Novel therapeutic approaches for treatment of CF patients with W1282X premature termination codon mutations

Study Protocol & Statistical Analysis Plan

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Title:	**Novel therapeutic approaches for treatment of CF patients with W1282X premature termination codon mutations**
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Background and Significance

Cystic Fibrosis is an autosomal recessive genetic disorder that most critically affects the lungs, but also the pancreas, liver, and intestine.[1–3] It is characterized by abnormal transport of chloride and sodium across the epithelium, leading to thick, viscous secretions and results from mutations in the gene for the protein cystic fibrosis transmembrane conductance regulator (CFTR).[3,4] This protein functions as a channel that transports chloride ions across the membrane of cells and is required to regulate the production and/or secretion of mucus, sweat, saliva, tears, and digestive enzymes. Most CF patients develop severe, chronic lung disease partly due to increased levels of sulfated mucins, inflammation, and recurrent bacterial infections that are eventually lethal; the median predicted survival age in the US in 2014 is 39.3 years.[5,6] There are approximately 30,000 individuals affected in the US and 70,000 worldwide.[1,5–8] Incidence varies widely according to country and ethnicity. In the US, estimates of incidence range from 1 in 1,900-3,700 white Americans.[1,7] CF is present, but less frequent, in other US groups: estimated incidences in Hispanic, Asian, and Black American populations have been reported to

be 1 in 9,200, 1 in 31,000, and 1 in 15,000, respectively.[9] There is wide country variability within Europe, ranging from 1 in 1,800 in Ireland to 1 in 25,000 in Finland.[10] CF is uncommon in Africa and Asia, with an incidence of 1 in 350,000 reported in Japan.[3]

Symptoms of CF often appear in infancy and childhood, the most frequent of which are respiratory symptoms, followed by failure to thrive, steatorrhea, and meconium ileus.[1,3] The lungs of individuals with CF are colonized and infected by bacteria from an early age. This leads to chronic airway infection and inflammation, progressing to bronchiectasis, gas trapping, hypoxemia, and hypercarbia. Pulmonary insufficiency is responsible for 70.5% of CF-related deaths in the US.[6] In the initial stage, common bacteria such as *Staphylococcus aureus* and *Haemophilus influenzae* colonize and infect the lungs. Eventually, *Pseudomonas aeruginosa* (and sometimes *Burkholderia cepacia*) dominates. Once within the lungs, these bacteria adapt to the environment and develop resistance to commonly used antibiotics.

Non-pulmonary complications of CF result from blockages of affected organs with thickened mucosal secretions. These blockages cause damage in the pancreas, liver, and intestines leading to exocrine pancreatic insufficiency, malabsorption, and recurrent pancreatitis; CF-related diabetes; chronic hepatobiliary disease and cirrhosis; and meconium ileus and distal intestinal obstructive syndrome.[1,3] Male infertility due to azoospermia secondary to congenital absence of the vas deferens is also a common complication of CF.

Specific Aims

Approximately 11% of CF patients have premature termination codons (PTC), causing truncated CFTR with little to no function. No approved therapies exist for patients with PTC mutations including W1282X, a unique mutation exhibiting partial CFTR activity even in its truncated form. CFTR modulators alone enhanced CFTR function in patient cells from W1282X/G542X CFTR. Several published studies including ours have shown CFTR modulators alone and/or in combination with readthrough (RT) agents benefit W1282X CFTR. Clinical studies further support an aspect of this notion, where two W1282X patients showed beneficial effect to Ivacaftor treatment. This sets the stage for studies to combine CFTR correctors, to improve truncated but partially active W1282X CFTR to the cell surface, with the CFTR potentiator ivacaftor.

Outcome Measures:

Change in Fev1

Participant Selection

Inclusion Criteria:

1. Evidence of singed and dated informed consent/assent documents indicating that the subject has been informed of all pertinent aspects of the trial
2. Age 18 years or older
3. Body weight > 16 kg.

4. Diagnosis of CF and documentation of the presence of a nonsense mutation of the CFTR gene, as determined by historical genotyping
5. Ability to perform a valid reproducible spirometry with FEV1 > 30% and < 90% predicted for age, gender, and height
6. If the subject is sexually active, willingness to abstain from sexual intercourse of employ a barrier or method of contraception during the study drug administration
7. Willingness and ability to comply with all study procedures and assessments.

Exclusion Criteria:

1. Any changes in a chronic treatment regimen for CF or for CF-related conditions within two weeks prior to screening
2. Evidence of pulmonary exacerbation or acute upper or lower respiratory tract infection within two weeks prior to screening.
3. Ongoing tobramycin immunosuppressive, warfarin, phenytoin, or tolbutamide therapy
4. History of solid organ or hematological transplantation.
5. A history of positive hepatitis B surface antigen test, hepatitis C antibody test, or human immunodeficiency
6. Major complications of lung disease within four weeks prior to screening
7. pregnancy or breast feeding
8. Current smoker or a smoking history of > 10 pack years
9. Prior or ongoing medical condition, medical history, physical findings, ECG, or laboratory abnormality that in the investigators opinion could adversely affect the safety of the subject, makes it unlikely that the course of treatment or follow-up would be completed or could impair the assessments of the study results

Recruitment Methods

Eligible participants will be recruited from the Principal Investigators Cystic Fibrosis specialty Care clinic the University of Alabama at Birmingham.

