg)	-1.4 (-4.4, 1.7) (p=0.38)	-1.2 (-5.8, 3.5) (p=0.62)	-1.2 (-5.8, 3.5) (p=0.62)	
LUM (600mg)	-2.6 (-5.7, 0.4) (p=0.091)	-2.6 (-6.7, 1.5) (p=0.21)	-3.1 (-7.7, 1.5) (p=0.18)	
*LUM (400mg)	-4.5 (-8.7, -0.4) (p= 0.032)	-4.5 (-8.7, -0.4) (p=0.032)	-6.4 (-12.7, -0.1) (p=0.045)	
Placebo	0.0 (-2.7, 2.6) (p=0.99)	--	1.9 (-2.1, 5.9) (p=0.35)	--

**LUM (600mg)	-3.8 (-7.0, -0.6) (p=0.020)	-3.8 (-7.0, -0.6) (p=0.076)	-5.5 (-10.3, -0.6) (p=0.03)	
Day 28-56				
LUM (200mg) + IVA (250mg)	2.0 (-0.8, 4.8) (p=0.17)	3.5 (-0.3, 7.4) (p=0.072)	3.1 (-1.3, 4.9) (p=0.16)	
LUM (400mg) + IVA (250mg)	2.0 (-0.9, 4.8) (p=0.17)	3.6 (-0.4, 7.5) (p=0.074)	3.0 (-1.5, 7.5) (p=0.19)	
LUM(600mg) + IVA (250mg)	6.2 (3.3, 9.0) (p<0.001)	7.7 (3.8, 11.7) (p<0.001)	9.7 (5.2, 14.2) (p<0.001)	
LUM(400mg) + IVA (250mg)	6.1 (2.0, 10.2) (p=0.004)	7.7 (2.7, 12.6) (p=0.003)	8.2 (1.8, 14.7) (p=0.01)	
Placebo	-1.6 (-4.2, 1.1) (p=0.25)	--	-2.1 (-6.3, 2.2) (p=0.34)	--
**LUM (600mg) + IVA (250mg)	2.3 (-0.8, 5.4) (p=0.15)	3.9 (-0.3, 8.0) (p=0.067)	4.3 (-0.6, 9.2) (p=0.084)	
Day 1-56				
LUM (200mg) + IVA (250mg)	1.8 (-1.3, 4.9) (p=0.25)	3.8 (-0.4, 8.1) (p=0.077)	2.5 (-2.2, 7.2) (p=0.29)	
LUM (400mg) + IVA (250mg)	0.6 (-2.5, 3.8) (p=0.69)	2.7 (-1.7, 7.0) (p=0.23)	1.7 (-3.1, 6.5) (p=0.48)	
LUM (600mg) + IVA (250mg)	3.6 (0.4, 6.8) (p=0.027)	5.6 (1.2, 10.0) (p=0.013)	5.6 (0.7, 10.4) (p=0.025)	
LUM (400mg) + IVA (250mg)	2.2 (-2.3, 6.7) (p=0.34)	4.2 (-1.3, 9.6) (p=0.13)	3.0 (-3.9, 9.8) (p=0.39)	
Placebo	-2.0 (-5.0, 0.9) (p=0.18)	--	-2.4 (-6.9, 2.1) (p=0.29)	--
**LUM (600mg) + IVA (250mg)	-1.7 (-5.1, 1.8) (p=0.33)	0.3 (-4.2, 4.9) (p=0.89)	-2.3 (-7.6, 2.9) (p=0.38)	
TRAFFIC				
LUM (600mg) + IVA (250mg)	3.6 (p<0.001)	4.0 (p<0.001)	6.4 (p<0.001)	6.7 (p<0.001)
LUM (400mg) + IVA (250mg)	2.2 (p<0.001)	2.6 (p<0.001)	4.0 (p<0.001)	4.3 (p<0.001)
Placebo	-0.44 (p=0.40)	--	-0.34 (p=0.71)	--
TRANSPORT				
LUM (600mg) + IVA (250mg)	2.5 (p<0.001)	2.6 (p<0.001)	4.4 (p<0.001)	4.4 (p<0.001)
LUM (400mg) + IVA (250mg)	2.9 (p<0.001)	3.0 (p<0.001)	5.3 (p<0.001)	5.3 (p<0.001)
Placebo	-0.15 (p=0.77)	--	0.0 (p=0.10)	--
* Cohort 3 Group which received Lumacaftor 400mg every 12 hours				
** Cohort 2 Heterozygous F508del group				

Table 2-3 – Sweat Chloride Concentration Results from F508del Trials		
	Mean Absolute Change From Baseline (mmol/L)	
Study	**Within-Group (95% CI)**	**Treatment Effect (95% CI)**
DISCOVER		
Part A		
IVA (150mg)	--	-2.9 (-5.6, -0.2) (p=0.04)
Placebo	--	--
Part B		
IVA (150mg)	2.2 (SD: 12.2)	--
Phase II Lumacaftor		
LUM (25mg)	-0.5	0.1
LUM (50mg)	-3.7 (-7.1, -0.28) (p=0.03)	-4.6
LUM (100mg)	-2.3	-6.13 (-12.3, -0.01) (p<0.05)
LUM (200mg)	-6.6 (-10.3, -2.8) (p=0.0008)	-8.21 (-14.3, -2.10) (p<0.01)
Placebo	2.2	
Phase II Lumacaftor-Ivacaftor		
Cohort 1		
Day 1-14		
LUM (200mg)	-4.8 (-8.6, 1.0) (p=0.02)	-3.1 (-8.7, 2.4) (p=0.26)
LUM (200mg)	-4.1 (-8.1, -0.1) (p=0.046)	-2.4 (-8.0, 3.2) (p=0.39)
Placebo	-1.7 (-5.6, 2.3) (p=0.41)	--
Day 14-21		
LUM (200mg) + IVA (150mg)	-2.1 (-5.4, 0.9) (p=0.19)	-2.7 (-7.5, 2.1) (p=0.27)
LUM (200mg) + IVA (250mg)	-9.1 (-12.9, -5.4) (p<0.001)	-9.7 (-14.8, -4.6) (p<0.001)
Placebo	0.5 (-3.0, 4.1) (p=0.75)	--
Day 1-21		
LUM (200mg) + IVA (150mg)	-6.7 (-11.1, -2.4) (p=0.003)	-5.0 (-11.6, 1.5) (p=0.13)
LUM (200mg) + IVA (250mg)	-12.6 (-17.2, -7.9) (p<0.001)	-10.9 (-17.6, -4.2) (p=0.002)
Placebo	-1.7 (-6.5, 3.1) (p=0.48)	--
Cohorts 2 & 3		
Day 1-28		
LUM (200mg)	-4.7 (-8.1, -1.4) (p<0.007)	-4.8 (-9.3, -0.2) (p=0.041)
LUM (400mg)	-8.2 (-11.7, -4.6) (p<0.001)	-8.2 (-12.9, -3.6) (p<0.001)
LUM (600mg)	-6.0 (-9.5, -2.5) (p<0.001)	-6.0 (-10.7, -1.4) (p=0.01)
*LUM (400mg)	-8.4 (-13.3, -3.4) (p=0.001)	-8.4 (-14.3, -2.6) (p=0.005)
Placebo	0.0 (-3.0, 3.1) (p=0.98)	--
**LUM (600mg)	-4.0 (-7.6, -0.3) (p=0.034)	-4.0 (-8.8, 0.8) (p=0.10)
Day 28-56		
LUM (200mg) + IVA (250mg)	0.3 (-4.2, 4.9) (p=0.89)	-1.3 (-7.6, 5.0) (p=0.68)
LUM (400mg) + IVA (250mg)	-1.0 (-5.8, 3.7) (p=0.66)	-2.7 (-9.1, 3.7) (p=0.41)
LUM (600mg) + IVA (250mg)	-2.9 (-7.5, 1.7) (p=0.22)	-4.5 (-10.9, 1.8) (p=0.16)
LUM (400mg) + IVA (250mg)	-2.2 (-9.2, 4.9) (p=0.54)	-3.8 (-12.0, 4.5) (p=0.37)
Placebo	1.6 (-2.7, 5.9) (p=0.45)	--
**LUM (600mg) + IVA (250mg)	-1.2 (-6.3, 3.8) (p=0.63)	-2.9 (-9.5, 3.8) (p=0.40)
Day 1-56		
LUM (200mg) + IVA (250mg)	-4.4 (-8.5, -0.3) (p=0.035)	-5.12 (-10.7, 0.5) (p=0.072)
LUM (400mg) + IVA (250mg)	-9.1 (-13.3, -4.9) (p<0.001)	-9.8 (-15.5, -4.2) (p<0.001)
LUM (600mg) + IVA (250mg)	-8.9 (-13.1, -4.7) (p<0.001)	-9.6 (-15.3, -4.0) (p=0.001)
LUM (400mg) + IVA (250mg)	-10.3 (-16.7, -4.0) (p=0.002)	-11.1 (-18.5, -3.7) (p=0.004)
Placebo	0.7 (-3.1, 4.5) (p=0.70)	--
**LUM (600mg) + IVA (250mg)	-5.2 (-9.8, -0.7) (p=0.025)	-6.0 (-11.9, -0.0) (p=0.05)
* Cohort 3 Group which received Lumacaftor 400mg every 12 hours		
** Cohort 2 Heterozygous F508del group		

