CHEST Online Supplement

Ivacaftor in Subjects With Cystic Fibrosis Who Are Homozygous for the *F508del-CFTR* Mutation

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e-Appendix 1.

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e-Appendix 2.

SUPPLEMENTAL METHODS

Selection of Study Population

Male or female subjects with a confirmed diagnosis of CF and who are documented to be homozygous for the *F508del-CFTR* mutation were considered for enrollment. CF diagnosis was considered confirmed if subjects had documentation in the subject's medical record of homozygosity for the *F508del-CFTR* mutation, and chronic sinopulmonary disease or gastrointestinal/nutritional abnormalities. Subjects were required to be at least 12 years of age or older and have a FEV_1 ≥40% of predicted normal for age, gender, and height (1) at screening. Females of child-bearing potential must have had a negative serum pregnancy test at screening and all subjects of child-bearing potential and who were sexually active must have met the contraception requirements. Subjects were required to have no clinically significant abnormalities in hematology, serum chemistry, coagulation, and urinalysis results at screening that, in the investigator's judgment, could interfere with the study assessments.

Subjects were excluded from the study if they had a history of any illness or condition that, in the opinion of the investigator, could have confounded the results of the study or posed an additional risk in administering study drug to the subject. Subjects were also excluded if they had an acute upper or lower respiratory infection, pulmonary exacerbation, or changes in therapy (including antibiotics) for pulmonary disease within 4 weeks before the first dose of study drug or any non-CF-related illness (defined as an acute [serious or non-serious] condition, such as gastroenteritis) within 2 weeks prior to first dose of study drug. Subjects who were pregnant, planning a pregnancy, breast-feeding, or not willing to follow contraception requirements were similarly excluded. Laboratory exclusions included hemoglobin <10 g/dL at screening, abnormal liver function test at screening (defined as ≥3× the upper limit of normal in 3 or more of the following: serum aspartate transaminase [AST], serum alanine transaminase [ALT], gamma-glutamyl transferase [GGT], serum alkaline phosphatase [ALP], total bilirubin), abnormal renal function at screening (defined as creatinine clearance <89 mL/min/1.73 m^2 using the Counahan-Barratt equation (2) for subjects aged 12 to 17 years or <50 mL/min using the Cockcroft-Gault equation (3) for subjects aged 18 years or older). Subjects with a history of prolonged QT/QT corrected for heart rate (HR; QTc) interval with Fridericia's correction (QTcF) >450 msec or QTcF >450 msec at screening were also excluded. Subjects could not have a history of solid organ or hematological transplantation or a history of alcohol, medication, or illicit drug abuse within 1 year prior to first dose of study drug. Colonization with organisms associated with a more rapid decline in pulmonary status (e.g. *B. cenocepacia*, *B. dolosa*, *M. abcessus*) at screening was an exclusion criterion.

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Subjects were not permitted to enroll if they were currently participating in another therapeutic clinical study or had participated in a trial of an investigational drug study (including prior studies with ivacaftor) without a wash-out period of ≥5 terminal half-lives of the previous investigational study drug or 30 days, whichever was longer, having elapsed before screening. Subjects who previously participated in a clinical trial of the CFTR corrector compound VX-809 at any time were not permitted to enroll. Subjects were excluded if they used inhaled hypertonic saline treatment, although subjects who had stopped inhaled hypertonic saline treatment and undergone a wash-out period of at least 4 weeks before the first dose of study drug were allowed to participate. Subjects were excluded in the event of concomitant use of any inhibitors or inducers of cytochrome P450 3A4 (CYP3A4), including consumption of herbal medications (e.g., St. John's Wort) and grapefruit or grapefruit juice, unless they stopped consuming these items at least 14 days prior to the first dose of study drug and did not consume these items during the study treatment period. Subjects who discontinued from the study after randomization were not eligible to re-enroll.

Randomization of Study Subjects and Blinding

Eligible subjects were randomized to a treatment group (ivacaftor or placebo) in a 4:1 ratio using a randomization code produced by Vertex. To protect the integrity of the blind, 2 statisticians were involved: a study statistician who was blinded to the actual treatment code and an unblinded statistician not otherwise associated with the study. The study statistician created the randomization specification and dummy randomization code, which were reviewed and approved by the unblinded statistician. After approval, the unblinded statistician generated the final randomization list that was provided to the Interactive Voice Response (IVRS)/Interactive Web Response System (IWRS).

