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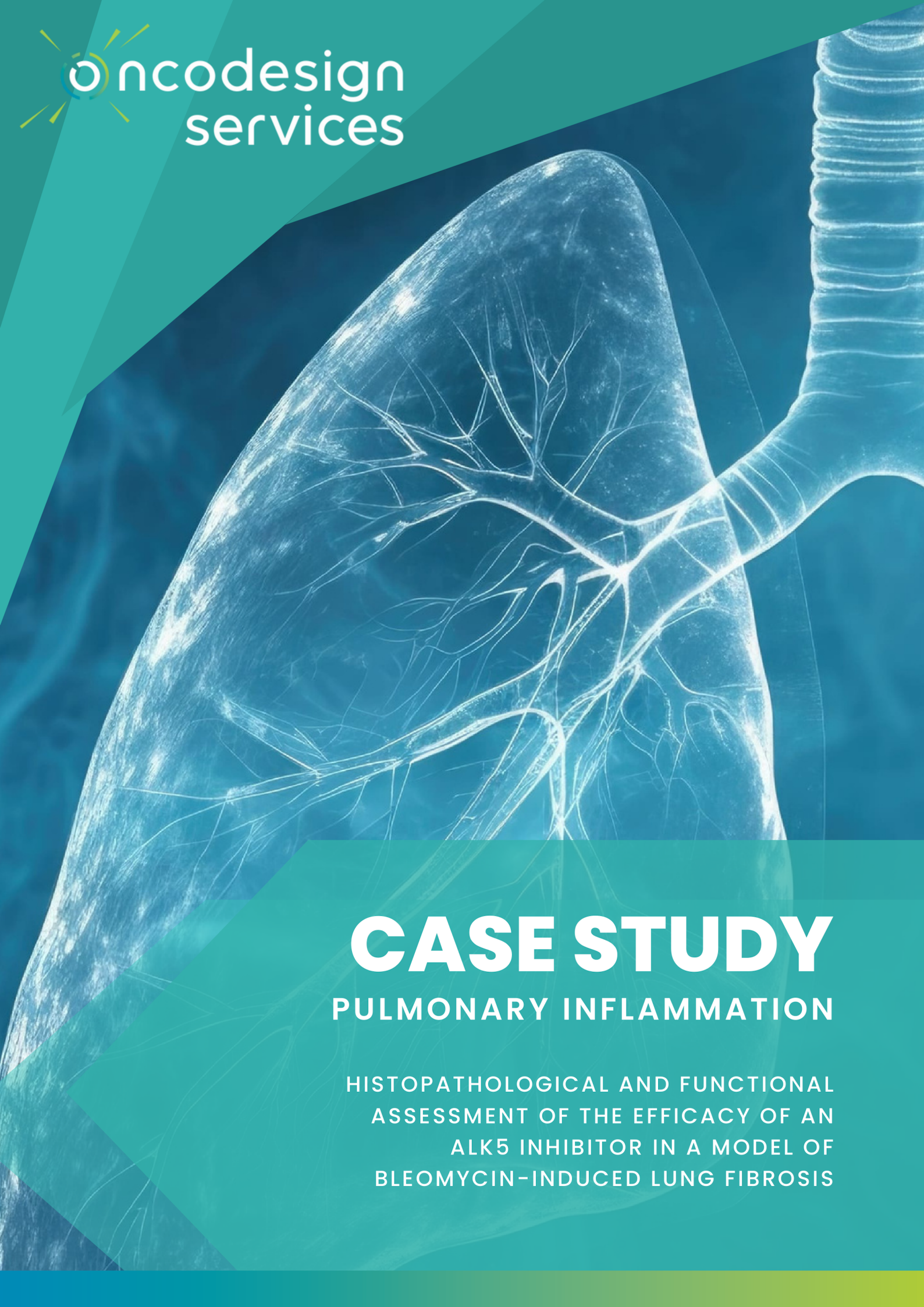


