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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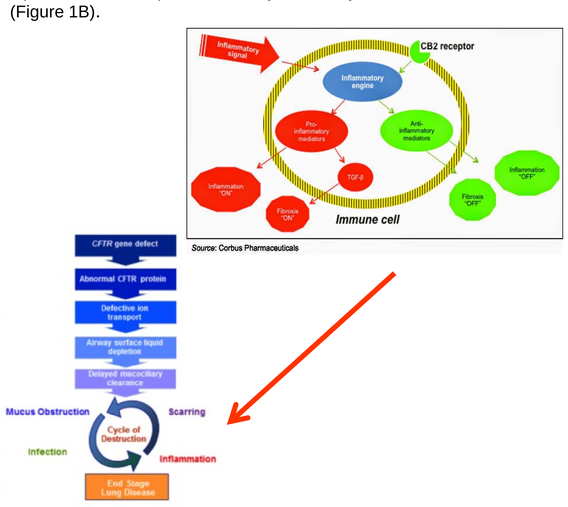
ABSTRACT

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 development of small molecule CFTR corrector and potentiators, there is still significant need to manage the inflammation that become constitutive from an early age in CF. Corbus Pharmaceuticals has developed an orally active synthetic CB2 agonist (JBT-101, 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 CFTR deficient mice. **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, Resunab™ was administered by gavage at 1 mg/kg or 5 mg/kg dose BID in 2% methylcellulose for 10 days starting 24 hours after establishing chronic *Pseudomonas aeruginosa* (PA) infection. In the first series of studies, 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 (congenic B6.129 Cftr^{tm1Kth} (FABPCFTR) 1Jaw/Cwr congenic mice, n=10) were evaluated for safety, toxicity and efficacy upon oral dosing of 5 mg/kg Resunab™ BID. 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 bacteria load (colony forming units, cfus), total and differential bronchoalveolar lavage (BAL) white blood cell counts (WBCs). In the first study in WT mice, Resunab™ was well tolerated and more efficient at resolving both inflammation and infection than vehicle. CF mice have a more robust inflammatory response to PA infection, and are very inefficient at resolve the bacterial burden. Further, post-infection CF mice loose significant weight and have higher clinical scores. In the second study which included 4 groups: 1) WT + vehicle (n=5), 2) WT +5 mg/Kg Resunab™ BID, 3) CF + vehicle (n=5), and 4) CF+ 5 mg/Kg Resunab™ BID, all animals were chronically infected with PA. All WT animals survived PA infection (both vehicle and Resunab™ treated). Resunab™ improve survival of CF mice from 3/5 (vehicle only) to 5/5 (5mg/Kg Resunab™ BID). Furthermore, treatment of CF mice with Resunab™ decreased weight loss (P<0.01), BAL wbc counts, and numbers of neutrophils (P<0.05) improved the ability of the animals to resolve pulmonary infection as assessed by lung CFUs ($P_{variance}$=0.002). These preliminary studies suggest that Resunab™ maybe effective to treat inflammation in CF and improves the ability to resolve bacterial infection.

INTRODUCTION

Cystic Fibrosis: While tremendous progress has been made in finding CFTR corrector and potentiator molecules, a cure is still elusive for patients with cystic fibrosis (CF). The combined effects of *Cftr* deficiency, inefficient mucocillary clearance, chronic infection and the ensuing inflammatory response are the major contributors to the underlying morbidity and mortality associated with CF. Further, the sequella associated with the chronicity of CF lung infection and inflammation necessitates the development of concurrent approaches to patient care, which include new and innovative therapeutics that can minimize the inflammatory response and the associated bacterial colonization with organisms such as *Pseudomonas aeruginosa* without enhancing the capacity for the pathogens to grow (Figure 1A). **Resunab™** is, a synthetic nonpsychoactive cannabinoid, agonist and lipoxin A_4 (LXA_4), an eicosanoid formed from sequential actions of 5- and 15-lipoxygenases (LOX), facilitate resolution of inflammation. Corbus Pharma has developed Resunab™, an orally bio-available synthetic anti-inflammatory / anti-fibrotic drug that has completed Phase 1 clinical testing. The drug binds to an important anti-inflammatory receptor on the cell surface of immune cells called CB2 that plays a role in terminating ("resolving") heightened inflammation. When Resunab™ binds to CB2 receptors on immune cells, it shifts the arachidonic acid metabolic pathways leading to the production of a unique spectrum of anti-inflammatory mediators such as PGJ2 and Lipoxin A4 that are part of the body's naturally "off-switch" for inflammation (Figure 1B).