Table 2-4 – CFQ-R Respiratory Domain Score from F508del Trials		
	Mean Absolute Change From Baseline (points)	
Study	**Within-Group (95% CI)**	**Treatment Effect (95% CI)**
DISCOVER		
Part A		
IVA (150mg)	-0.1	
Placebo	-1.4	--
Part B		
IVA (150mg)		--
Phase II Lumacaftor		
LUM (25mg)	-5.2	-9.8 (p<0.05)
LUM (50mg)	-6.3 (p<0.05)	-10.9 (p<0.05)
LUM (100mg)	-1.3	-5.8
LUM (200mg)	2.2	-2.3
Placebo	4.5	--
Phase II Lumacaftor-Ivacaftor		
Cohorts 2 & 3		
Day 1-28		
LUM (200mg)	5.2 (-1.5, 12.0) (p=0.13)	2.3 (-6.7, 11.3) (p=0.61)
LUM (400mg)	-2.3 (-9.3, 4.6) (p=0.51)	-5.3 (-14.4, 3.9) (p=0.26)
LUM (600mg)	-9.5 (-16.4, -2.6) (p=0.007)	-12.4 (-21.6, -3.3) (p=0.008)
*LUM (400mg)	-8.8 (-18.1, 0.5) (p=0.065)	-11.7 (-22.8, -0.6) (p=0.040)
Placebo	2.9 (-3.1, 8.9) (p=0.34)	--
**LUM (600mg)	-9.9 (-17.2, -2.7) (p=0.008)	-12.8 (-22.3, -3.4) (p=0.008)
Day 28-56		
LUM (200mg) + IVA (250mg)	3.3 (-3.6, 10.2) (p=0.35)	11.8 (2.5, 21.2) (p=0.013)
LUM (400mg) + IVA (250mg)	7.9 (0.8, 14.9) (p=0.030)	16.4 (6.9, 26.0) (p<0.001)
LUM (600mg) + IVA (250mg)	8.9 (1.9, 15.9) (p=0.014)	17.4 (7.9, 27.0) (p<0.001)
LUM (400mg) + IVA (250mg)	11.2 (1.3, 21.1) (p=0.028)	19.8 (7.9, 31.6) (p=0.001)
Placebo	-8.6 (-14.9, -2.2) (p=0.009)	--
**LUM (600mg) + IVA (250mg)	5.5 (-2.1, 13.1) (p=0.15)	14.1 (4.1, 24.1) (0.006)
Day 1-56		
LUM (200mg) + IVA (250mg)	7.9 (0.5, 15.3) (p=0.037)	15.9 (5.8, 26.0) (p=0.002)
LUM (400mg) + IVA (250mg)	5.5 (-2.2, 13.2) (p=0.16)	13.5 (3.2, 23.9) (p=0.011)
LUM (600mg) + IVA (250mg)	-0.9 (-8.5, 6.7) (p=0.81)	7.1 (-3.3, 17.4) (p=0.18)
LUM (400mg) + IVA (250mg)	4.0 (-6.8, 14.8) (p=0.46)	12.0 (-0.8, 24.9) (p=0.066)
Placebo	-8.0 (-14.9, -1.1) (p=0.023)	--
**LUM (600mg) + IVA (250mg)	-4.9 (-13.2, 3.4) (p=0.24)	3.1 (-7.7, 13.9) (p=0.57)
TRAFFIC		
LUM (600mg) + IVA (250mg)	5.0 (p<0.001)	3.9 (p=0.02)
LUM (400mg) + IVA (250mg)	2.6 (p=0.03)	1.5 (p=0.36)
Placebo	1.1 (p=0.34)	--
TRANSPORT		
LUM (600mg) + IVA (250mg)	5.0 (p<0.001)	2.2 (p=0.17)
LUM (400mg) + IVA (250mg)	5.7 (p<0.001)	2.9 (p=0.07)
Placebo	2.8 (p=0.02)	--
* Cohort 3 Group which received Lumacaftor 400mg every 12 hours		
** Cohort 2 Heterozygous F508del group		

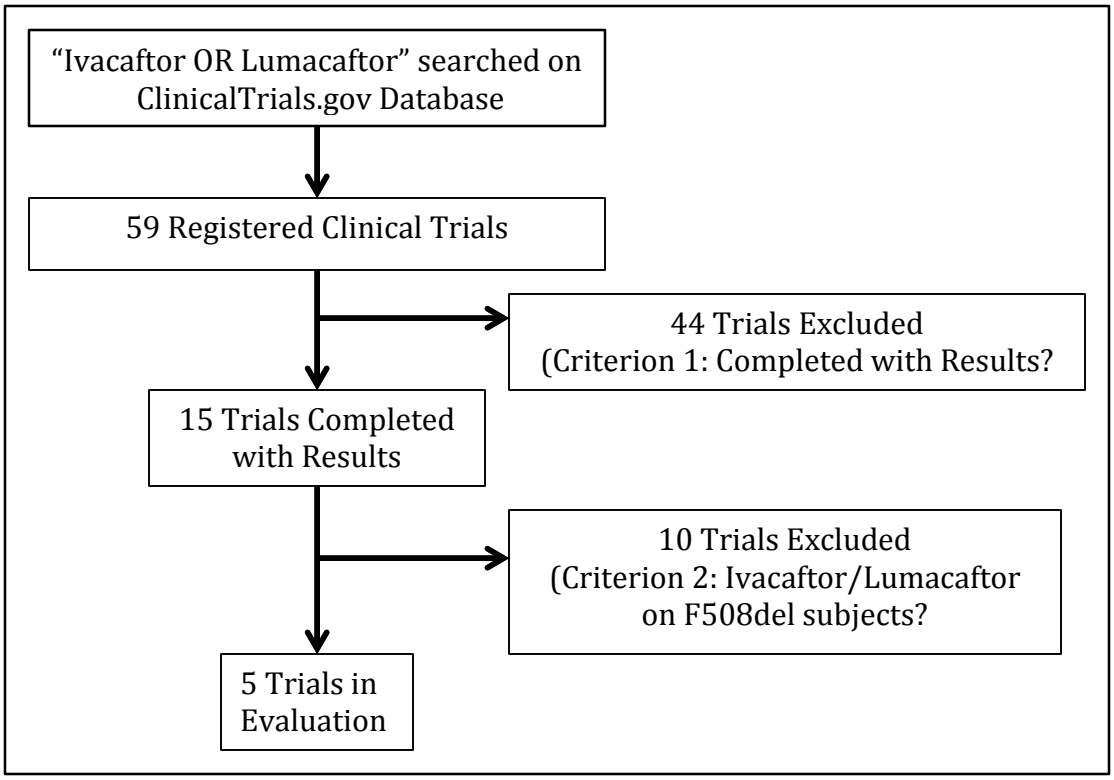

Figure 2-1: Flow Diagram of Trials Included In Study

DISCOVER

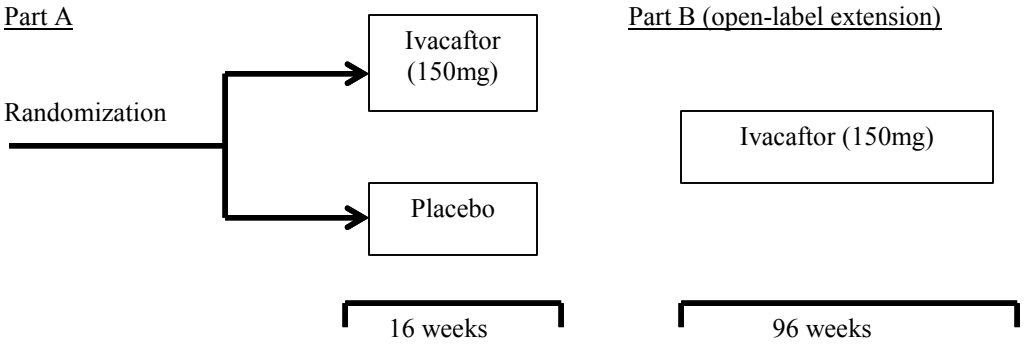

Figure 2-2 – Study Design of Clinical Trial DISCOVER. Note: DISCOVER was a Phase II Investigation of Ivacaftor Monotherapy on Patients Homozygous for the F508del Mutation.

Phase II: Lumacaftor Monotherapy

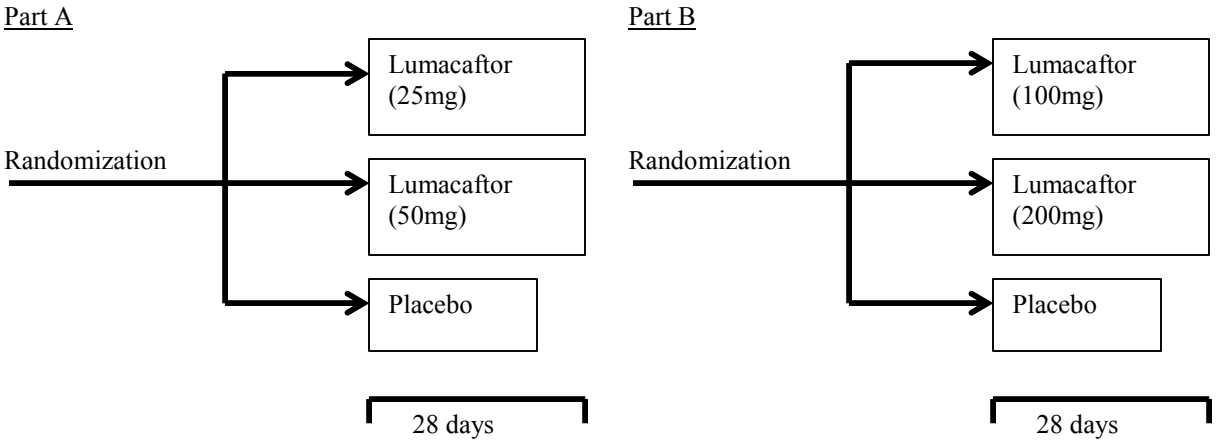

Figure 2-3 –Phase II Clinical Trial of Lumacaftor Monotherapy.

