PRECLINICAL INTERLEUKIN PROFILE OF RSBT-001: A FIRST-IN-CLASS, STEROID-FREE IMMUNE MODULATOR IN DEVELOPMENT FOR IDIOPATHIC PULMONARY FIBROSIS

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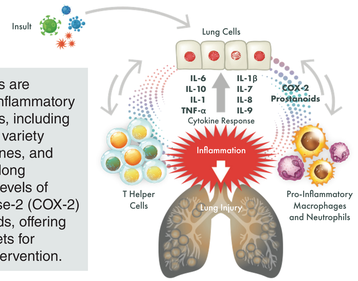
INTRODUCTION

RS BioTherapeutics is a preclinical-stage pharmaceutical company developing ***a first-in-class, multi-targeted immune modulator (RSBT-001)*** for the treatment of a broad range of respiratory diseases characterized by pulmonary inflammation.

Many serious respiratory diseases are characterized by pulmonary inflammation, including Chronic Obstructive Pulmonary Disease (COPD), Idiopathic Pulmonary Fibrosis (IPF), and Acute Respiratory Distress Syndrome (ARDS) to name a few (**Figure 1**). In many of these life-threatening diseases current treatment options offer benefits only to subsets of patients[1] and often do so at the expense of serious adverse effects, and/or have patterns of misuse/overuse.[2] With inflammation playing a key role in their complex etiologies, anti-inflammatory therapies are often part of the therapeutic regimen.

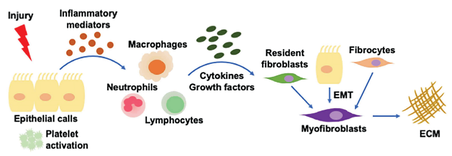
Figure 1: Multiple cell types, cytokines/chemokines, and pathways play important roles in inflammatory lung conditions such as COPD, IPF, and ARDS[3-7]



Multiple factors are implicated in inflammatory lung conditions, including increases in a variety of cells, cytokines, and chemokines along with elevated levels of cyclooxygenase-2 (COX-2) and prostanoids, offering attractive targets for therapeutic intervention.

Idiopathic Pulmonary Fibrosis (IPF) is a major health concern that has multifactorial etiology. The prevalence of IPF has been estimated to be 10–20 per 100,000 individuals in the United States and Europe,[8,9] but the true epidemiology is difficult to determine because IPF initially presents with nonspecific symptoms, often delaying diagnosis for years. Complex interactions coordinated by multiple mediators, including interleukins, have been implicated in the pathophysiology of IPF, and reflect the interplay of inflammatory and fibrotic mediators as seen in **Figure 2**.[10-13]

Figure 2: Pathophysiology of IPF[11]



i, Injury
- Epithelial/endothelial cell damage
- Platelet activation
- Clotting and coagulation

ii, Inflammation
- Cytokine, chemokine, growth factor release
- Fibroblast migration, proliferation, and activation
- EMT
- Fibrocyte recruitment

iii, Tissue repair
- Wound contraction
- Re-epithelialization
- Tissue remodeling

The pressing need for the development of novel IPF therapies is reflected in the poor prognosis for individuals with IPF — the median survival after diagnosis is estimated to be only 3–5 years. In 2014, two drugs—pirfenidone and nintedanib—were approved by the FDA for the treatment of pulmonary fibrosis. Despite the availability of these 2 options, there is an unmet need for new approaches to treatment. Whether due to low efficacy, adverse effects or high costs, under 30% of IPF patients are taking one of these approved agents, leaving room for additional, more targeted therapies.[14]

Evidence in IPF research is leading to an understanding that a multi-factorial disease will likely benefit from a multi-targeted therapeutic approach. In line with this, many drugs with single target approaches have failed clinical trials for IPF.

RATIONALE

RSBT-001 is a first-in-class, multi-targeted immune modulator for the treatment of a broad range of respiratory diseases characterized by pulmonary inflammation.

RSBT-001 is a proprietary, metal-coordinated micellar combination of a botanically derived cannabinoid complex and a GRAS metal. The micellar construct consists of a water-soluble outer layer containing the lipophilic cannabinoid payload (**Figure 3**).