(A) CF Lung Infection and Inflammation **(B) Corbus:Resunab™ Mechanism.**

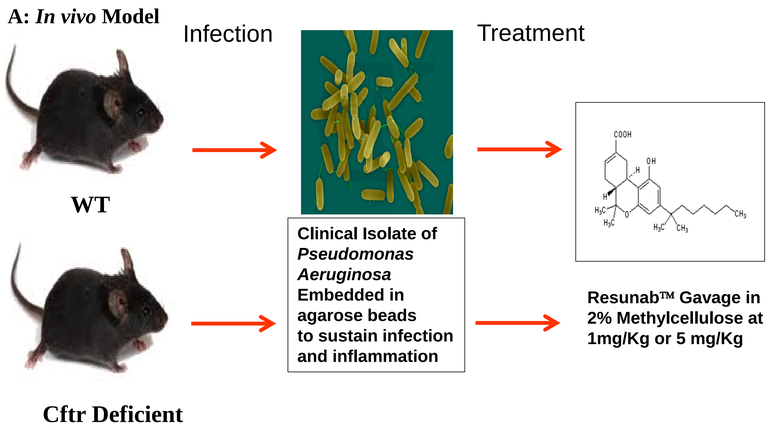
Figure 1: The Theory for Resunab™ as a CF Therapeutic. Deficient CFTR function, results in chronic infection which sets up a vicious circle of infection-inflammation and lung damage and destruction (1A). Resunab™ binds to the CB2 receptor of immune cells which re-directs the inflammatory response towards the "resolving inflammation" mode (1B). Resunab™ has the capacity to redirect the inflammatory response which may ultimate break the vicious triad of "infection-inflammation and lung destruction".

MATERIALS & METHODS

Application: Since patients with CF not only have inflammation, but they are also chronically colonized with bacteria, pre-clinical studies were designed to demonstrate the anti-inflammatory potential of Resunab™ and the overall impact on *Pseudomonas aeruginosa* colonization. The murine model of CF in which the *Cftr* gene is either knocked out or dysfunctional can provide a consistent and reproducible model in which to measure the differences in the CF host's inflammatory response to pathogens relative to controls with functional *Cftr* (6,7) providing an ideal window for studying anti-inflammatory drugs in the context of ongoing chronic infection similar to what is seen in patients with CF (8,9). As part of the Cystic Fibrosis Foundation's Pre-Clinical Modeling Core Center, we explored the potential of Resunab™ as a new therapeutic for CF, evaluating both its anti-inflammatory potential as well as the impact on pathogen resolution.

Specifics: We used the C*ftr* gut corrected mouse B6.129 *Cftr^{tm1Kth}* Tg(FABPCFTR)1Jaw/Cwr (gut corrected F508del) and controls. There were two different studies: Study 1- wild type mice to evaluate the safety and efficacy of Resunab™. Study 2- wild type and Cftr deficient animals to determine the impact of Resunab™ on inflammation and infection resolution in CF. For each species (WT and Cftr deficient) there were at least two groups: *Pseudomonas areuginosa* infection (10^5 viable-CFUs enpregnated into agarose beads) with vehicle (2% methylcellulose), and infection *Pseudomonas aeruginosa* infection with Resunab™ at either dose 1 (1mg/Kg Resunab™) or dose 2 (5 mg/Kg Resunab™) BID by gavage (Figure 2A). Each dose given BID, 8 hours apart. Resunab™ treatment began 24 hours post infection (Figure 2B)

Figure 2:Resunab™ in the CF Anti-Inflammatory Pre-Clinical Models. WT and *Cftr* deficient mice were infected with 10^5 of PAM-5715 (Clinical Strain of *Pseudomonas aeruginosa)* using the agarose bead model followed 24 hours later with Resunab™ gavage BID at either 1 or 5 mg/Kg Resunab™ (2A). A subset of mice were euthanized at day 3 to assess the earlier impact of Resunab™ on the infection/inflammation process with the rest terminated on day 10 to measure the effects on infection/inflammation resolution.