Phase II Lumacaftor-Ivacaftor Therapy

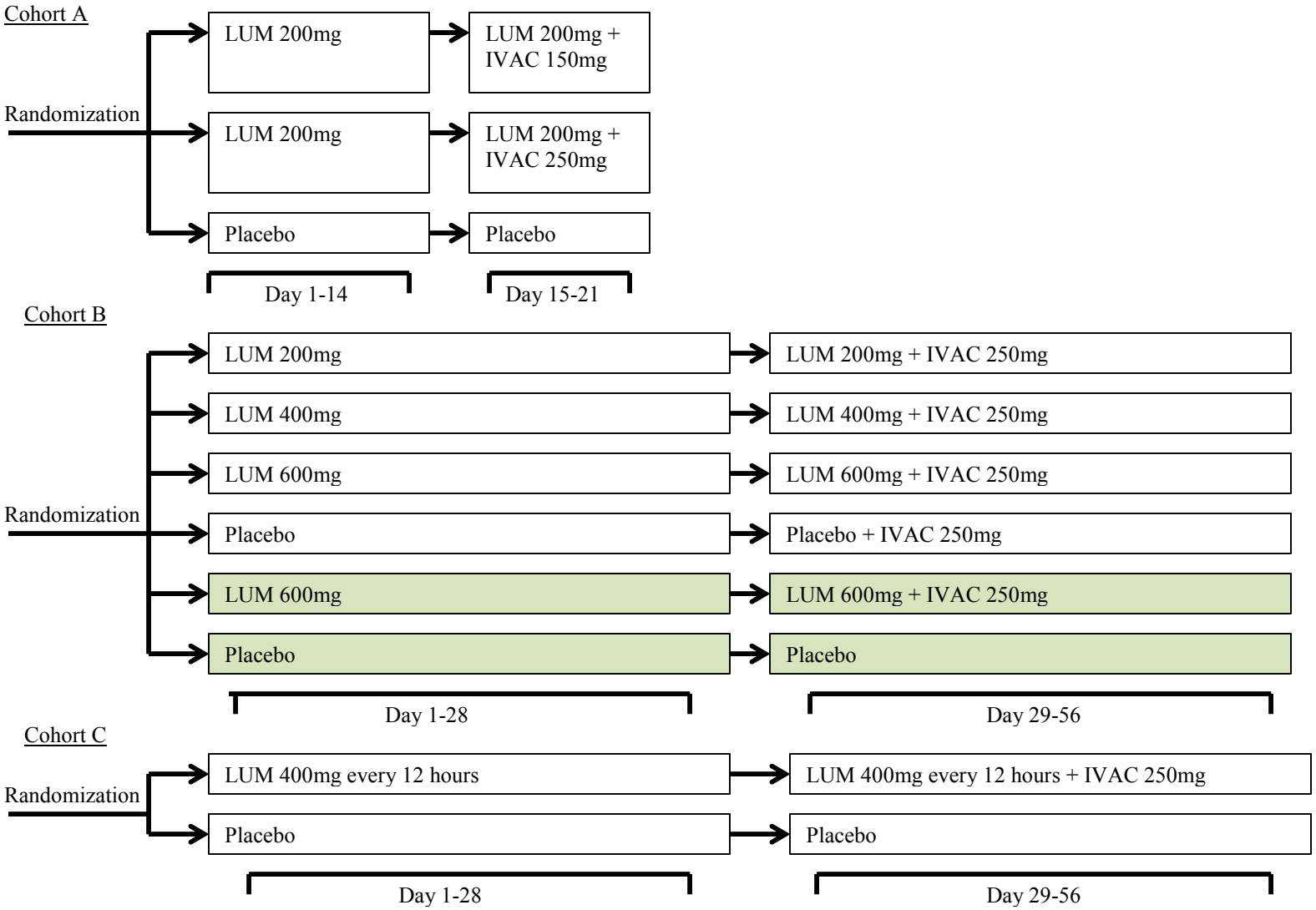

Figure 2-4 – Phase II Clinical Trial of Lumacaftor-Ivacaftor Therapy. Note: LUM and IVAC represent Lumacaftor and Ivacaftor, respectively. Unless indicated otherwise, Lumacaftor therapy was administered once daily and Ivacaftor was administered once every 12 hours. Light green boxes indicate a group of patients with one F508del allele.

TRAFFIC/TRANSPORT

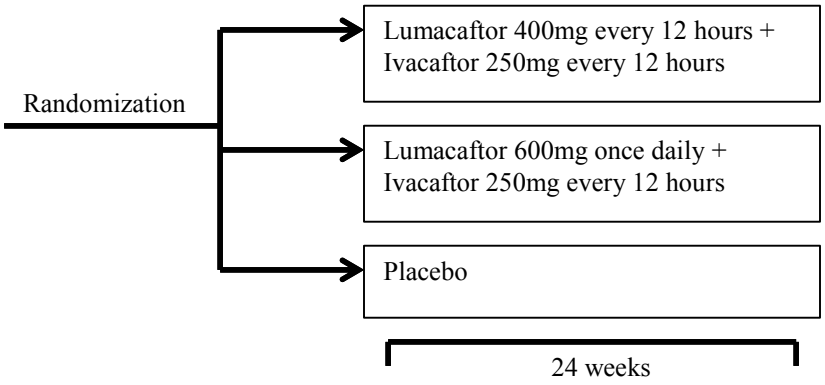

Figure 2-5 – Clinical Trial Design of TRAFFIC and TRANSPORT.

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CHAPTER 3

Gaps in Cystic Fibrosis Treatment Which Existed When Kalydeco First Received Regulatory Approval and Where We Stand Today

3.1 Introduction

Ivacaftor, trade named Kalydeco, is a therapeutic agent for the treatment of Cystic Fibrosis (CF) which first received regulatory approval in 2012 by the United States Food and Drug Administration (FDA) [1], followed by the European Medicines Agency (EMA) [2], Health Canada [3] and other regulatory bodies. Vertex Pharmaceuticals, the developer of the drug, priced Kalydeco at approximately $300,000 USD per patient per year [4]. The high price is attributed to, among other things, the cost of development which took 14 years of internally-funded research [5]. Recently, regulatory approval was granted to a second drug, Lumacaftor-Ivacaftor (Orkambi) [6-7], priced at approximately $259,000 USD per patient per year [8].

Kalydeco was initially approved for CF patients with the G551D mutation, present on at least one allele in approximately 4% of patients [9]. Meanwhile, Orkambi is aimed at treating patients homozygous for the F508del mutation, responsible for approximately half of all observed CF cases [9-11]. There has been concern regarding the regulatory approval of these therapeutic agents on at least two fronts: 1) the high cost of treatments and the impact this will have on providing cost-effective healthcare to patients and the societal impact on the healthcare system; and 2) the relative amount of clinical benefits seen among CF patients receiving these treatments [12].

Moreover, during its initial approval for patients with the G551D mutation, the majority of the CF patient population did not have access to treatment options, such as

Kalydeco. Even among patients with the G551D mutation, there were certain subpopulations, such as pediatric patients, for whom no data regarding the clinical benefits of Ivacaftor existed, given that clinical trials were conducted on patients that met more specific eligibility criteria. In light of the recent approval of Orkambi, which now makes treatment options available to a much larger patient population, we felt it would be important to determine if and how the generation of evidence regarding the benefit of these therapies has evolved over time, and whether important data gaps were addressed.

This paper has several aims: 1) to provide a list of key gaps that existed at the time Kalydeco was initially approved by regulatory bodies; and 2) to address, based on clinical trial information, consultation with stakeholders and experts, as well as a survey of regulatory body documents and other relevant literature, which key gaps have been addressed and to what degree. Findings from this work can be useful to determine which gaps remain in the treatment of patients eligible to receive Kalydeco, and what can be expected for emerging therapies such as Orkambi.

3.2 Methods

Our research methodology consisted of two main steps: 1) Derivation of a list of gaps; and 2) Determination of which of the gaps have or have not been filled (Figure 3-1).

3.2.1 Step 1 – Deriving a List of Gaps

We began by reviewing relevant critical trials pertaining to Kalydeco's first regulatory approval. In order to determine which clinical trials were relevant, we turned to approval body evaluations conducted by the FDA, EMA and Health Canada, and included only those clinical trials that were part of their initial approval decision evaluations for Kalydeco. Clinical trial information was subsequently accessed through clinicaltrials.gov by inserting trial identification numbers (obtained from the approval body documents) in the search bar.

Information accessed on the clinicaltrials.gov website contained clinical trial registration information as well as references to relevant publications. All background information, relevant publications, including supplementary materials and protocols of clinical trials, were reviewed.

Clinical trials were initially studied using CONSORT (Consolidated Standards of Reporting Trials) [13], which aims to improve the quality of reporting of randomized controlled trials. Specifically, the CONSORT checklist was used as a guide to determine areas where the reporting of clinical trial conduct was not fully clear and to evaluate any weaknesses in the general clinical trial conduct.

Next, approval body documents for the FDA and EMA were accessed using their respective websites and searching them by using search terms "Ivacaftor" and "Kalydeco". All approval body documents for Kalydeco pertaining to its initial approval (2012 for United States FDA and EMA, 2014 for Health Canada) were reviewed. Approval body documents were used because they contain information pertaining to clinical trials that may otherwise be been inaccessible by only studying the clinical trial publications, such as certain data given by the clinical trial investigators to the regulatory bodies and appraisals conducted by regulatory body experts, such as statisticians and clinicians.

Using both the CONSORT evaluation results and regulatory body documents, an initial list of gaps was created by the lead investigator, SM. A gap was generally defined as any lack of knowledge pertaining to Kalydeco which may be considered substantial. Specifically, issues surrounding clinical endpoints used in clinical trials, uncertainty surrounding the clinical benefit of Kalydeco, and discussions of cost-effectiveness were key points of focus when creating a gap.

After creating of a list of gaps, a short-list of gaps was finalized. This was done

through discussions with members of the PRISM (Promoting Rare Disease Innovation through Sustainable Mechanisms) workgroup, which aims to improve decision-making regarding the development, introduction and funding of treatments for rare diseases, and Dr. Neil Brown, a respirologist with substantial CF expertise, Dr. Brown was also involved in patient care for CF patients through centers in the province of Alberta in Canada that contributed CF patients to some of the clinical trials in the study and provided clinical expertise and study-specific insight that may otherwise have been inaccessible. The determination of which list of gaps should be short-listed for further study was based on those gaps, which if filled, would have the largest CF-patient and societal impact.

3.2.2 Step 2 – Evaluation of Gaps

The evaluation of gaps was aimed to see which gaps that were short-listed during Step 1 had been filled up until the initial regulatory approval of Orkambi. For this reason, regulatory documents from those bodies where Orkambi had been approved were reviewed. Firstly, this included the main regulatory body approval document from the FDA and EMA for Orkambi. This further included all documents that had been released on the FDA and EMA websites since the regulatory approval of Kalydeco and up until the regulatory approval for Orkambi. These latter documents generally pertained to the approval of Kalydeco for new groups of patients. All clinical trials mentioned in these documents were subsequently accessed using their identification numbers on clinicaltrials.gov, and all content, including relevant publications, supplementary materials, and study protocols, were accessed.

The final evaluation of gaps involved required using information from three sources. Firstly, just as in Step 1, all clinical trials were evaluated using the CONSORT checklist with special attention given to the short-list of gaps. Secondly, all approval body documents that had been released by the FDA and EMA were studied, particularly in relation to the key

gaps. Thirdly, a general survey of existing literature specific to the list of gaps was conducted through the PubMed database. For example, if a gap pertained to a clinical endpoint, the PubMed database would be searched for the clinical endpoint in relations to CF. Using available information from all three sources, the study investigators conducted an evaluation of the gaps to determine the extent to which they had been filled.

3.3. Results

3.3.1 Relevant Studies and List of Gaps

Three studies were most heavily relied on in the original regulatory approval of Kalydeco; one Phase II study on adult patients aimed at evaluating the safety and adverse-event profile of Ivacaftor [14], and two Phase III studies evaluating the efficacy of Ivacaftor in patients aged 12 and older (STRIVE) [15] and aged 6 and older (ENVISION) [16].

Based on CONSORT evaluations of these three clinical trials, approval body documents, PRISM workgroup discussions, and a discussion with a CF expert, Dr. Neil Brown, a short-list of 4 key gaps that existed at the time Kalydeco was initially approved were finalized.