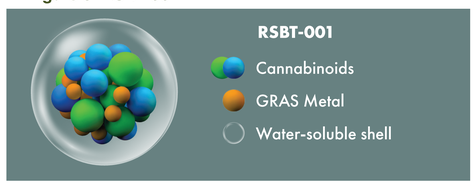
RSBT-001 harnesses the immune modulating power of these anti-inflammatory, non-intoxicating cannabinoids which are delivered by a polarity adaptive transport mechanism.

RSBT-001 is expected to provide therapeutic benefit by modulating multiple upstream and downstream pathways relating to inflammation.

As a multi-targeted immune modulator, RSBT-001 is uniquely positioned as a platform for respiratory drug development, creating potential development opportunities in Chronic Obstructive Pulmonary Disease (COPD), Idiopathic Pulmonary Fibrosis (IPF), Pulmonary Arterial Hypertension (PAH), Interstitial Lung Disease, Asthma, Bronchitis, Acute Respiratory Distress Syndrome (ARDS), and Medical Chemical Countermeasures.

RSBT-001 has been shown to impact multiple cytokines and pathways in two preclinical studies, teeing up its potential impact on a multitude of respiratory diseases characterized by pulmonary inflammation. To determine the therapeutic potential for IPF, we characterized the existing preclinical interleukin data relevant to IPF.

Figure 3: RSBT-001



METHODS

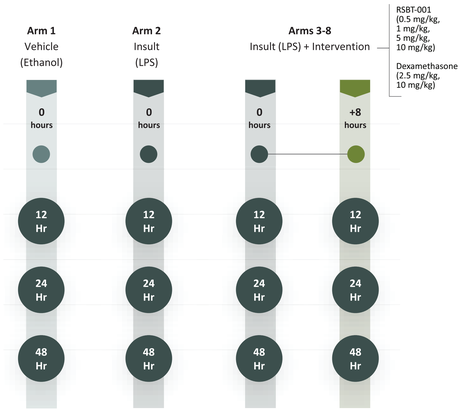
A lipopolysaccharide (LPS; 0.8 mg/mL) model (N=96; 8-week-old CF-1 mice) was used to investigate the biomarker impact of four doses of RSBT-001 (0.5, 1, 5, and 10 mg/kg) as compared with two doses of dexamethasone (2.5 and 10 mg/kg). Control arms included LPS and vehicle (10% ethanol) alone (**Figure 4**).

Mice were lightly anesthetized with isoflurane in an induction chamber. Control substances (LPS, vehicle) were administered into the lungs by allowing the mice to breathe in a 50 microliter drop dispensed by pipette into the nasal orifice, with RSBT-001 or dexamethasone administered in a similar fashion to the arms that received LPS eight hours later.

Mice were humanely sacrificed by cervical dislocation after administration of isoflurane anesthesia and harvested at 12, 24, and 48 hours. Lungs were removed and processed into homogenate for a 32-plex biomarker analysis by Eve Technologies. The 32 biomarkers examined include Eotaxin, G-CSF, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, IP-10, KC, LIF, LIX, MCP-1, M-CSF, MIG, MIP-1α, MIP-1β, MIP-2, RANTES, TNF-α, VEGF.

The study was performed at Marshall University.

Figure 4: Study design



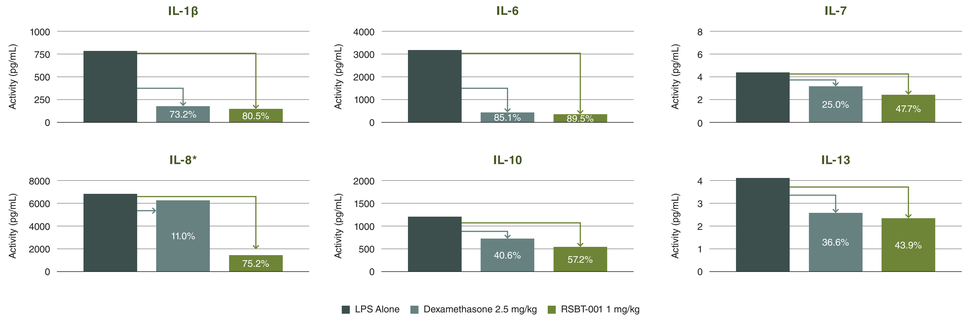
RESULTS

RSBT-001 delivered directly to the mouse lung demonstrated a substantial impact on LPS-induced, acute inflammatory activity in the mouse lung across nearly all key biomarkers studied in the 32-plex analysis, many of which are associated with respiratory diseases characterized by pulmonary inflammation. Greater reductions were observed as compared with the most potent corticosteroid. RSBT-001 1 mg/kg was determined to be most effective.