CASE STUDY

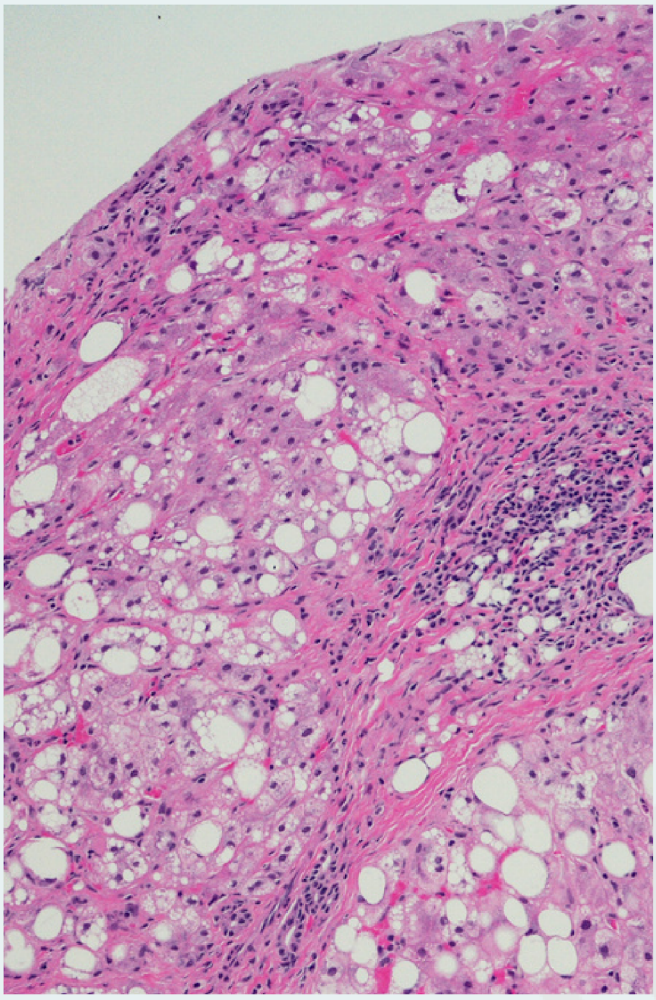
PULMONARY INFLAMMATION

HISTOPATHOLOGICAL AND FUNCTIONAL ASSESSMENT OF THE EFFICACY OF AN ALK5 INHIBITOR IN A MODEL OF BLEOMYCIN-INDUCED LUNG FIBROSIS

INTRODUCTION

The causes of idiopathic pulmonary fibrosis (IPF) are still poorly known and inadequately addressed with current medicines. Developers of novel treatments typically use the mouse bleomycin lung fibrosis model for preclinical efficacy testing, with collagen staining of the lungs post-mortem to reveal and score fibrosis in the lung tissue.

However, functional readouts of lung function are more translational and becoming more popular for preclinical Proof Of Concept evaluation. Plethysmography is defined as the measurement of mechanical parameters, **the flexiVent®** device is marketed specifically to deliver these plethysmographic data, and Oncodesign Services has recently added this instrument to our platform.



In humans, one of the most relevant parameters for following IPF patients and providing prognosis, besides imaging, is plethysmography, i.e. functional evaluation of lung function by asking the patient to perform different respiratory maneuvers into a machine measuring parameters such as air pressure and volume. Such methods can now be transferred to mice, using custom systems performing complex lung recruitment, inflation and deflation maneuvers requiring no voluntary breathing from the animal.

To address this need for more translational models, we aimed at establishing functional readouts for testing the efficacy of compounds against lung fibrosis, in the bleomycin-induced lung fibrosis rodent model. Specifically, we compared the efficacy of a known reference compound on two complementary readouts:

- **histology**, i.e. lung tissue modifications,
- **plethysmography**, i.e. measurements of mechanical parameters associated to the lung tissue

STUDY DESIGN, ADMINISTRATION OF BLEOMYCIN TO INDUCE FIBROSIS IN MICE

In this study, on D0, we administered **bleomycin** intratracheally to C57BL/6 male mice, causing acute lung inflammation, lasting for about a week, which in turn led to fibrosis developing in about 3 weeks *(Figure 1)*. One group received no Bleomycin, serving as a negative control *(G1 in Table 1)*.