Since the initial approval of Kalydeco until the initial approval of Orkambi, at least ten clinical trials were conducted and evaluated in regulatory body decisions to extend CF treatment to more patients; six clinical trials of Ivacaftor [17-22], one clinical trial of Lumacaftor [23], and 3 clinical trials of Lumacaftor-Ivacaftor [24-25]. All of these clinical trials were reviewed along with relevant approval body documents and existing literature to derive conclusions about the list of 4 gaps (see Figure 3-2 for a summary)

3.3.2 Gap 1 – Extension of treatment options beyond G551D

CF is caused by mutations in the gene encoding the Cystic Fibrosis Transmembrane Regulatory (CFTR) [26], an ion channel responsible for salt and water transport through the

epithelia of organs, such as the lungs [27]. CFTR mutations have historically been categorized into a Class nomenclature [28]. The G551D mutation is a Class III gating mutation, where CFTR fails to open normally when activated [28-29]. Ivacaftor, works mechanistically through potentiation, thereby prolonging channel opening following activation. Theoretically, the action of a potentiator should benefit other mutations categorized as Class III [30]. Additionally, even for mutations that are not categorized as Class III, there may be ion channel defects that could be improved through potentiation. A major gap, then, at the time Kalydeco was approved, existed in the need to test Ivacaftor on other CF sub-populations that could potentially benefit from its use.

Since the initial approval of Kalydeco for the G551D mutation, treatment options have been expanded to other CF populations. Firstly, Kalydeco received regulatory approval [31] for use in 9 non-G551D Class III gating mutations which account for approximately 1% of CF patients [32]: G178R, G551S, S549N, S549R, G970R, G1244E, S1251N, S1255P, or G1349. Additionally, Kalydeco was approved for the treatment of the R117H mutation which is present in approximately 3-4% of CF patients [9, 30]. Although categorized as Class IV, some patients with the R117H mutation nonetheless show some gating defects [33]. Lastly, although Ivacaftor showed little/no benefit in patients homozygous for the F508del mutation [18], it was combined with a second "corrector" compound, Lumacaftor. Corrector's work by increasing the amount of CFTR present at the cell surface [28]. The combination of Lumacaftor-Ivacaftor was deemed to provide sufficient benefit in patients homozygous for the F508del mutation, resulting in the regulatory approval of Orkambi for the treatment of these patients [34-35]. Due to the approval of Orkambi, targeted therapeutic options are now available for approximately half of the existing CF population.

Given that there are over 2,000 mutations in CFTR [36], one major gap in the

treatment of CF, in the context of our study, has been the expansion of these treatment options for more CF sub-populations, especially patients homozygous for the F508del mutation. Questions of clinical efficacy aside, the availability of treatment options for the large subset of the CF population has filled a major gap which existed at the time Kalydeco was first approved.

3.3.3 Gap 2 – Limited Information in Certain G551D Populations

Kalydeco was initially approved for patients with the G551D mutation over the age of 6. However, there existed numerous G551D sub-populations for which little information regarding clinical efficacy was available. Firstly, there was no information about the benefit of Kalydeco in patients under the age of 6 or over the age of 65. Although clinical trials were conducted in patients over 6, patients over the age of 65 were not evaluated. Also, eligibility criteria for inclusion in clinical trials required that FEV1% predicted values be within a certain range (40-90% FEV1% predicted in STRIVE, and 40-105% FEV1% predicted in ENVISION), while FEV1% predicted ranges for patients with one G551D allele can theoretically span 25-128% [37]. Other groups of patients not eligible in clinical trials included pregnant and lactating women. A full list of inclusion/exclusion criteria can be found in the study protocols of these trials.

Secondly, according to the Canadian Drug Expert Committee (CDEC), approximately 25% of patients with the G551D mutation in clinical trials did not show a percent predicted improvement of at least 5% in a key measure of lung function [38], and the primary efficacy endpoint for clinical trials, percent predicted forced expiratory volume in 1 second (FEV1% predicted).

With respect to the first point, a Phase III clinical trial, KIWI, was conducted on patients aged 2-5 years [21]. The study results supported the safety and efficacy of Ivacaftor

in this patient population and subsequently led to the approval of Kalydeco at a lower dose for pediatric patients aged 2-5 years old with the G551D mutation or other Class III gating mutations. Ivacaftor in patients under 2 years of age or in the population over 65 years of age have not been evaluated. Also, the inclusion/exclusion criteria in clinical trials means groups of patients like pregnant women or those with FEV1% values below or above a certain threshold have never been studied.

With respect to the second point, 25% of patients with the G551D mutation who did not show an absolute improvement of at least 5% in FEV1% predicted in clinical trials may be labeled, by some accounts, as non-responders. It is important to note that it is unclear whether a 5% improvement is clinically meaningful, since there is no established minimal clinically important difference (MCID) for FEV1% predicted [39]. However, improvement in lung function by even a few percentage points may arguably clinically benefit CF patients; one study found that patients can have progressive lung function loss of as much -3.89% (± 4.11%) per year [40]. However, there exists little information as of yet about the characteristics of those patients classified as non-responders, according to an FEV1% predicted response of 5% criterion, signaling the need to determine appropriate discontinuation criteria to identify non-responders [38], especially in order to aid resource utilization.

One study showed that majority of patients with clinical characteristics similar to those in STRIVE/ENVISION could potentially benefit from Kalydeco [41]. Despite this, although clinical trials have shown benefit of Ivacaftor in patients aged 2-5 with the G551D mutation, there still exist populations of patients (<2 years, 65+ years, potential non-responders, those not meeting eligibility criteria) for whom little or no data exists regarding Ivacaftor's benefit. As such, although this gap has been mitigated, it still remains to be fully

filled.

3.3.4 Gap 3 – Usefulness of Sweat Chloride Concentration Reductions

Sweat chloride concentration was initially proposed as the primary efficacy endpoint in clinical trials evaluating Ivacaftor, but the FDA advised against its use since it was not a fully established clinical endpoint for CF [42]. Sweat chloride testing continues to be the gold standard of CF diagnosis [43] with diagnostic cut-off values that are generally agreed upon [44]. It was not until initial clinical trials of Ivacaftor, however, that sweat chloride concentration began to be used as a clinical endpoint and biomarker for CFTR activity. There are at least two points of contention with respect to sweat chloride concentration in this regard: 1) the reliability and validity of sweat chloride concentration as a biomarker of CFTR activity; and 2) the translation of sweat chloride concentration reduction changes to clinical benefits. Arguably, the use of sweat chloride concentration during initial evaluations of Ivacaftor presented some uncertainty regarding the meaningfulness of results, and thus was an important gap in our evaluation.

In general, the validity of sweat chloride as a biomarker of CFTR function has been demonstrated but the reliability of this measure is not fully supported [45]. For example, one study focusing on the variability of sweat chloride concentration among patients with the G551D mutation found a within-subject standard deviation of 8.1mmol/L (95% CI 7.5-8.7) [46]. To put this in perspective, a recent Phase II clinical trial evaluating Lumacaftor-Ivacaftor set its power threshold for sweat chloride reduction detection to 10-16mmol/L. A reduction of 10mmol/L is close to the within-subject standard deviation of sweat chloride concentration by some accounts.

Furthermore, according to both United States FDA and EMA evaluations [47-48] and existing literature [49-50], changes in sweat chloride concentration have not correlated

well with important measures that are considered clinically relevant, such as FEV1% predicted. One study, however, using data from STRIVE and ENVISION, demonstrated that changes in sweat chloride concentration showed some predictive potential in identifying individuals that showed improvements in pulmonary function [51]. The study found that sweat chloride concentration reductions after 15 days of treatment had a positive predictive value of 86.3%, negative predictive value of 65.5%, a sensitivity of 73.9% and a specificity of 80.9% for an FEV1% predicted improvement of 5% or greater from baseline to week 16.

Despite having promise and support for use as a biomarker for CFTR activity, there exists considerable uncertainty regarding the clinical significance of sweat chloride concentration. As such, this gap has gone largely unfilled since Kalydeco's initial approval.

3.3.5 Gap 4 – Cost-Effectiveness of Kalydeco

Initially priced at $294,000 USD per patient per year, costs of Kalydeco subsequently increased to $311,000 USD with individual patient charges reaching up to an estimated $373,000 USD per year [52]. To increase patient access to Kalydeco, Vertex Pharmaceuticals made Kalydeco free to uninsured patients in the United States with household incomes less than $150,000 [53], helped with up to 30% of copayments for some patients with insurance [53], and even offered free treatment for a small number of patients in the UK [54]. Despite this, legitimate concerns regarding the cost of Kalydeco have been raised.

Firstly, initial estimates for the 320 or so eligible patients with the G551D mutation in the UK put the annual cost of Kalydeco as much as £55-60 million [54, 55]. To put this in context, the UK's annual healthcare budget for all CF patients is approximately £110 million [55]. Given that the treatment will need to be taken throughout a patients lifetime, the estimated cost for the entire eligible cohort of G551D patients in the UK could be £438-479 million based on some accounts [56].

Several issues pertaining to the cost of Kalydeco have been raised. Kalydeco was developed through what is being coined "venture philanthropy" [54], specifically the partnership between the CF foundation and Vertex Pharmaceuticals. In 2000, this charity invested in drug development for CF and as of 2015, had received a $3.3 billion return on their initial investment of $150 million from sales of Kalydeco [57]. This, in turn has spurred debate about the ethics of such high levels of return for a non-profit foundation as well as societal considerations such as the sustainability of such a model for personalized drug development, especially for orphan diseases [54, 57, 58]. Additionally, there has been a lack of transparency about how the cost for Kalydeco was determined. As one author put it, "Is this legitimate profit or a huge gravy train"? [53].

Secondly, there are concerns about the cost-effectiveness of Kalydeco, especially when compared to other CF treatments. Improvements in outcomes like FEV1% predicted, and frequency of pulmonary exacerbations are similar to those seen with rhDNAse [59], hypertonic saline [60], and azithromycin [61, 62], except Kalydeco costs substantially more [55]. The "wow-factor" with Ivacaftor treatment is that it targets the underlying defect in CF and represents a breakthrough which may one day lead to a cure, but is currently by no means curative [55].