RESULTS

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

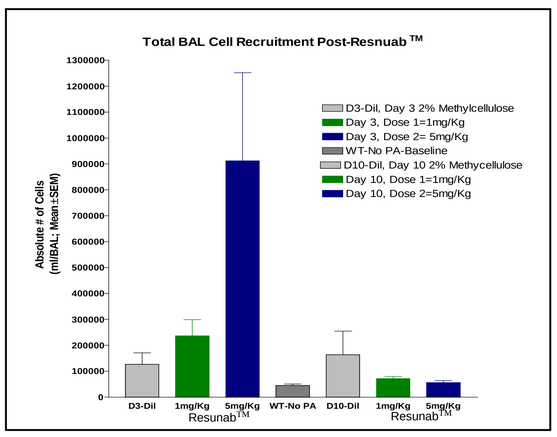


Figure 3: White Blood Cell Response to Infection. Animals were infected on day 0 and Resnuab™ was started at day 1 BID. Animals were euthanized at day 3 and day 10. The white blood cell response was elevated in the Resnuab™ treatment groups (green= 1mg/kg, blue =5mg/kg) at day 3 relative to controls infected (blue) or not infected (black). All groups normalized at day 10, without elevation of white blood cells compared to controls.

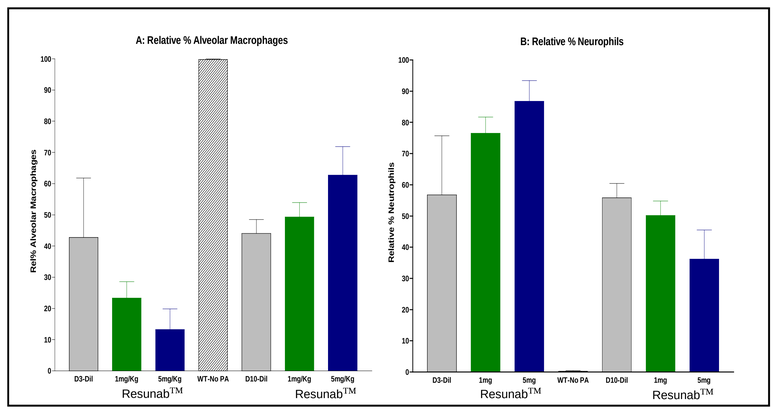


Figure 4: BAL Differentials in Response to Pseudomonas aeruginosa Chronic Infection. BAL was performed on mice at day 3 and day 10. Differentials were performed for each group at day 3 and day 10 with a focus on macrophages (A), neutrophils (B). Consistent with the white blood cell counts, there were elevated levels of neutrophils in all treatment groups relative to the non-treated control. By day 10, there was a shift in the treatment groups towards an increase in alveolar macrophages (A) and decreased neutrophils (B). There was no significant difference or levels of lymphocytes or eosinophils in any of the groups (data not shown).

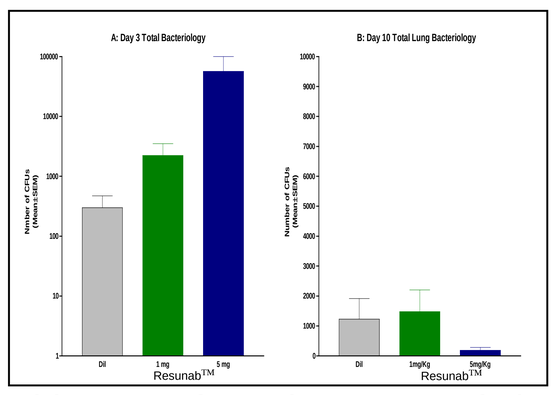
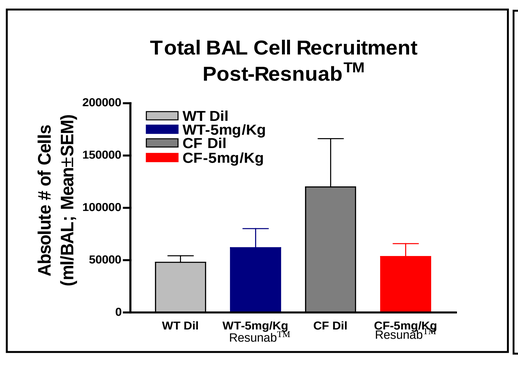


Figure 5: Bacterial Burden *Post-Pseudomonas aeruginosa* Infection and Resunab™ Treatment. There were differences in the overall level of infection in the groups at day 3- (A) but all groups normalized and had no significant differences in the bacterial burden at day 10,(B) with 5 mg/kg dose having the least level of infection.