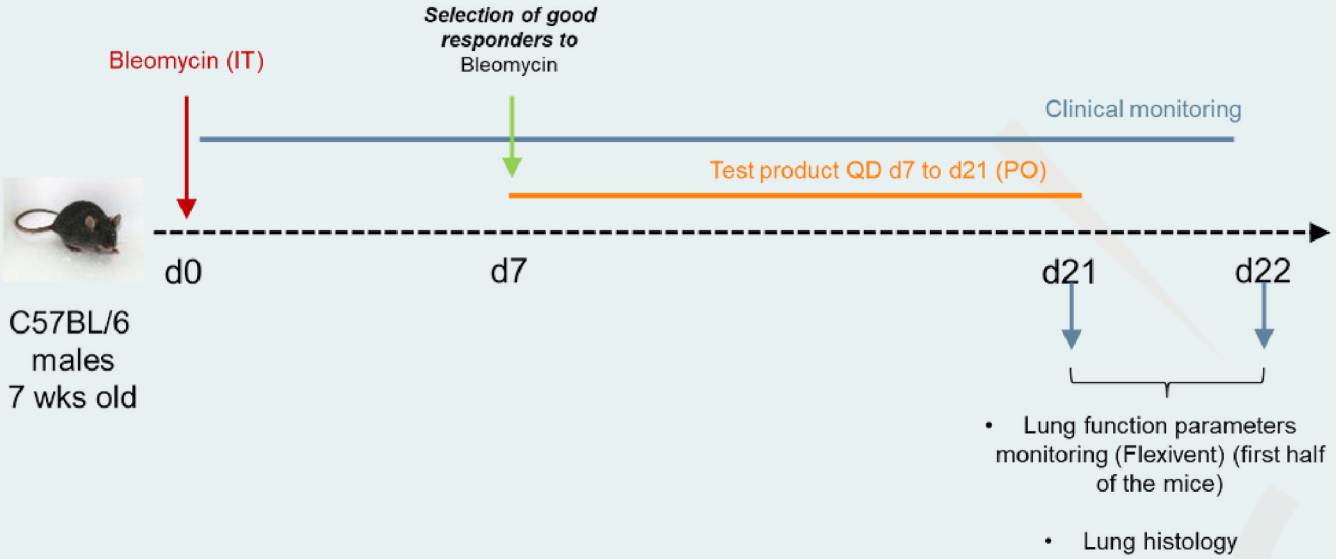


Figure 1: Study design

Group	Disease induction	Treatment	Treatment Schedule	Dose (mg/kg/inj) Volume (mL/kg/inj)	Route
G1	Saline IT at D0 (negative control)	Vehicle	Daily from D7 to D20/21	N/A 10mL/kg	PO
G2	BLM IT at D0	Vehicle	Daily from D7 to D20/21	N/A 10mL/kg	PO
G3	BLM IT at D0	ALK5 inhibitor [SB-525334]	Daily from D7 to D20/21	60mg/kg/day 10mL/kg	PO

Table 1: Study groups

Given that the inflammatory phase manifests by transient body weight loss *(Figure 2)*, peaking around D7 after challenge, we selected good responders based on body weight change (-5% to -20% weight loss range) on D7. Among the selected animals, two groups were constituted (G2 and G3 in Table 1).

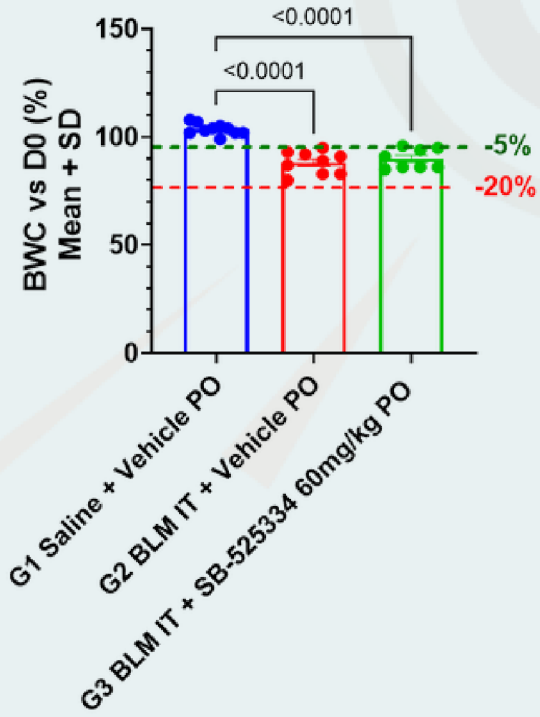


Figure 2: Randomization

One of them received vehicle, while the other received an ALK5 inhibitor (SB-525334, 60mg/kg, PO, QD). Treatment started on D7 and lasted until D21.

