KEYSTONE SYMPOSIA

Fibrosis Pathogenesis and Resolution: From Mechanisms to Therapies
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Comparison of the Histological Phenotypes of Lung Fibrosis induced by Oropharyngeal and Subcutaneous Bleomycin Administration in Mouse

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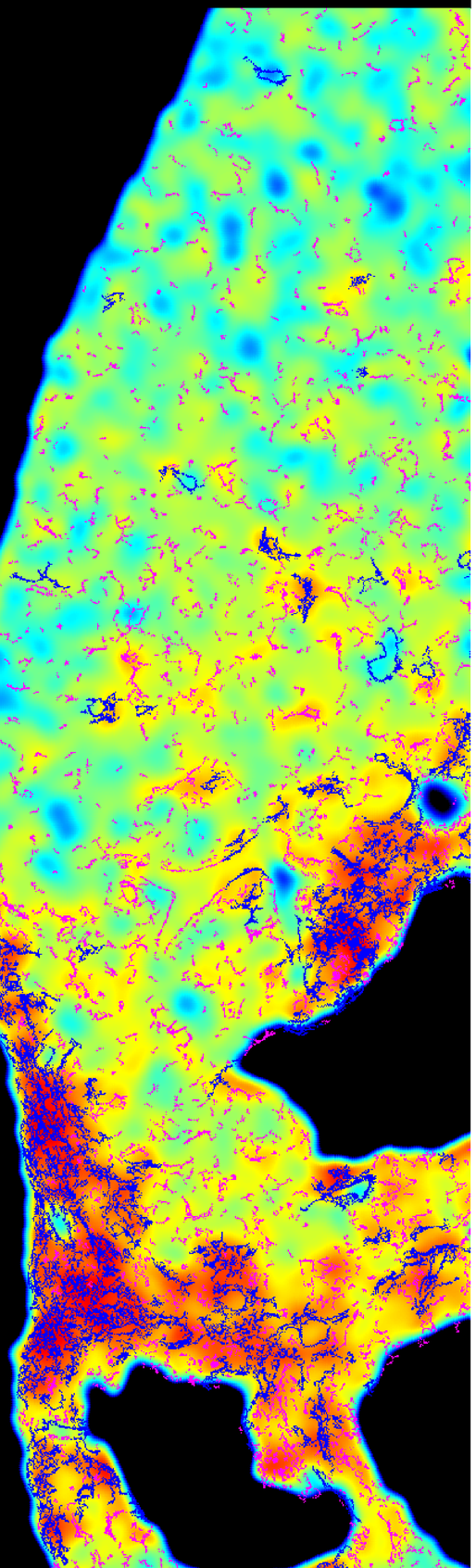
TNO innovation for life



1 Introduction

Bleomycin (BLM) is commonly used for the induction of fibrosis in the lungs of animal models. Administration of a single dose of BLM results in a transient fibrosis development which peaks around 21-28 days and returns to baseline at day 35.

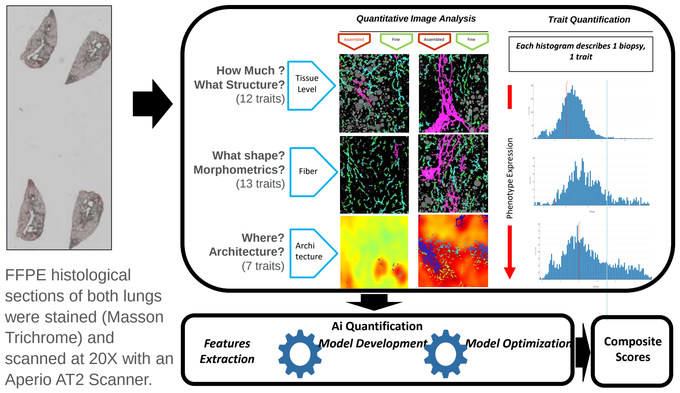
2 Aim

Here, we compare the histological outcomes between a single oropharyngeal (OP) administration and multiple (3x per week) subcutaneous (SC) injections of BLM. To detect subtle differences, we use a novel Digital Pathology quantitative image analysis and AI platform, FibroNest™ (Pharmanest, Princeton, USA) to generate and measure quantifiable Fibrosis Traits (qFTs). These parameters are used to describe the fibrosis severity and histological differences between the two methods of administration.

3 Method

Two similar fibrosis progression studies were conducted, both including a control-PBS group sacrificed on day 21 and three BLM exposed groups sacrificed on day 7, 21 and 35.