Module II: Impact of Resunab™ on CF Lung Infection and Inflammation



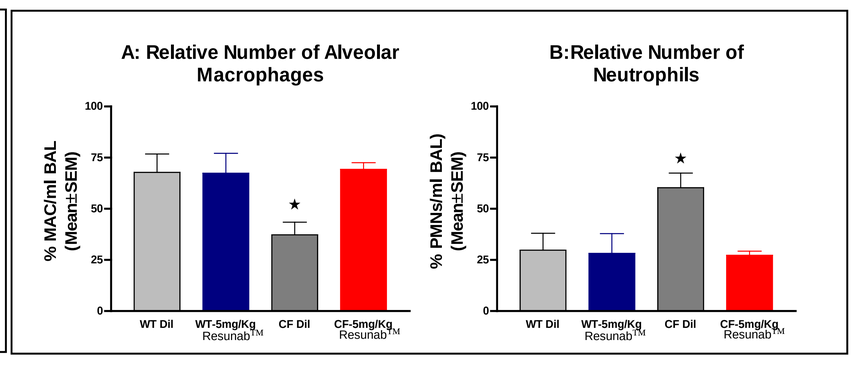


Figure 6: White Blood Cell Response to Infection. Animals were infected on day 0 and Resunab™ was started at day 1 BID. Animals were euthanized at day 10. The white blood cell response was elevated in the CF group compared to controls (dark grey bars). The Resunab™ treated CF group (red), had less cellular recruitment than CF animals without Resunab™. Total white blood cell recruitment in the WT animals treated with Resunab™ (blue) were comparable to the wild type vehicle control group (2% methylcellulose, light grey.)

Figure 7: BAL Differentials in Response to Pseudomonas aeruginosa Chronic Infection. BAL was performed on mice at day 10. Differentials were performed for each group with a focus on macrophages (A) and neutrophils (B), Consistent with the white blood cell counts, there were elevated levels of neutrophils in the CF group treated with 2% methylcelluose. By day 10, there was a shift in the treatment groups towards an increase in alveolar macrophages (A) and decreased neutrophils (B). There was no significant difference or levels of lymphocytes or eosinophils in any of the groups (data not shown).

Figure 8: Bacterial Burden Post-*Pseudomonas aeruginosa* Infection and Resunab™ Treatment. All groups normalized and had no significant differences in the bacterial burden at day 10, with 5 mg/kg dose having the least level of infection.

SUMMARY OF RESULTS

- In the first study in WT mice, Resunab™ was well tolerated and more efficient at resolving both inflammation and infection than vehicle. In the first phase of the study with analysis at Day-3, it appeared that the Resunab™ initially increased total cell counts (Figure 3, P<0.05) related to increased numbers of neutrophils (not lymphocytes or eosinophils, data not shown) which was quickly resolved by Day-10 associated with increased numbers of alveolar macrophages (P<0.05). This goes along with the proposed function of Resunab™ as an initiator of the resolvin pathway. Further, although the level of bacterial burden at Day 3 was higher in both the 1mg/Kg and 5 mg/Kg dose of Resunab™, there was significant resolution of the bacterial burden by Day 10 (with the 5mg/Kg does being significant at P<0.05).
- CF mice have a more robust inflammatory response to PA infection, and are very inefficient at resolve the bacterial burden. Further, post-infection CF mice loose significant weight and have higher clinical scores. In the second study which included 4 groups: 1) WT + 2% methycellulose (n=5), 2) WT +5 mg/Kg Resunab™ BID, 3) CF + 2% methylcellulose (n=5), and 4) CF+ 5 mg/Kg Resunab™, all animals were chronically infected with PA. All WT animals survived PA infection (both vehicle and Resunab™ treated). Resnuab™ improve survival of CF mice from 3/5 (vehicle only) to 5/5 (5mg/Kg JBT-101 BID). Furthermore, treatment of CF mice with Resunab™ decreased weight loss (P<0.01, Data not shown),. Additionally, treatment with Resunab™ resulted in an increase in BAL total white blood cell counts, 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 decreased the total number of BAL PA CFUs ($P_{variance}$=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 beneficial in scenarios of difficult to treat pulmonary infections.
3. The mechanistic 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 inflammation resolution component of the host response to infection.
4. These studies also implicate that inflammation resolution is defective in CF, and that aiding in the resolution component of the host response ultimately may provide a pathway for infection resolution in CF. This is consistent with our previous work demonstrating that defective immunity in CF contributes to inefficient inflammation and infection resolution.

SUMMARY

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

ACKNOWLEDGEMENTS

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