Once fibrosis had been allowed to develop, animals were terminated on D21 or D22 after plethysmography measurements, for lung collection and histological analysis.

Throughout the experiment, body weight loss was monitored daily, first to select good responders, then to monitor the clinical state of the animals and putative fibrosis development *(Figure 3)*. Statistical analysis revealed that the body weight change of challenged animals became significantly different from that of the non-challenged animals (G1) after 6 days.

For G2 (vehicle), this effect lasted until the end of the experiment, while it disappeared for the group receiving the ALK5 inhibitor from D12 (G3).

Taking the results together, we can suppose that fibrosis was induced. Besides, the clinical improvement of the animals receiving the ALK5 inhibitor points towards efficacy against fibrosis development.

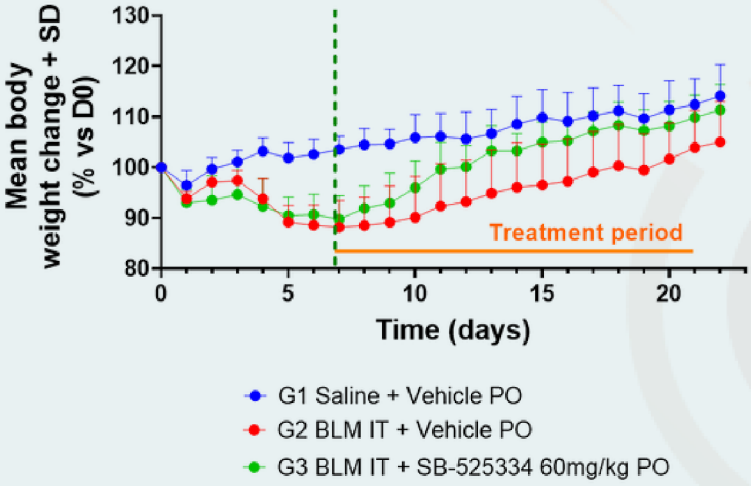


Figure 3: Evolution of body weight change (% of D0 weight)

PLETHYSMOGRAPHY READOUTS

As a terminal procedure, **plethysmography** was performed on all the animals. Briefly, animals were deeply anesthetized, and received strong opiate analgesia. Tracheotomy was performed to insert a canula, and airtightness was obtained by ligaturing the trachea on the canula. Animals were connected to the **flexiVent®** apparatus, ventilation was initiated, and pancuronium bromide was administered to obtain total absence of voluntary ventilation. Perturbations were then initiated to measure the relevant parameters. Importantly, the signal was inspected online to detect invalid perturbations, and all maneuvers were repeated to obtain three valid reads. For the sake of simplicity, only a selected panel of parameters extracted from the maneuvers is described below *(Figure 4)*.

Based on the literature in the bleomycin model and in humans with IPF, the most relevant parameters for lung fibrosis monitoring are as follows:

- **Tissue elastance (H)**: reflects the elastic energy conservation in the alveoli i.e. ability of the tissue to retract and revert to its original shape. Index of tissue stiffness.
- **Static compliance (Cst)**: reflects the intrinsic elastic properties of the respiratory system (i.e. lung + chest wall) at rest.
- **Work of Breathing (WOB)** is the amount of energy required to overcome the elastic and resistive qualities of the respiratory system; thereby producing tidal ventilation. To accurately represent the mechanical parameters of the lung tissue, WOB has to be normalized by the lung capacity (or one of its estimates). WOBn thus represents the energy required to move a fixed volume of air.