Study Methods:

This study will be an open-label, single-center study, where up to 5 CF patients homozygous for W1282X CFTR or W1282X mutation in-trans with other minimal function mutations will initiate Symdeko for 28 days followed by Ivacaftor for 28 days OR Ivacaftor for 28 days followed by a 28 day off period.

This assigned alternation will occur for 168 days for 9 study visits across a 6-month period.

Subjects may continue to receive existing therapy/prophylaxis for treatment of CF or CF-related conditions. The two treatment intervals will be summarized and evaluated for inter-individual and between treatment group analyses.

Statistical Analysis

Results will also be compared to historical run-in data already collected. Clinical efficacy of Symdeko as compared to baseline CFTR modulator therapy will be evaluated by lung function (spirometry).

Costs

The experimental procedure will be done at no cost to the subject or his/her insurance company. If any complications arise, they will be billed to the subject's insurance company.

Biostatistical Analysis

The within-subject change in FEV1 over baseline CFTR modulator therapy will be the primary efficacy outcome measure. Based on the design, the primary analysis will be a descriptive analysis of within-subject changes in outcome measures from week 1 to 24 of CFTR function. These data also will be used to test the null hypothesis of no change using the non-parametric Wilcoxon signed-rank test (due to small numbers). Change with washout (if conducted; this can be omitted for safety considerations) also will be informative. AE reporting will be conducted via listing tables and classified according to causality, per protocol. All statistical tests will be two-sided and will use $\alpha=0.05$.

Risk and Discomforts

CFQR Questionnaire:
There is a possible risk of loss of confidentiality in questionnaires due to medical history not remaining private. This potential risk is guarded against by maintaining completed questionnaires in a locked filing system in a locked room at the Child Health Research Center and in password-protected computers located at the center.

Blood Draw
Drawing blood from the arm may cause pain, bruising, light-headedness, and, on rare occasions, infection.

Spirometry:
Some people may have some light-headedness or chest soreness from the hard blowing. The chest soreness usually goes away by itself. It can also be helped with non-prescription pain-relievers.

Sweat Test:

The Macroduct System is a frequently used system in hospitals and clinics worldwide. This is considered to be a very safe painless procedure, but rare small burns have been reported at the site of the electrode even though the participant showed no discomfort during the test.

Nasal Potential Difference Test:
Local irritation of the skin where the needle is placed and local infection at the site are possible but unlikely. Nasal irritation, tearing and sneezing may occur because of the tubing placement in the nose. Nose bleeding is possible, but very unlikely. Infusion of amiloride, isoproterenol and ATP into the nose will have no foreseeable risks or discomforts.

HNE Collection:
The brushings and/or scrapes inside the nose can be mildly painful and may be associated with a small amount of bleeding. The risk of infection at the site of the brushing and/or scrape is very small.

Rectal Biopsy:

The rectal biopsy will be mucosal and not transmural, acquiring approximately 2-3 mm of tissue
per biopsy from the very distal rectum (5 cm cephalad). Risks associated with the sub-study procedures include:
• Potential discomfort related to saline enema
• Potential risks of sedation- Conscious sedation will include use of intravenous midazolam and/or morphine as determined by the Gastroenterologist and based on UAB policies regarding conscious sedation (attached). Both medications may cause serious or life-threatening breathing problems such as shallow, slowed, or temporarily stopped breathing. This GI will monitor heart and lungs and to provide life-saving medical treatment quickly if needed.
• Potential risks of local bleeding and infection
• Very small risk of bowel perforation

Bleeding would be predicted to be self-limited and infection would be treatable with antibiotics.
Bowel perforation would be treated with antibiotics, repeat endoscopy and, in severe cases, with
surgical intervention. These risks are minimized by performing biopsies under direct visualization, not including subjects with diseased tissue in the distal rectum, and are small relative to the potential future benefits of developing new CF therapeutics to address patient specific CFTR mutation defects.

Risks of optional conscious sedation:
Nausea and vomiting, although very uncommon, may occur.
Slight risk of respiratory slowing and respiratory arrest

Risk will be minimized through continuous monitoring throughout participation in the study, including comprehensive laboratory assessments, and physical exams. Participants will undergo blood tests during this study to look for any possible changes in liver function > than 3X upper limit of normal and creatinine kinase.

Potential Benefits

There may be no direct medical benefits to the study participants. data acquired during the study may help inform how to treat people with cystic fibrosis in the future.

Monitoring and Quality Assurance

Dr. Rowe is Principal Investigators at the UAB study site and will be directly responsible for the conduct of all study-related procedures performed at UAB. Dr. Rowe will be responsible for ensuring patient safety and will directly perform or supervise the performance of the study procedures described per protocol. Dr. Rowe has weekly meetings with his research team in which all facets of the protocol will be discussed and reviewed. Dr. Rowe is also responsible for reporting any unexpected or adverse events to the UAB IRB within the required time frame according to guidelines.

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