According to the CDEC, with respect to cost-utility analysis comparing Ivacaftor plus standard of care (SoC) to just SoC alone, Vertex Pharamaceuticals reported that Ivacaftor plus SoC led to an increase of 4.6 life years and 4.6 quality-adjusted life years (QALY) at an incremental cost of approximately $700,000 CAD per QALY. The CDEC pointed to a lack of transparency regarding cost and utility derivation, and after performing its own, more conservative analysis, found that an incremental cost per QALY of about $2 million for Ivacaftor plus SoC compared to SoC alone (which could potentially exceed $9

million per QALY depending on assumptions) [38]. Based on estimates in the UK, the estimated QALY cost for Kalydeco was between £335,000 and £1,274,000 [56]. Once again, to put this into context, NICE (the National Institute for Clinical Excellence) generally sets a threshold for treatments between £20,000-30,000 per QALY [58].

Owing to this, it is clear that the high cost as well as issues surrounding the cost-effectiveness of Kalydeco were major gaps that existed when it initially received a regulatory approval. As of yet, there have been no changes in the costs of Kalydeco, and this gap, remains largely unfilled and may remain unfilled until 2025, which is when Vertex Pharmaceuticals' patent on Kalydeco expires.

3.4 Discussion

In addition to these key gaps, there existed several gaps which were not included as a main focus but are still relevant to this research. Firstly, during Kalydeco's initial approval, there was no long-term data supporting its use. However, a clinical trial PERSIST [63] has since demonstrated both the long-term safety and sustainability of treatment effect in G551D patients. Secondly, clinical trials evaluating Ivacaftor generally utilized the CFQ-R respiratory domain score to assess quality of life improvements dealing with respiratory parameters. The CFQ-R questionnaire is a validated quality of life questionnaire [64] and an MCID of 4 points has been established for the respiratory domain [65]. Improvements in CFQ-R respiratory domain scores have not correlated well with FEV1% predicted changes, meaning that it likely measures different aspects of respiratory health [66].

One potential limitation of this research pertains to gaps that currently exist but were not captured in our research. Specifically, although the PRISM workgroup collaborates with numerous stakeholders, including patients, and Dr. Brown was involved in the care of CF patients, we did not directly interact with CF patients in this study. Collaborating with CF

patients who are receiving Kalydeco or interested in Kalydeco or Orkambi might point to additional gaps not considered in our initial evaluation. One way we tried to do this was to survey online communities of CF patients; unfortunately, due to limitations imposed by the University of Alberta Research Ethics Board, we were unable to carry out this aspect of our research. Including CF patients, thus, is important to address this potential limitation of our study.

Although the focus of this paper was on evaluating how the generation of evidence has evolved since Kalydeco's approval, it will be interesting to see how important gaps for Orkambi evolve in the coming years. As mentioned previously, there exists no established MCID for FEV1% predicted improvement and the benefit seen in clinical trials for Orkambi are considerably less than for Kalydeco in patients with the G551D mutation, reaching at best, a mean absolute improvement of 3.6% in FEV1% predicted [67, 68]. Whether or not the improvements seen are actually clinically meaningful is pertinent to the discussion surrounding the cost-effectiveness of Orkambi.

Moreover, while 25% of patients with the G551D mutation in clinical trials with did not show an improvement of at least 5% in absolute FEV1% predicted improvement [38], there are arguably far more non-responders in the case of studies on patients homozygous for the F508del mutation. According to TRAFFIC/TRANSPORT results, two large-scale Phase III studies most heavily relied on by approval bodies in approving Orkambi, at best 46.2% of patients show a relative improvement of 5% in FEV1% predicted compared to 22% in the placebo group [68]. This means that, under controlled clinical trial conditions, over half of the patients receiving Lumacaftor-Ivacaftor did not show at least this improvement. The improvement mentioned is relative and not absolute, and will be smaller in magnitude if considered in terms of absolute improvement.

Regarding cost and cost-effectiveness, Orkambi, which is available to a much larger subset of patients with CF, is priced at $259,000 USD per patient year [8], amounting to annual revenue of approximately $2 billion for Vertex Pharmaceuticals in the United States alone [12]. The clinical benefit seen for the CF patients with the G551D or other gating mutations, has never been seen in clinical trials of Lumacaftor-Ivacaftor for the F508del homozygous population. Recently, NICE recommended against funding Orkambi which it deemed too costly given its relative benefit [69].

Apart from these considerations, the safety of both Kalydeco and Orkambi have been well-demonstrated. For example, reductions in pulmonary exacerbations, a key determinant of quality of life and short-term CF mortality, through treatment with Kalydeco and Orkambi have been shown. Also, in contrast to non-responders, there are patients who will receive a much larger clinical benefit through the use of Kalydeco and Orkambi than the mean values observed in clinical trial results. Determining the characteristics of those patients who are most likely and those who are unlikely to benefit from these treatments is an important gap moving forward.

3.5 Conclusion

There has been tremendous hype surrounding the regulatory approval of Kalydeco and Orkambi, and for good reason. For the first time since the discovery of the CFTR channel, a breakthrough targeted therapeutic agent has been developed, and has potentially paved the way towards finding a cure. There still exists uncertainty about the cost-effectiveness of Kalydeco, the usefulness of clinical endpoints like sweat chloride concentration, and the benefit to some patients, even those with the G551D mutation. Given that CF is a rare disease, ethical considerations regarding the appropriateness of withholding treatments from the CF population are at the heart of discussions surrounding

these novel personalized therapeutic agents. The appropriateness and sustainability of what appears to be a new paradigm in orphan treatment development will be better elucidated in the coming years.

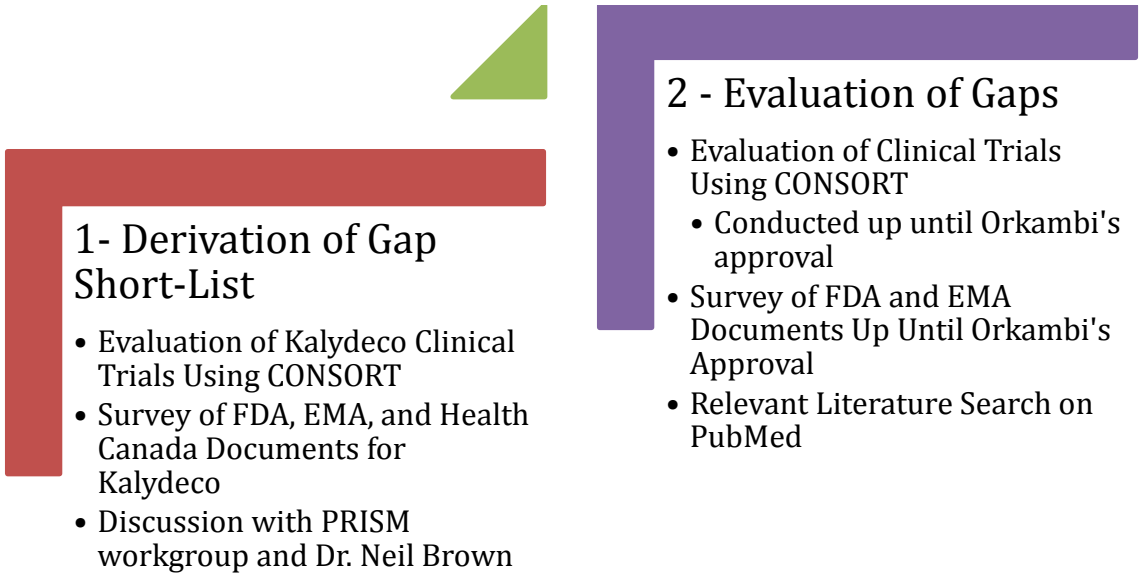

Figure 3-1 – Summary of Two-Step Methodology.

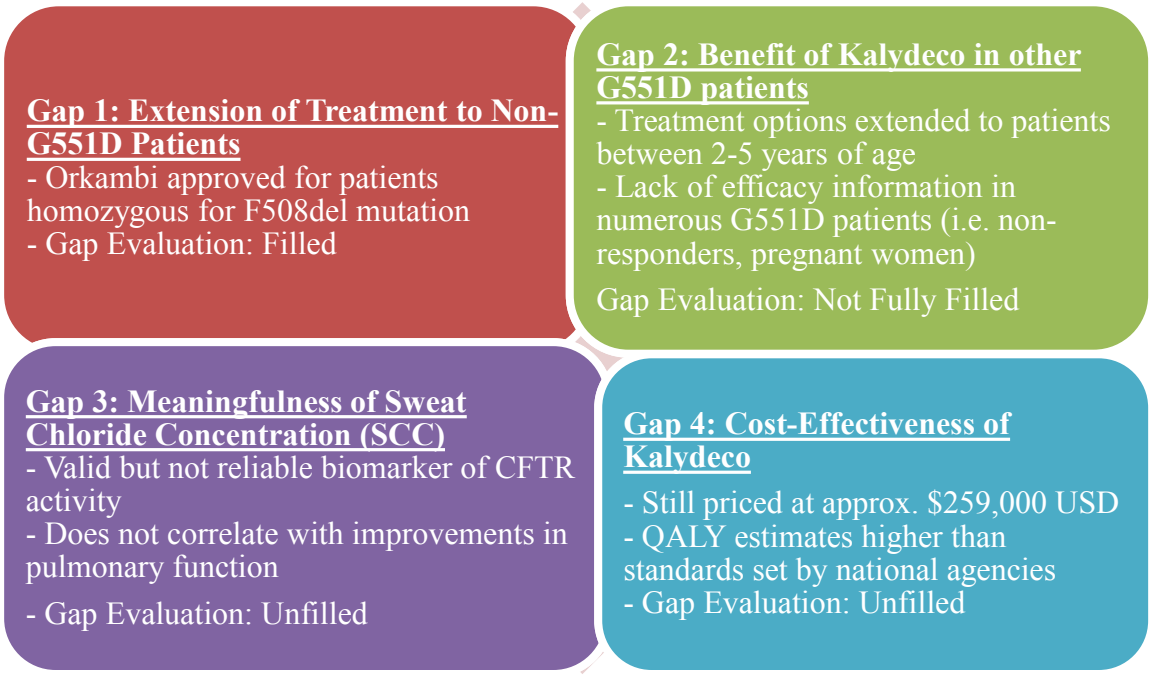

Figure 3-2 – Overview of Results of Gap Short-List Evaluation

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APPENDIX

Stem Cell Portrayal on a Popular Online Community for Amyotrophic Lateral Sclerosis (ALS)

A.1 Introduction

Amyotrophic Lateral Sclerosis (ALS) is a debilitating and rapidly progressive neurodegenerative disease with a median survival of 2-5 years from disease onset [1]. Riluzole, which first received regulatory approval in 1995, remains the only approved treatment for ALS and only slightly slows disease progression, prolonging median survival by approximately 2-3 months [2]. In the past decade alone, at least 18 different drugs for ALS treatment have been studied in large phase 2 and 3 randomised controlled trials (RCTs), none of which have shown positive results [3].

