Resunab benefits in the murine model of CF lung infection and inflammation without jeopardizing resolution of *Pseudomonas aeruginosa* (PA) colonization in the lung.

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**ABSTRACT**

Introduction: Pulmonary infection and inflammation continue to be the major contributors to the morbidity and mortality in CF. Although great strides have been made in the development of small molecule CFTR corrector and potentiators, there is still significant need to manage the inflammation that become constitutive in an early age. Corbus Pharmaceuticals has developed an orally active synthetic CB2 agonist (JB-T101, Resunab™) with significant anti-inflammatory and anti-fibrotic properties that may be of benefit in CF. Hypothesis: Our studies tested the hypothesis that Resunab™ will provide anti-inflammatory benefit in the murine model of CF lung infection and inflammation without jeopardizing resolution of *Pseudomonas aeruginosa* (PA) colonization in the lung.

**Specific Aims**

1) determine the safety and potential toxicity of the Resunab™ in the murine model of chronic PA lung infection and inflammation; 2) establish the therapeutic potential of Resunab™ in CF lung infection using chronically infected murine models.

**METHODS & RESULTS**

As a collaboration between Corbus Pharmaceuticals Inc, and the Cystic Fibrosis Foundation Anti-Inflammatory Pre-Clinical Modeling Core Center at Case Western Reserve University, with the Cystic Fibrosis Foundation Modeling Core Center. *Pseudomonas aeruginosa* (PA) was administered by gavage at 1 mg/kg or 5 mg/kg dose BID in 2% methylcellulose for 10 days to establish chronic *Pseudomonas aeruginosa* (PA) infection. In the first study, WT (C57BL/6J) animals were utilized to evaluate oral dosing, safety and toxicity of Resunab™. In the second series of studies a limited number of both WT (n=10) and CF mice (cystic B6.129 Chrmdm (FADBCPCTR) 1/2ER congenic mice, n=10) were evaluated for safety, toxicity and efficacy upon oral dosing of 5 mg/kg Resunab™. As controls, PA infected WT and CF mice were given the 2% methylcellulose vehicle. CF and WT animals in this study were followed daily for clinical score and weights for 10 days. At day 10, animals were euthanized and evaluated for bacterial load (colonies forming units, cfu), total and differential bronchoalveolar lavage (BAL) white blood cells (WBCs). In the first study in WT mice, Resunab™ was well tolerated and more efficient at resolving both infection and inflammation than vehicle. CF mice have a more robust inflammatory response to PA infection, which includes increased number of BALWBCs and leukocytes. There were differences in the overall level of infection in the groups at day 3-10. In WT mice, CF mice had less CFUs at day 10 at either 1 mg/kg or 5 mg/kg Resunab™ dose. In CF mice, the difference in CFUs was greater than that seen in WT mice at day 10 (Figure 2A). A subset of mice were euthanized at day 3 to assess the earlier impact of Resunab™ on the infection/inflammation process with the resultant data from day 10 to determine the effects on infection/inflammation resolution.

**RESULTS**

**Module I: Resunab™ Safety and Toxicity in the Murine Model of Pneumonia**

**Material & Methods**

Resunab was delivered to animals via oral gavage (Figure 2A). Each dose given BID, 8 hours apart. Resunab™ (2A). A subset of mice were euthanized at day 3 to determine the safety and potential toxicity of the Resunab™ in the murine model of chronic PA lung infection and inflammation. For each species (WT and Cftr deficient) there were at least two groups, the control group was given the 2% methylcellulose vehicle, and the treatment group was given 1 mg/kg or 5 mg/kg Resunab™ (PA) colonization in the lung. There were differences in the overall level of infection in the groups at day 3-10. In WT mice, CF mice had less CFUs at day 10 at either 1 mg/kg or 5 mg/kg Resunab™ dose. In CF mice, the difference in CFUs was greater than that seen in WT mice at day 10 (Figure 2A). A subset of mice were euthanized at day 3 to assess the earlier impact of Resunab™ on the infection/inflammation process with the resultant data from day 10 to determine the effects on infection/inflammation resolution.

**Conclusions**

Consistent with the white blood cell counts, there were elevated levels of neutrophils (not lymphocytes or eosinophils) as assessed by lung CFUs (P<0.01, Data not shown). Additional, treatment with Resunab™ resulted in a decrease in BAL total while white blood cells, which changed phenotype shifting away from neutrophils (which is the usual CF response to PA infection) to macrophages (P<0.05). Importantly, the ability to shift the inflammatory response away from the usual neutrophil infiltrate also correlated with an improved ability of the animals to resolve pulmonary infection. The decrease of CFUs was not seen in BAL PA CFUs (P<0.002). These preliminary studies suggest that Resunab™ may be effective to treat inflammation in CF and improving the ability to resolve bacterial infection.

**CONCLUSIONS**

1. These preliminary studies suggest that Resunab™ may be effective to treat inflammation in CF as well as improve the ability to resolve bacterial infection.

2. Treatment of the pneumonia model (WT animals infected with *Pseudomonas aeruginosa*) demonstrated that Resunab™ may be sufficiently effective to enhance the resolution of the host response to infection.

3. The mechanismic effects of Resunab™ in chronic *Pseudomonas aeruginosa* pneumonia appears to be on the shifting of the pulmonary inflammatory cell infiltrate which is likely related to the impact of the Resunab™ to enhance the inflammasome resolution component of the host response to infection.

**SUMMARY**

These preliminary studies suggest that Resunab™ may be effective to treat inflammation in CF and has the potential of improving the resolution of CF. Current on-going studies are utilizing larger numbers of CF deficient animals as well as determining mechanisms of Resunab™ effectiveness in infection and inflammation resolution.

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**REFERENCES**


Bonfield TL, Hodges CA, Drumml ML. Cystic Fibrosis, the gut corrected mouse B6.129 gut corrected F508del and controls. There were differences in the overall level of infection in the groups at day 3-10. In WT mice, CF mice had less CFUs at day 10 at either 1 mg/kg or 5 mg/kg Resunab™ dose. In CF mice, the difference in CFUs was greater than that seen in WT mice at day 10 (Figure 2A). A subset of mice were euthanized at day 3 to assess the earlier impact of Resunab™ on the infection/inflammation process with the resultant data from day 10 to determine the effects on infection/inflammation resolution.

**Figure 1:** The theory for Resunab™ as a CF Therapeutic. Defined CFTR deficient, results in chronic infection which results in a vicious cycle of infection and inflammation. Inflammation and infection results in the recruitment of leukocytes which release inflammatory mediators. These inflammatory mediators recruit and activate more leukocytes which results in the inflammatory response toward the "resolving inflammation", a cycle that may ultimately lead to lung destruction. Resunab™ has the capability to shift this vicious cycle toward "resolving inflammation" and ultimately breaks the vicious triad of "infection-inflammation and lung destruction."