Of key interleukins related to IPF, RSBT-001 reduced LPS-induced inflammatory activity to a greater extent than dexamethasone at 2.5 mg/kg at 24 hours (**Figure 5**).

RESULTS

Figure 5: 24-hour interleukin activity (pg/mL)



*KC, the mouse ortholog of human IL-8, was evaluated.

These data offer a compelling basis for use of RSBT-001 as a novel multi-targeted immune modulator that may play an important role as a future treatment option for the management of IPF.

Similar results were found in a prior proof-of-concept study (N=30).

CONCLUSIONS

RSBT-001 is a proprietary, metal-coordinated cannabinoid complex. **RSBT-001** has been shown to modulate multiple upstream and downstream pathways relating to inflammation and fibrosis in two preclinical studies. It is THC-free and therefore non-intoxicating.

RSBT-001 is neither a single- or dual-target agent but rather a multi-targeted immune modulator, offering it the unique ability to mitigate a broad array of the inter-related inciting factors that drive the development of respiratory diseases characterized by pulmonary inflammation. While this fact might bring up the potential concern of off-target effects (which will be rigorously tested in future pre-IND studies), it should be noted that cannabinoids have been used by the masses for a very long time, and that Epidiolex, a purified, botanical cannabinoid extract, was approved by the FDA in 2018.

RSBT-001 will be delivered by a Dry Powder Inhaler (DPI), which offers a more targeted therapeutic modality that could also reinforce safety by reducing the therapeutic dose range. Fewer off-target effects are expected due to direct delivery to the lungs. Preliminary data have confirmed minimal systemic exposure.

In IPF, fibrosis typically results from tissue injury that leads to immune activation (inflammation) followed by dysregulated tissue remodeling and repair (fibrosis). RSBT-001 offers a novel type of immune modulation that may play an important role as a future treatment option for the management of IPF.

In this study, RSBT-001 delivered directly to the mouse lung demonstrated a substantial impact on LPS-induced biomarker expression in the mouse lung across a spectrum of key interleukins associated with IPF, with greater reductions observed as compared with the most potent corticosteroid.

Pursuant to this, an ongoing research collaboration with NIH is exploring both a preventive and treatment paradigm in a bleomycin mouse model to further define the impact of RSBT-001 on inflammation and fibrosis.

DISCLOSURES

This study was funded by RS BioTherapeutics, Inc.

Michelle L. Shuffett, MD (RS Biotherapeutics: employee, stock options)

Eric Blough, PhD (RS Biotherapeutics: study funding)

REFERENCES

1. Agusti A, et al. *Am J Respir Crit Care Med*. 2023;207(7):819-837.
2. Alabi FO, et al. *BMC Pulm Med*. 2023;23(1):216.
3. Barnes PJ. *Nat Rev Immunol*. 2008;8(3):183-192.
4. McMillan DH, et al. *Am J Pathol*. 2011;179(1):125-133.
5. Rodrigues SO, et al. *Pharmaceuticals (Basel)*. 2021;14(10):979.
6. Rossi F, et al. *Int J Mol Sci*. 2020;21(11):3809.
7. Rumzhum NN, Ammit AJ. *Clin Exp Allergy*. 2016;46(3):397-410.
8. Ley B, Collard HR. *Clin Epidemiol*. 2013;5:483-492.
9. Nalysnyk L, et al. *Eur Respir Rev*. 2012;21(126):355-361.
10. She YX, et al. *Cell Death Discov*. 2021;7(1):52.
11. Ishida Y, et al. *Int J Mol Sci*. 2023;24(4):3149.
12. Desai O, et al. *Front Med (Lausanne)*. 2018;5:43.
13. Lederer DJ, Martinez FJ. *N Engl J Med*. 2018;378(19):1811-1823.
14. Dempsey TM, et al. *Ann Am Thorac Soc*. 2021;18(7):1121-1128.

Poster PDF

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