Given the lack of treatments available through standard clinical routes, as well as the rapid nature of disease progression, many ALS patients have turned to non-standard routes towards finding cures and symptom management, such as self-experimentation, alternative medicine, and off-label drug use which may carry significant risk [4]. For example, approximately half of all ALS patients take unproven supplements [5]. Additionally, off-label drug use generally surges following initial positive clinical trial results. After minocycline, a wide-spectrum antibiotic, was shown to slow disease progression in animal models [6, 7] and was found to be safe in subsequent phase 1 and 2 RCTs [8], ALS patients rushed to obtain the drug off-label. However, results from a large phase 3 study showed that minocycline actually accelerated disease progression by 25% compared to placebo [9].

Perhaps no prospective ALS treatment has been surrounded by as much hype as stem cell therapy. Stem cells can theoretically restore function in ALS through several

mechanisms [3, 10, 11]. Recently, an open-label study of stem cells administered to 12 ALS patients found the treatment to be well-tolerated and found a 25% improvement after 6 months in the slope of disease progression based on one of two criteria [12]. Despite this, stem cells have yet to be tested rigorously and remained unapproved/unavailable for ALS patients. Some patients wishing to receive stem cell treatments have been offered them at substantial financial costs [13] and with cases of serious side-effects reported for patients travelling to countries such as China to receive them [14, 15].

An emerging and ever-growing avenue for communication among patients with ALS are online communities. These include chat rooms, discussion boards, forums, or other online avenues for open dialogue between groups of patients, their families/caregivers, and anyone else who may have interest in the community. However, a legitimate criticism of online communities has to do with the quality of information being provided. For example, membership on such communities can often be obtained simply through a valid email address, making the information prone to contamination and manipulation. Certain online communities such as patientslikeme.com [16] have numerous safeguards in place to protect both the integrity of content and privacy of users, but this is not always the case for other forums.

The extent to which online communities exacerbate patients' desire for self-experimentation or to receive treatments such as stem cells is currently unknown. For this project, we sought to explore the portrayal of stem cell therapy, including what kinds of therapies were being discussed and members' attitude towards stem cell therapy, on popular online communities for ALS. Findings from this study can be useful to preliminarily understand the kinds of discussion occurring on online communities for ALS as they pertain

to stem cells and stem cell therapy and the influence, if any, of these discussions on patients' behaviors to seek such therapies.

A.2 Methods

Detailed information regarding our methodology is provided below. We used a systematic approach to identity online communities and to select relevant content for our research. Upon identification of relevant material, all content was extracted and analyzed through the use of a coding frame developed specifically for this research.

A.2.1 Development of Search Terms

We searched Orphanet [17], a rare disease registry, to find synonyms for the keyword "amyotrophic lateral sclerosis". We then used a Wikipedia page on online communities [18], to identify synonyms for the key word "online community". All possible combinations between "amyotrophic lateral sclerosis" synonyms and "online community" synonyms were searched directly on Google [the first 10 websites for each combination were accessed]. We opted to use Google as our search engine of choice since it holds the majority of the current desktop search engine market share worldwide and is by far the dominant search engine used in North America [19]. According to Google's search algorithm, over 90% of the traffic for a given search term is directed through the first page of results (the first 10 results) [20].

A.2.2 Website Search and Inclusion/Exclusion

Three inclusion/exclusion criteria were applied to select websites that were relevant ALS online communities for our study. Firstly, the website needed to be an online community. For this reason, websites where there was no back-and-forth communication occurring or where members could not start their own discussions, such as blogs, were excluded. Secondly, the forum needed to be specific to ALS. General discussion forums where only a few topics were dedicated to ALS were excluded. Health forums where major

subsections were dedicated to ALS discussion were included. Thirdly, using the search option directly on the forum, the term "stem cell" was searched. If no relevant results were found for this search term, the website was excluded.

A.2.3 Use of Online Communities for Research

Although there is no consensus on what constitutes a website being "publically available", it is generally agreed that if a website has no barriers to accessing content, such as the need for registration, the website is publically available [21]. However, even among websites considered publically available, the terms of use may explicitly or non-explicitly prevent the use of data for research purposes of any sort. Also, users may have a reasonable expectation of privacy in public internet forums [21]. For this reason, we contacted website administrators of each forum that initially passed our inclusion/exclusion requirements and included only those forums from whom we received a positive response regarding our specific research objectives, regardless of whether they were public forums or not.

A.2.4 Identification of Search Terms and Content Extraction

The search term "stem cell" was used to identify general content on the forum pertaining to stem cells. All resultant posts from this search were accessed directly on the website and scanned for instances where the term "stem cell" was used. Based on a survey of posts from the search, a list of terms used in conjunction with "stem cell" was compiled. A list of specific more search terms were finalized based on those terms that were commonly used in conjunction with the term "stem cell".

Each specific search term was subsequently searched on the forum and all resultant posts were accessed and manually extracted into a Microsoft Excel file as well as relevant accompanying content including the topic name, number of replies, and date of the post.

A.2.5 Coding Frame and Content Coding

Using a similar approach as the development of search terms, all posts for the search term "stem cell" were accessed directly on the forum. An initial coding frame was created based on content that was accessed from this general search. Specifically, this was done through a reading all posts resulting from the general search and making notes of recurring themes as well as any interesting aspects/discussions that stood out. Next, a final iteration of the coding frame was done after reading all extracted posts pertaining to the specific search terms leading to a final, refined, coding frame.

Using the coding frame, one coder read and coded all extracted content consistently. A second coder then independently coded approximately 10% of the extracted content (every tenth post for each search term). A Cohen's kappa for inter-coder reliability was calculated using Graphpad, an online software [22]. Any disagreements between the raters were resolved through discussion and consensus.

A.3 Results

A.3.1 Google Search Terms and Website Inclusion/Exclusion

A total of 20 search terms were developed based on combinations of synonyms for the terms "amyotrophic lateral sclerosis" and "online community". A full list of these search terms is included in Table A-1.

Overall, a total of 200 URL's were accessed for our search (10 for each search term); 108 of these were unique URL's and 68 were unique domains. Based on the inclusion/exclusion criteria, 52 domains were excluded because of Criterion 1 (website must be a forum), 6 domains were excluded because of Criterion 2 (forum must be dedicated to ALS), and 2 domains were excluded because of Criterion 3 (forum must have relevant discussion about stem cells). This left a total of 7 relevant forums for further study (Table A-2).

We contacted administrators for all 7 websites and received permission from 2 websites to conduct research for our study purpose (alstdi.org/forum and ehealthforum.com). However, almost no relevant content for our study (<5 posts) existed on ehealthforum.com, limiting our research to a single online forum, alstdi.org/forum or the ALS Therapy Development Institute (ALSTDI) Forum.

A.3.2 Specific Website Search Terms and Coding Frame

Nine specific search terms were identified and searched on the website; stem cell transplant, stem cell treatment, stem cell therapy, stem cell clinic, stem cell injection, pluripotent stem cell, iPSC (induced pluripotent stem cell), neural stem cell, and mesenchymal stem cell (Table A-3). The ALSTDI search results are limited to 100 of the most recent posts for each search term, so a maximum of 100 posts per search term, if available, were accessed and extracted. The latest possible date for an extracted post was April 30, 2016.

With respect to the coding frame, we initially devised 10 questions for our coding frame based on 100 posts pertaining to the general term "stem cell". Following a reading of all extracted posts from the 9 specific search terms, a final coding frame of 11 questions for coding was finalized.

Overall, 540 posts were coded by the first coder and 59 posts by the second coder. The Cohen's kappa for inter-coder reliability produced a mean score of k=0.606, indicating 'substantial' inter-rater agreement according to standards for interpreting kappa [23] (Table A-4).

A.3.3 Content Analysis

The average post length of the 540 posts was 503 words. Posts followed a historical distribution as follows: 0.37% in 2005, 1.85% in 2006, 1.85% in 2007, 5.00% in 2008, 2.96%

in 2009, 4.81% in 2010, 10.56% in 2011, 6.48% in 2012, 10.93% in 2013, 25.56% in 2014, 13.70% in 2015, and 15.93% in 2016 (Figure A-1). The majority of search terms led to forum content posted as early as 2008, with the exception of the search terms "iPSc" (earliest post 2011), "stem cell treatment" (earliest post 2013) and "stem cell therapy" (earliest post 2014"), likely due to the high usage of these terms in recent years. Table A-3 further highlights the proportion of all posts belonging to each search term. We were able to determine the identities of posters for approximately 35% of all posts (188 posts); the vast majority of these posts belonged to ALS patients (80.3%), followed by family members of ALS patients (11.7%), ALS patient group advocates (6.4%), and ALS researchers (1.6%). We classified posts into 8 main categories (Figure A-2).

A.3.3.1 Stem Cell Clinics

The primary topic of discussion in exactly 15% of all content (81 posts) were stem cell clinics. Topics ranged from news reports about newly operating clinics, questions from patients about obtaining therapy from such clinics, patient experiences with clinics, as well as responses to questions from users. We found the sentiment regarding stem cell clinics to largely be negative, with 49.4% (40 posts) of posts portraying a negative attitude towards stem cell clinics and 22.2% (18 posts) displaying a neutral attitude. Out of the 28.4% (23 posts) of posts that displayed an optimistic or positive attitude, 39.1% (9 posts) still urged some sort of caution with respect to stem cell clinics (Figure A-3). Countries touted to offer stem therapies included China, India, Israel, Italy, Mexico, Turkey, Ukraine, United Arab Emirates, and the United States with costs ranging from as little as $5,000USD to as much as $100,000USD for the procedure.

In 7 posts, patients or family members of patients who obtained stem cell therapy through unapproved clinics described their experience. In all 7 of these posts, the sentiment

regarding stem cells was negative, with only one patient describing a temporary improvement in leg strength and a return to baseline state after a 2-week period. None of the posters recommended obtaining stem cell therapy in this manner and often referred to these operations as "scams" and described the "terrible" experience they had at facilities they visited for stem cell therapy. A separate post involved a news story about a patient with motor neuron disease who recovered after obtaining stem cell therapy from India; the link led to a webpage that had since been removed.

In several instances, family members of patients who had recently been diagnosed with ALS directly sought feedback regarding stem cell clinics. In every case, responses from forum users discouraged stem cell clinics and often referred to them as "scams" which were preying on "desperate" people. Forum users also pointed to alternative starting points in terms of medication protocols, designed by ALS patients, and recommended enrolling in reputable clinical trials through websites such as clinicaltrials.gov which were enrolling ALS patients if one wanted to obtain stem cell therapy via a trustworthy avenue.