The subjects, all site personnel including the investigator, the study monitor, and the Vertex study team were blinded with the exception of site personnel for whom this information was important to ensure the safety of a subject in the event of life-threatening medical emergency or in the event of a pregnancy, Vertex global patient safety personnel to satisfy serious adverse event processing regulations, the unblinded statistician preparing the final randomization list, the IVRS/IWRS vendor, the Vertex clinical supply chain, the independent Data Monitoring Committee (DMC), and the vendor preparing unblinded analyses for the DMC. Sweat chloride laboratory personnel and the monitor who was reviewing the sweat chloride results were unblinded to the sweat chloride results but remained blinded to treatment assignment. Vertex Drug Metabolism and Pharmacokinetics laboratory personnel or their designee were unblinded to the bioanalysis results but remained blinded to treatment assignment. A clinical pharmacologist not involved in the conduct of the study reviewed the bioanalysis results on an ongoing

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basis, but remained blinded to the subject's identity number and treatment assignment. Subjects and their parent/caregiver should not have been informed of their study-related spirometry results.

Treatment Compliance

To ensure treatment compliance, the investigator or designee supervised all study drug dosing that occurred at the site. At each visit, site personnel reviewed that the subject was compliant with study drug dosing and reminded the subject of study drug dosing requirements. Compliance was also confirmed by ongoing drug accountability.

Safety Analyses

Assessment of safety of ivacaftor was a primary objective of this study. Treatment-emergent adverse events are defined as adverse events with a start date or increased severity on, or after, the first dose of study drug through the follow-up visit. Adverse event summaries are presented using preferred terminology and system-organ classification according to the Medical Dictionary for Regulatory Activities (MedDRA) version 12.0. Subjects with multiple occurrences of the same adverse event, or a continuing adverse event, were counted once, and only the maximum severity level (mild, moderate, or severe), as determined by the investigator, was presented. The definition of a serious adverse event is an adverse event that was any of the following: life-threatening or fatal, resulted in unanticipated inpatient hospitalization or prolongation of hospitalization, caused persistent or significant disability or incapacity, resulted in congenital anomaly/birth defect, or was otherwise judged to be an important medical event that could jeopardize the subject and/or require surgical or medical intervention.

Vital signs assessed at each visit were systolic and diastolic blood pressure (mmHg), oral body temperature (°C), heart rate (beats per minute), and respiratory rate (breaths per minute). Physical examination was performed by medically qualified personnel and included assessment of the following body systems: head, neck, thyroid, eyes, ears, nose, throat, respiratory system, cardiovascular system, lymph nodes, abdomen, skin, musculoskeletal, and neurological. Electrocardiogram (ECG) monitoring was performed using standard 12-lead digital and 24-hour ambulatory ECG. Standard ECG assessments were PR, QT, and QTc intervals (Fridericia's correction [QTcF = QT/RR0.33] and Bazett's correction [QTcB = QT/RR0.50]), QRS duration, and HR. Ambulatory ECG monitoring was conducted at Day -14 and Week 16 visits; ambulatory ECG data were interpreted by a cardiologist at the Central ECG laboratory.

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Efficacy Analyses

Analyses of change from baseline in pulmonary function were based on a mixed-effects model for repeated measures that included absolute change from baseline in percent predicted forced expiratory volume at one second (FEV_1) as the dependent variable, treatment (ivacaftor versus placebo), visit (Day 15, Week 8, and Week 16), and treatment by visit interaction as fixed effects, subject as a random effect, with adjustment for continuous age, and continuous baseline percent predicted FEV_1. In the model, visit was treated as class variable and an unstructured covariance matrix was assumed to model the within-subject variances. This model imposed no assumptions on mean trend and correlation structure and was considered robust. Denominator degrees of freedom were estimated using the Kenward-Roger approximation (4). There was no imputation of missing data. The main effect of treatment obtained from the model was interpreted as the average treatment effect (effect of ivacaftor) across all post-baseline visits. The estimated mean treatment effect, a 95% confidence interval (CI), and a 2-sided P value were calculated. Analyses for change in sweat chloride concentration were similar to that for pulmonary function.

Additional analyses were performed for responders based on FEV_1 and sweat chloride separately to explore the subject characteristics associated with these responses. A responder based on FEV_1 was defined as having a ≥10% relative increase from baseline in percent predicted FEV_1 at 1 or more time points from Day 15 through Week 16, inclusive. A sweat chloride responder was defined as a ≥15 mmol/L absolute decrease from the baseline in average sweat chloride at both Day 15 and Week 8 visits. In addition, similar responder analyses were performed on responses based on both ≥10% relative increase in mean percent predicted FEV_1 from Day 15 through Week 16 and ≥15 mmol/L absolute decrease in mean average sweat chloride from Day 15 through Week 16. Differences between treatment groups were compared using Pearson Chi-square test if all expected frequencies were no less than 5 or Fisher's exact test if at least one expected frequency was less than 5.