While tissue elastance increased in animals that had received bleomycin and no treatment (G2 in Figure 4A), its counterpart static compliance decreased in these animals (G2 in Figure B). That trend was corrected by the treatment with the ALK-5 inhibitor (G3). These results confirm the higher difficulty to expand the lungs in the challenged animals, and provides proof of functionally-relevant efficacy of the ALK-5 inhibitor. In coherence with this, Work of Breathing (WOBn) increased following bleomycin challenge, indicating that the energy expenditure associated with air displacement was increased (Figure 4C). These effects are summarized by the pressure-volume curves obtained, Which means that higher pressure was generated by identical air volumes when lungs have been previously exposed to bleomycin, a trend that is quite corrected by the ALK-5 inhibitor treatment (Figure 4D).

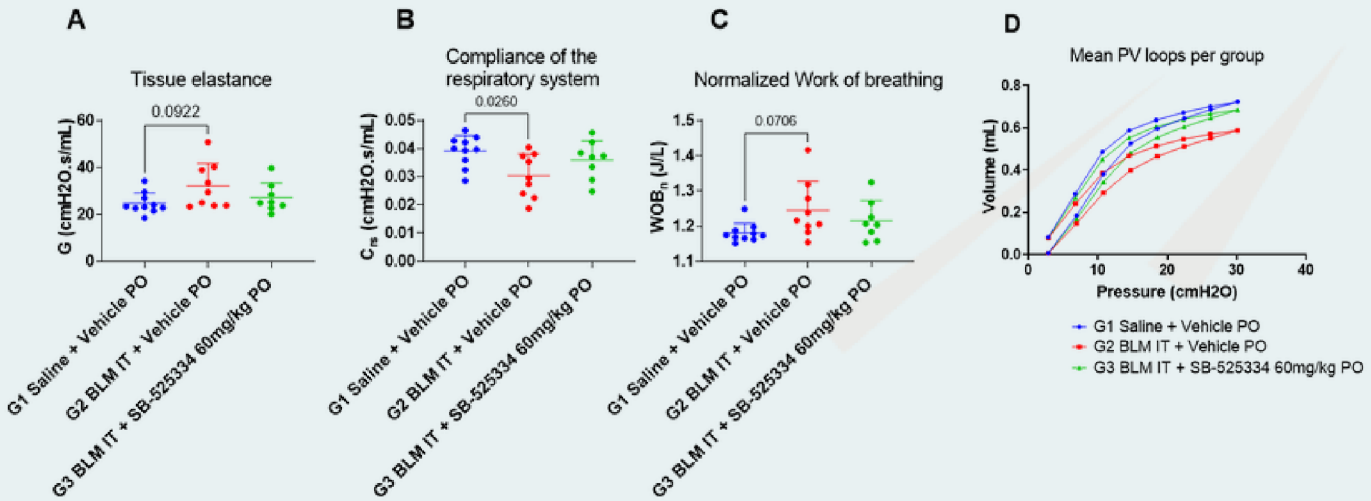


Figure 4: Lung mechanical parameters measured by the flexiVent® device

Overall, the flexiVent® assessment of the lungs of the animals provided a thorough picture of the functional deficits caused by the bleomycin aggression, and evidence that the ALK-5 inhibitor could partly correct some of the functional deficits.

HISTOLOGY READOUTS

Histology was performed on the lungs of the animals, collected post mortem. Specifically, the slides were colored with Hematoxilin and Phloxin, and with Sirius Red, to evaluate the extent and severity of fibrosis, 3 weeks after bleomycin aggression.