A.3.3.2 Clinical Trials and Case Reports of Patient Improvement

A total of 111 posts (20.6%) were in relation to clinical trials on stem cell therapy, and included information about upcoming trials, results from clinical trials, experience of patients who received stem cell therapy through a trial, as well as case reports of at least two patients who had displayed "remarkable" improvements following treatment. The large majority of posts regarding clinical trials, 63.1% (70 posts), viewed them with optimism, but even amongst these, approximately 32.9% (23 posts) still urged caution, especially with respect to being overly-optimistic about positive findings (Figure A-3). Primarily, neural stem cells (46.0%, 51 posts) and mesenchymal stem cells (34.2%, 38 posts) were the main topics of discussion when it came to clinical trials (15.3% (17 posts) were designated "other", while

4.5% (5 posts) were on induced pluripotent stem cells (iPSC's)).

Two companies, Neuralstem [24] and Brainstorm [25], have been at the forefront of clinical trial research on neural stem cells and mesenchymal stem cells, respectively, and were a major point of discussion among forum users. Importantly, both companies have reported at least one case of what has been deemed "remarkable" improvement in outcomes for a patient receiving their treatment. In both cases however, many forum users were critical of improvements that were seen.

In the case of Ted Harada, treated with Neuralstem's therapy, several forum users suggested alternative explanations for the improvement seen, such as a high dose of anti-inflammatory medication that Ted Harada was receiving combined with anesthetics from the surgery. One forum user specifically mentioned that the hype surrounding Ted Harada could be mis-used by illegitimate stem cell clinics hoping to exploit ALS patients for profit. With respect to Brainstorm, a patient known as "The Rabbi" was purported to have improvement following treatment and once again, forum users pointed to the fact that this patient had both Myasthenia Gravis and ALS, and this confounded any benefits that were seen.

Overall, results from these clinical trials were viewed with optimism but forum users mentioned on several occasions that both Neuralstem and Brainstorm's trials were intended to test the safety and not the efficacy of the treatment, and wanted to await results from future studies before making conclusions about the treatments effectiveness.

A.3.3.3 Usage of Scientific Publications in Posts

We deemed 135 posts (25%) of all posts as a discussion of a scientific finding or theory about stem cells in relation to ALS. Ninety eight (72.6%) of these posts were direct links to or direct copy-pastes of peer-reviewed journal articles pertaining to mouse models, biological mechanisms, derivation of cell-lines, methodology, and reviews, all pertaining on

stem cells. Nineteen posts (14.1%) were news reports that summarized or discussed new scientific findings that pertained to stem cells and stem cell therapy. As such, 117 of 135 posts (86.7%) were either direct or indirect references to peer-reviewed scientific publications on stem cells. The remaining 18 posts were summaries of conferences/symposiums on stem cells or links to educational videos on stem cells and stem cell therapy.

A.3.3.4 Right To Try Discussions

A recurring point of discussion among forum users were ethical questions pertaining to patients' perceived "right to try". Even among discussions about unregulated stem cell clinics, forum users still believed, despite potential risks from treatment and the financial cost, that ALS patients had the right to proceed with treatment in hopes of improving their condition. We found at least 10 posts that primarily focused on ethical discussions pertaining to patients' right to self-experimentation. Several of these posts expressed their frustration and mis-trust of regulatory bodies such as the United States Food and Drug Administration which they viewed as "corrupt" and only chasing profits. In several separate posts, a discussion about the difference in the urgency surrounding treatment for Ebola and ALS took place. One user remarked that perhaps if ALS was "contagious", there would be more urgency in finding a cure while another user argued that financial incentive was the primary distinguishing feature between Ebola and ALS treatment. Despite this, numerous other users, although in favor of patients right to try, mentioned that the lack of approved treatment options for ALS was not due to a lack of effort, as countless research incentives on both stem cell therapy and other treatments were actively under way and showing promise.

A.4 Discussion

Online communities are a major hub for discussion among patients with ALS. To our knowledge, with the exception of ALS research on patientslikeme.com, this is the first research to explore stem cells portrayal, including patient sentiment and the kinds of discussions taking place, on an online community for ALS which is largely open to any member of the general public. During our survey of posts, we came across a post from a user who, commenting on ALSTDI's forum, remarked that it was the only forum which they frequented due to the relatively higher quality of content and discussion among forum users compared to other websites. In several other posts, forum users praised another forum user and patient who, also a statistician, found a major flaw in a clinical trial that was conducted and described it to be a fabricated study. As such, due to the need to respect the privacy of users on other forums where we could not carry out this research, it is unclear whether the findings from this paper are specific only to the ALSTDI forum or to other ALS forums which may display similar characteristics.

Based on the results presented in this paper, these authors argue that the discussions that pertain to stem cells and stem cell therapy on the ALSTDI forum should be considered to be, at minimum, of good quality. Forum users often used peer-reviewed journals to share novel scientific findings, were critical of extravagant claims and findings, and often urged caution even when displaying optimism about novel developments in stem cell therapy. In contrast, similar studies which have looked at the portrayal of stem cell therapy in popular media, including social networks such as twitter and prominent newspapers, have found the portrayal of stem cell therapy to be overly optimistic [26, 27]. Discussions on the ALSTDI forum thus present a source which may help counteract many of the extravagant claims about stem cell therapy for ALS that may be made in popular media.

There are several limitations for this research which are important to consider.

Firstly, we limited our study to 540 posts, and did not directly access any of the individual threads related to each of the 540 posts. In some cases, threads ranged well into hundreds of pages and were infeasible to review given that our study objective was an introductory and exploratory survey of posts regarding stem cell portrayal. The ALSTDI search option limits results to the most recent 100 posts, and this might be overcome by using an Internet archiving software such as "WayBack Machine" [28] which creates a time-stamp of websites at different times. We opted not to do this in order to respect the website's reasons for limiting search results to the most recent 100 posts. Secondly, we obtained data from one of several popular forums for ALS and the translation and relevance of findings from this research to other ALS forums in unclear. Lastly, automation of data extraction using software such as "ParseHub" [29] for larger datasets and the creation of algorithms to perform automated sentiment analysis could expand this research from 540 posts to tens of thousands. However, as we discovered, even during manual analysis, it was not always clear whether the author's stance towards stem cell therapy was positive, negative, or neutral.

Our Kappa statistic for inter-rater reliability produced a mean score of 0.606 indicating 'substantial' strength of agreement. However, we obtained a Kappa score of 0.191 ('slight' or 'poor') pertaining to a question on the identity of the poster. This was due to the fact that the first coder also retrospectively inputted information as it became available. For example, whenever the identity of a poster was mentioned either directly by the poster or by another user, the first coder would fill in all the instances where that user had posted something. Since the second coder only coded every tenth post, they were only able to code based on the information available from the posts they had access to. In all instances of disagreement, the second coder had coded the post as "unknown" while the first coder had filled in the posters identity. We obtained a Kappa score of 0.915 for poster

attitude/sentiment, indicating 'almost perfect' strength of agreement. Given that this was a major focus of this research, our sentiment analysis is a strong point in our study and adds support for our findings.

An important and arguably unique aspect for ALS surrounds patients' right to self-experimentation. ALS, unlike many other neurodegenerative diseases, has only one approved treatment which slightly slows disease progression and also has a very poor prognosis with a relatively quick progression from disease onset to death. In one study (using information from the online community, patientslikeme.com) an analysis of self-reported data provided by patients was able to determine that a treatment for ALS, lithium carbonate, was ineffective, years before clinical trial results concluded the same [30]. In another case, it was discovered that ALS patients enrolled in a clinical trial were communicating with each other on an online forum to try to un-blind themselves to their treatment administration [31]. ALS patients are highly motivated, especially given the nature of their disease, and will continue to self-experiment in order to gain access to therapies that carry hope for slowing disease progression or disease reversal. The usage of online community content from ALS patients, especially in a structured format, may be invaluable for identifying novel targets for pharmacological intervention, developing new theories for disease etiology, and for testing promising therapeutic agents in clinical trials.

A.5 Conclusion

This study presents a systematic approach to using information from online communities for research purposes. The approach outlined in this paper can be used by investigators to study patient sentiment regarding a large number of topics pertaining to diseases with a burgeoning online presence. We found the ALSTDI forum to have a variety of discussions pertaining to stem cells and stem cell therapy including stem cell clinics,

clinical trials, ethical discussions like patients' right to try, and the sharing of new scientific findings from peer-reviewed publications. An important step forward involves increasing the amount of content used in the study and automating processes, like data extraction. Forums like ALSTDI provide important insight, especially with respect to patients, and their approach to treatments like stem cells, and may prove an invaluable tool for improving both the experience of patients afflicted with diseases like ALS and for elucidating novel therapeutic targets to study in the near future.

Table A-1 – Google Search Engine Search Terms		
ALS Forum	ALS Chat Room	ALS Discussion Board
ALS Message Board	ALS Online Community	Amyotrophic Lateral Sclerosis Online Community
Amyotrophic Lateral Sclerosis Forum	Amyotrophic Lateral Sclerosis Chat Room	Amyotrophic Lateral Sclerosis Discussion Board
Amyotrophic Lateral Sclerosis Message Board	Charcot Disease Chat Room	Charcot Disease Online Community
Charcot Disease Forum	Charcot Disease Message Board	Charcot Disease Discussion Board
Lou Gehrig's Disease Forum	Lou Gehrig's Disease Chat Room	Lou Gehrig's Disease Discussion Board
Lou Gehrig's Disease Message Board	Lou Gehrig's Disease Online Community	

Table A-2 – Online Communities Passing Inclusion/Exclusion Criteria
http://www.alsforums.com/
http://www.als.net/forum/
http://neurotalk.psychcentral.com/forum6.html
http://www.patientslikeme.com/conditions/9-als-amyotrophic-lateral-sclerosis
http://www.healthboards.com/boards/amyotrophic-lateral-sclerosis-als/
http://ehealthforum.com/health/lou_gehrigs_disease.html
https://www.inspire.com/groups/als-association/discussion/als-chatroom/

Table A-3 – Specific Search Terms and Proportion of Dataset		
Search Term	**Number of Posts**	**Proportion (of all posts)**
Stem Cell Transplant	57	10.6%
Stem Cell Treatment	26	4.8%
Stem Cell Therapy	13	2.4%
Stem Cell Clinic	28	5.2%
Stem Cell Injection	41	7.6%
Pluripotent Stem Cell	78	14.4%
iPSC	100	18.5%
Neural Stem Cell	97	18.0%
Mesenchymal Stem Cell	100	18.5%
Total	**540**	**100%**

Table A-4 – Inter-Rater Agreement On Key Coding Frame Variables		
Variable	**Kappa Score**	**Agreement**
Role of Stem Cells	0.609	Substantial
Discussion Topic	0.663	Substantial
Attitude/Sentiment Toward Stem Cells	0.915	Almost Perfect
Is Caution Urged?	0.513	Moderate
Identity of Poster	0.191	Slight
Type of Stem Cell	0.745	Moderate
* Level of agreement is based on Landis & Koch 1977 Benchmark Scale. The Kappa statistic values are suggested to denote the following strength of agreement: <0=poor, 0.01-0.20=slight, 0.21-0.40=fair, 0.41-0.60=moderate, 0.61-0.80=substantial and 0.81-1=almost perfect.		