Pulmonary exacerbation in this study was defined using a modified Fuchs criteria (5) of new or a change in antibiotic therapy (modified to include inhaled and oral antibiotics as well as IV antibiotics) for any 4 or more of the following symptoms: new or increased 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%; radiographic changes indicative of pulmonary infection.

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Signs/Symptoms of Sinopulmonary Disease

Administration of new or change in antibiotic therapy (intravenous [IV], inhaled, or oral) in response to signs/symptoms of sinopulmonary disease was documented. The following are the sinopulmonary signs/symptoms:

- Change in sputum
- New or increased hemoptysis
- Increased cough
- Increased dyspnea
- Malaise, fatigue, or lethargy
- Temperature above 38°C (equivalent to approximately 100.4°F)
- 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%
- Radiographic changes indicative of pulmonary infection

The protocol definition of pulmonary exacerbation for this study was new or changed antibiotic therapy (IV, inhaled, or oral) for any 4 or more sinopulmonary signs/symptoms.

Time-to-first Event Analyses

Time-to-first event of pulmonary exacerbation was analyzed using Cox regression with adjustment for age group (<18 year versus ≥18 years) and percent predicted FEV_1 (<70%, 70 to 90%, and >90% predicted) and Kaplan-Meier methods. A subject without exacerbation before withdrawal or completion of the study period was considered censored at the time of withdrawal or completion of the study period.

Open-label Extension Period (Part B)

At the scheduled interim analysis at 40 weeks (Week 24 of ivacaftor in Part B), the most common adverse events were pulmonary exacerbation (44.7%), cough (21.1%), abdominal pain (10.5%), bronchitis (7.9%), and nausea (7.9%). Most were mild or moderate in severity. One subject discontinued due to adverse events.

There was no further improvement in FEV_1 with continued treatment with ivacaftor; the mean (SD) change from Week 16 to Week 40 was –3.5 (11.7) percentage points. The change in sweat chloride observed with ivacaftor

through Week 16 was not sustained for the population of subjects who continued treatment; the mean (SD) change from Week 16 to Week 40 was 2.2 (12.2) mmol/L. There was no apparent change in lung function in those patients who had a sweat chloride response meeting eligibility for the Part B extension (Figure E2) although the number of patients is small.

There was no evidence for a reduced rate of pulmonary exacerbation for subjects treated with ivacaftor through 40 weeks. The annualized rate of pulmonary exacerbations for ivacaftor subjects who entered Part B was 0.29 per subject from baseline to Week 16 (Part A) and 0.80 per subject from Week 16 to Week 40 (Part B). For the 5 placebo subjects, the rate of exacerbations was 0.65 per subject through Week 16 and 0.88 per subject during ivacaftor treatment in Part B.

IRB Committee Names and Project Approval Numbers

- IRB No. 090709: Akron Children's Hospital Institutional Review Board
- IRB No. 090880: Vanderbilt University Institutional Review Board
- CHRMS # 10-039: Committee on Human Research in the Medical Sciences at The University of Vermont
- Study Number 2009014: The Children's Medical Center of Dayton Institutional Review Board
- Study #1113128: Western Institutional Review Board at The Children's Hospital of Alabama Translational Research Unit
- Study #1112987: Western Institutional Review Board at University of Rochester Medical Center
- Study #1111295: Western Institutional Review Board at Virginia Commonwealth University
- R09-08-007: Atlantic Health Institutional Review Board
- IRB #09 06-126: Children's Mercy Hospital Pediatric Institutional Review Board
- 2009-1424: Institutional Review Board at Cincinnati Children's Hospital Medical Center
- IRB # 09-052: Connecticut Children's Medical Center Institutional Review Board
- 09-00491-FB: The University of Tennessee Institutional Review Board
- Project No. 9110566: Drexel University College of Medicine Institutional Review Board
- 0908-10: Indiana University – Purdue University Indianapolis Institutional Review Board
- 2009-P-001399: Partners Human Research Committee at Brigham and Women's Hospital
- 2009-164 Spectrum Health Research and Human Rights Committee
- CPHS# 22029: Committee for the Protection of Human Subjects at Dartmouth-Hitchcock Medical Center
- Study #1112093: Western Institutional Review Board at Providence Health and Services Washington