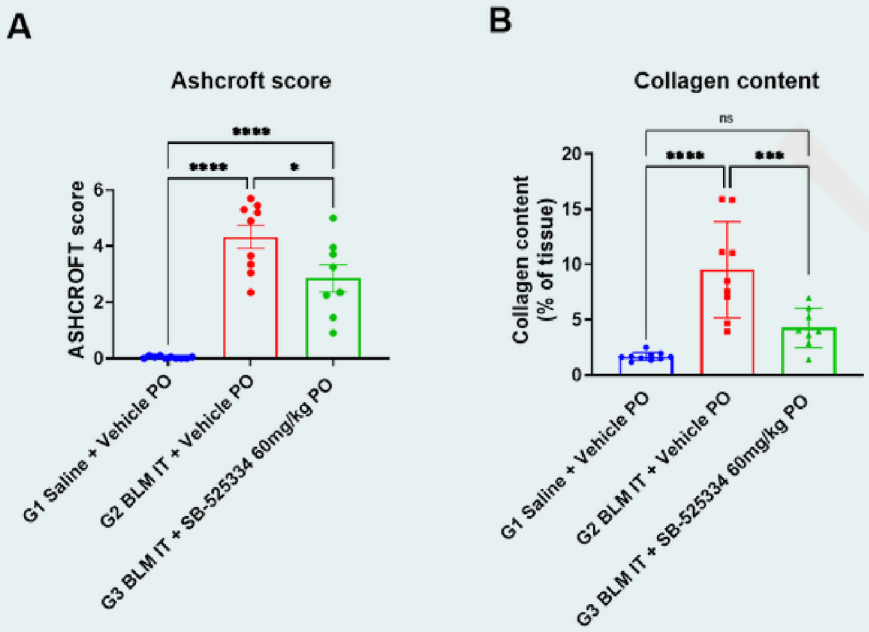
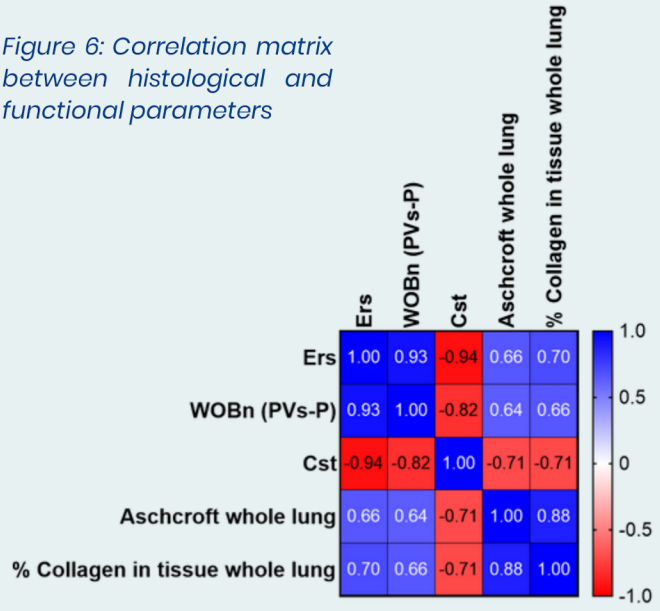


Figure 5: Lung histological parameters

Two measures were computed: the collagen content in lung sections, indicating the extent of collagen deposition *per se*, and the modified Ashcroft score (Ashcroft et al., 1988; Hübner et al., 2008), recapitulating the extent and severity of the fibrotic lesions. Both were drastically increased in the lungs of the mice that had received Bleomycin compared to the negative control group *(Figure 5)*. In comparison, this effect was partly mitigated by the treatment with the ALK-5 inhibitor.

Figure 6: Correlation matrix between histological and functional parameters



Functional (plethysmography) and histological parameters were strongly correlated with one another *(Figure 6)*, with increased collagen content and Ashcroft lesion score associated to higher elastance and work of breathing, and lower static compliance. Overall, these findings indicate increased lung stiffness i.e. lesser lung function when lesions were more severe. This confirms the coherence of the readouts while reproducing published results (Philips et al., 2012).

CONCLUSION

Overall, this study provided us with valuable insight on the impairments of lung function caused by bleomycin injury and subsequent lung fibrosis. Specifically, **flexiVent®** measurements highlighted the increased lung stiffness resulting in increased energy expenditure for ventilation. These modifications were strongly correlated with histological modifications, highlighting the coherence and complementarity between such readouts. Finally, we observed the efficacy of the reference compound, an ALK-5inhibitor, which was able to partly relieve clinical (body weight), functional (plethysmography) and histological signs of the development of lung fibrosis.

Oncodesign Services has over 25 years of experience in pharmacology services, initially in oncology but then extended to other therapeutic areas such as inflammatory diseases. A large range of *in vivo* inflammation models are readily available or can be developed to your specific needs.

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Who is Oncodesign Services?

Oncodesign Services is a Contract research organization (CRO) specializing in **drug discovery** and **preclinical services**. From target identification to IND filing, the company contributes to the development of innovative therapies in oncology, inflammation and infectious diseases.

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