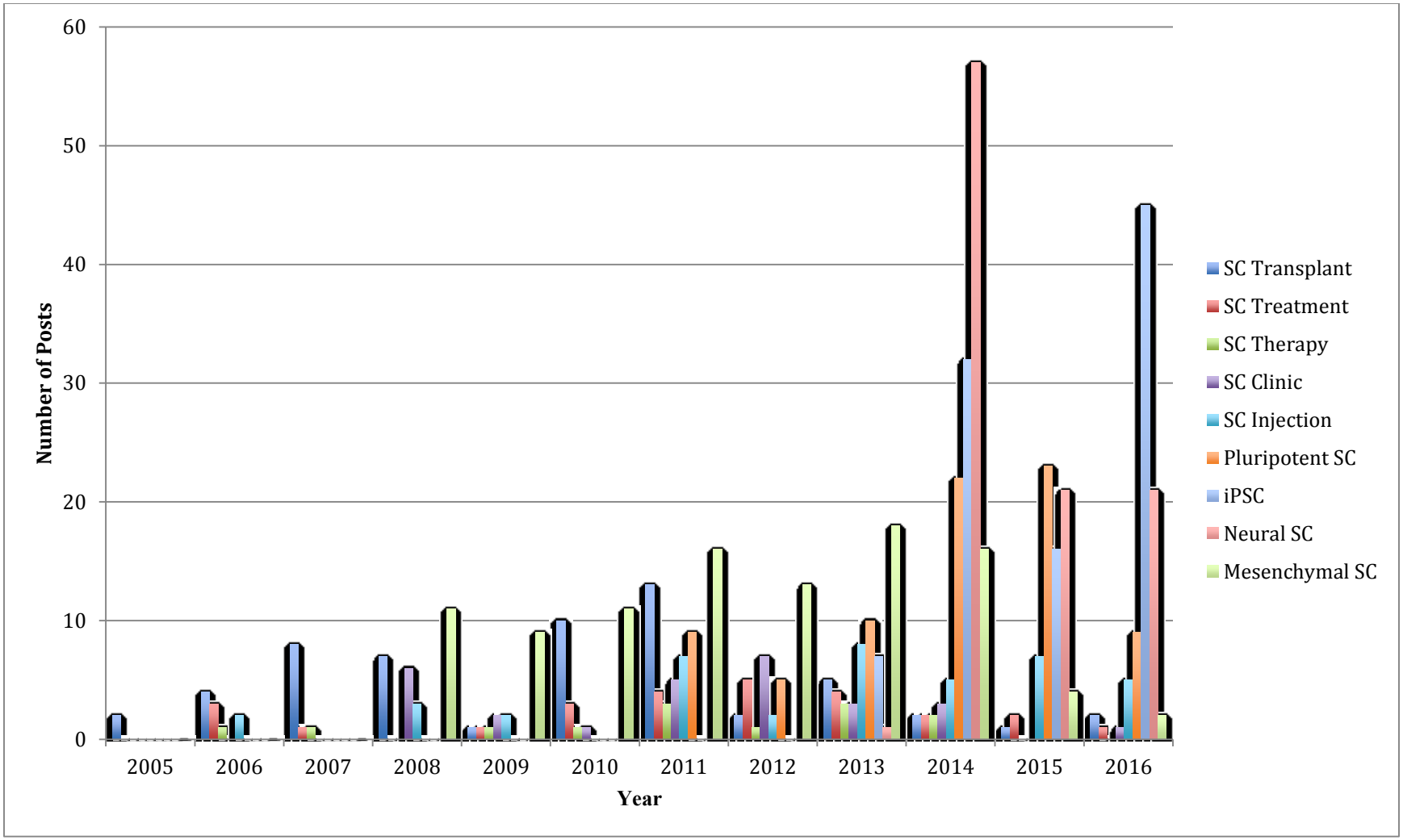

Figure A-1 – Distribution of Posts by Year and Search Term. Note: SC is an abbreviation for Stem Cell.

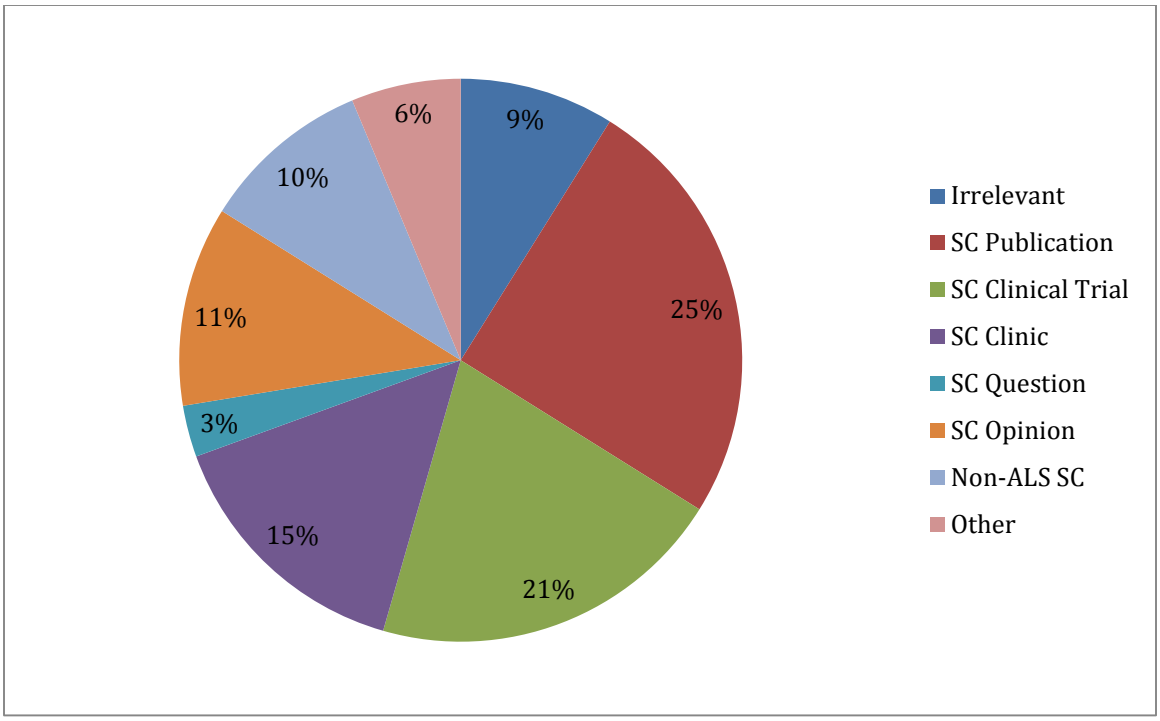

Figure A-2 – Primary Topic of Discussion in All Posts. Note: SC is an abbreviation for Stem Cell.

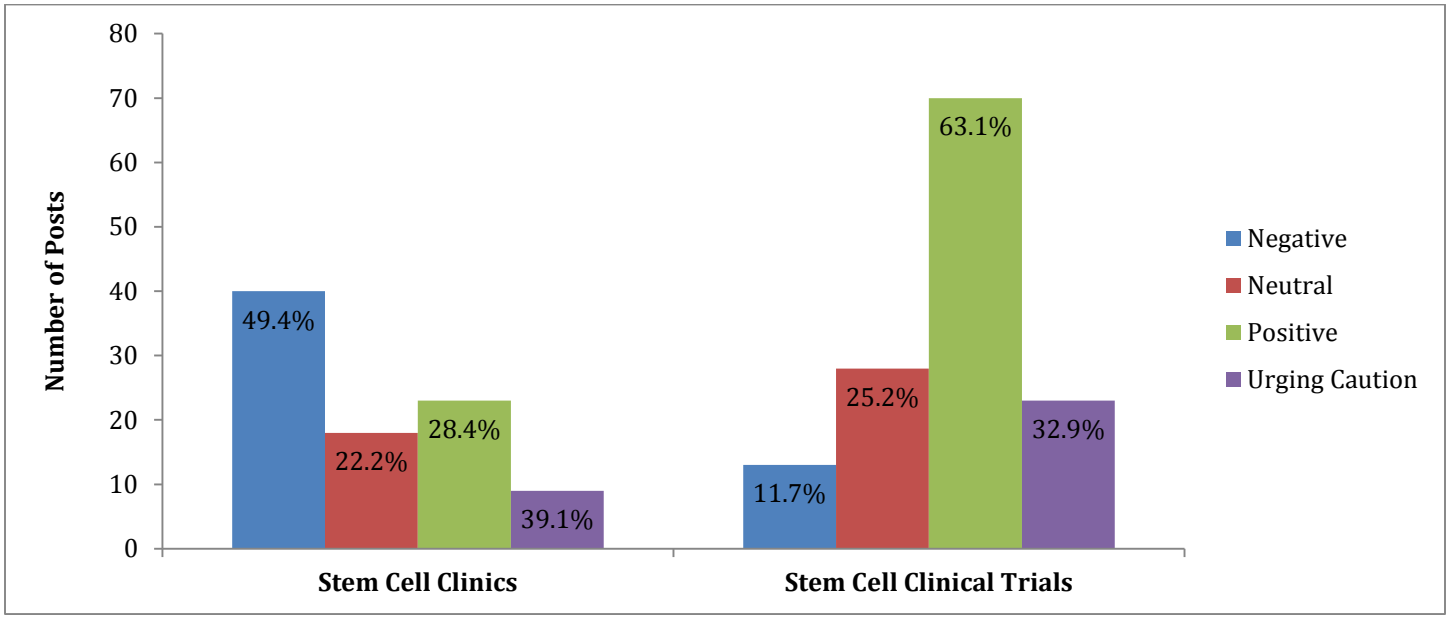

Figure A-3 – Sentiment Pertaining to Stem Clinics and Clinical Trials. Note: "Urging Caution" is only in relation to posts labeled "Positive" (i.e. the proportion of posts among those labeled "Positive" where caution is urged).

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