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- 09-061-B: The University of Chicago Biological Sciences Division Institutional Review Board
- 20090762: University of Miami Medical Sciences Institutional Review Board
- IRB Number 14777: The University of Oklahoma Health Sciences Center Institutional Review Board
- 09-0071BM St. Luke's Health System Institutional Review Board
- IRB #09-050: ProMedica Health System Institutional Review Board
- IRB Study # 09-023 Institutional Research Review Board at Saint Barnabas Health Care System at Monmouth Medical Center
- IRB #3162 Maine Medical Center Research Institute Institutional Review Board
- Study #1111985 Western Institutional Review Board at Albany Medical College
- IRB #2009-036 Cook Children's Health Care System Institutional Review Board
- IRB-AAAE4396 Columbia University Institutional Review Board
- IRB Protocol No. 31707: Penn State Hershey College of Medicine Institutional Review Board
- ORCT #09-040: Saint Vincent Catholic Medical Centers Integrated Scientific and Ethical Review Board
- 19315: Institutional Review Board for Human Research at Medical University of South Carolina
- 0036515: University of Utah Institutional Review Board
- 2424: University at Buffalo Children & Youth Institutional Review Board
- IRB #09-120 Institutional Review Board at Nemours
- 13449: University of Massachusetts Medical School Institutional Review Board

e-Table 1. Number of subjects achieving response level for average change from baseline across all treatment time points.

Response criterion	**Placebo (n = 28) n (%)**	**Ivacaftor (n = 112) n (%)**	***P***
Mean decrease ≥ 5 mmol/L	6 (21.4)	42 (37.5)	0.11
Mean decrease ≥ 10 mmol/L	0	17 (15.2)	0.02
Mean decrease ≥ 15 mmol/L	0	8 (7.1)	0.36
Mean decrease ≥ 20 mmol/L	0	2 (1.8)	1.00
Mean decrease ≥ 30 mmol/L	0	1 (0.9)	1.00

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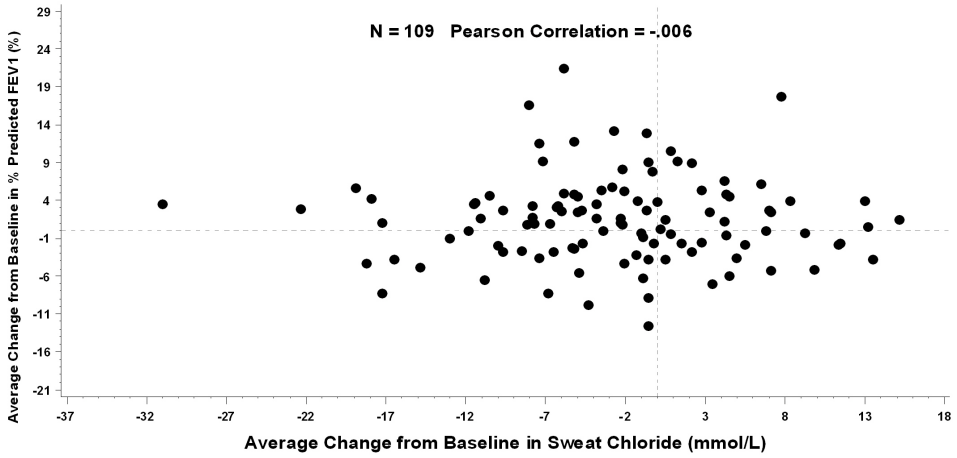
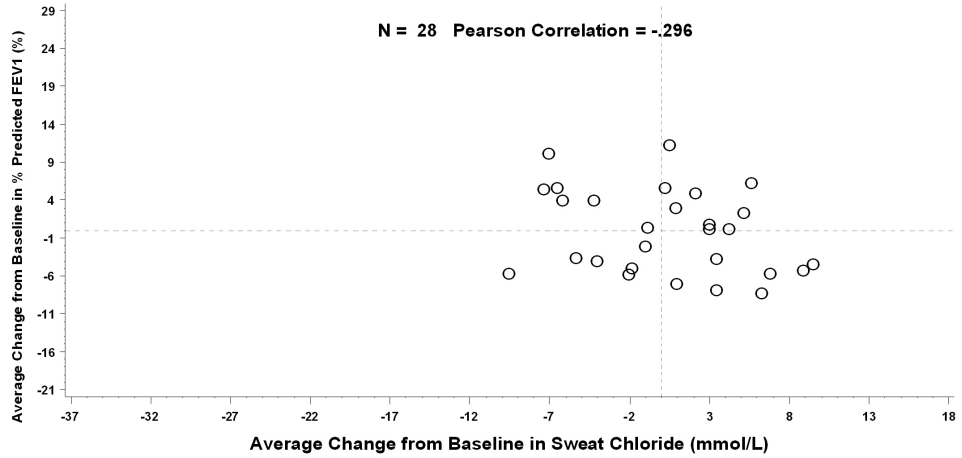
e-Figure 1. Scatter Plots of Average Change from Baseline in Percent Predicted FEV_1 versus Average Change from Baseline in Sweat Chloride from Baseline Through Week 16.
A correlation was not observed between the absolute change in percent predicted FEV_1 and the change in sweat chloride values over the 16 weeks of treatment in either the ivacaftor group (Pearson correlation coefficient: -0.006) or the placebo group (Pearson correlation coefficient:
–0.296). Panel A shows the ivacaftor group overall. Panel B shows the placebo group overall.

E1A



E1B



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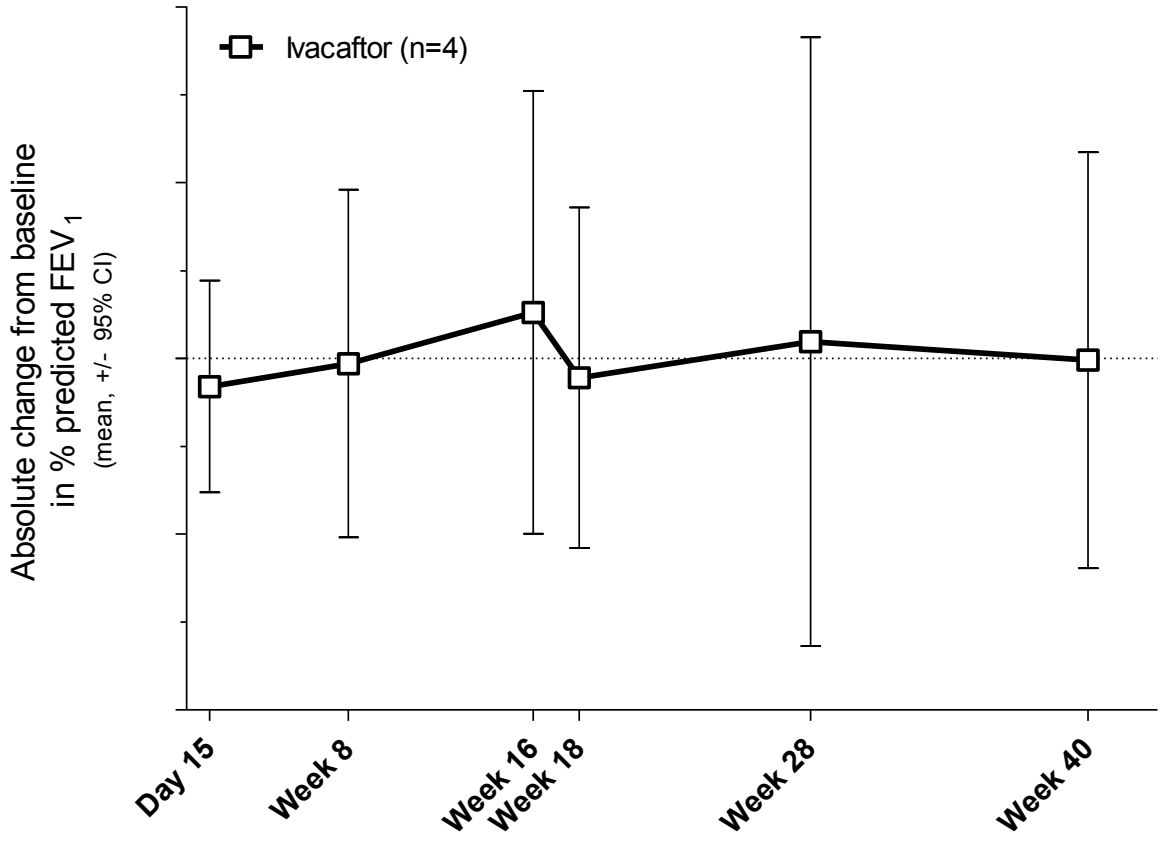
e-Figure 2. Change from Baseline in Percent Predicted FEV_1 in subjects that met sweat chloride eligibility requirements (sweat chloride concentration reduction from baseline ≥15 mmol/L at both the Day 15 **and** Week 8 visits).



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