Group Name	Intervention		Day 7	Day 21	Day 35
Control	Single oropharyngeal (OP) PBS administration	N (mice count)		6	
"OP" Intervention	Single oropharyngeal (OP) BLM (0.05unit) administration		6	6	5
Control	Single oropharyngeal (OP) PBS folowed by multiple administration subcutaneous (SC) injections of PBS (3x per week)			8	
"OP + SC" Intervention	Single oropharyngeal (OP) BLM folowed by multiple administration subcutaneous (SC) injections of BLM (3x per week, 01 unit)		8	8	8



FFPE histological sections of both lungs were stained (Masson Trichrome) and scanned at 20X with an Aperio AT2 Scanner.

FibroNest™, was used to quantify the fibrosis histological phenotype including 32 traits for collagen content, fiber morphometry, and architecture, generating 315 quantitative fibrosis traits (qFT).

For each study, principal qFTs were automatically detected to best describe the progression of the Fibrosis histological phenotype and combined into a normalized Phenotypic Composite Fibrosis Score (Ph-FCS).

A Venn Diagram is constructed to identify the qFTs that are common in the two models (co-qFTs). The co-qFTs are used to create a unique composite score (co-FCS) that describe the progression of Fibrosis severity regardless of the bleomycin administration method.

4 Representative Images and Results

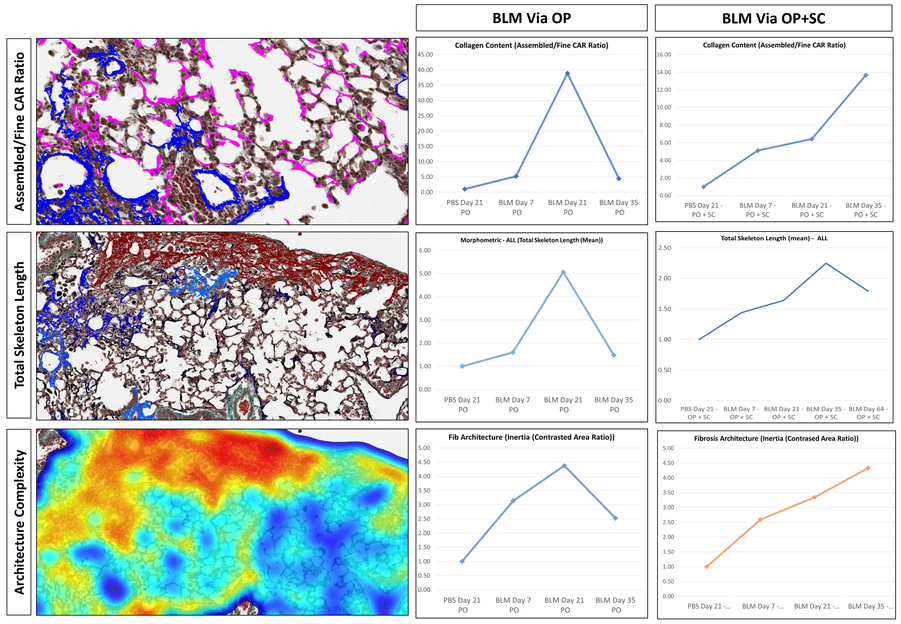
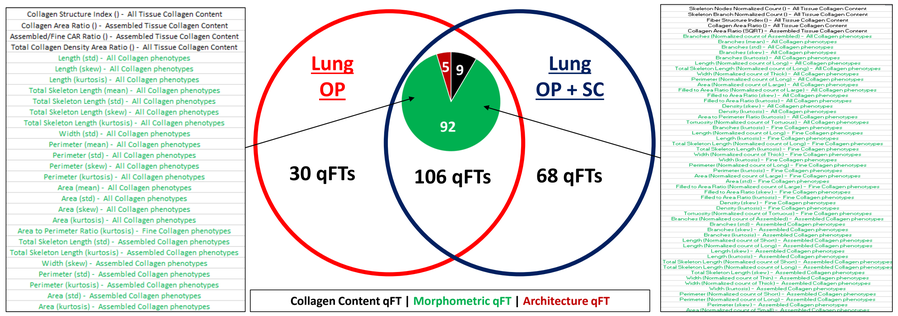
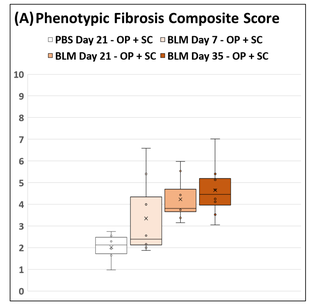


Fig. 1: Illustrative images: the three panel illustrate both visually (images and Image analysis layers) and quantitatively (charts) the evolution of three chose qFTs (Ration of assembled to fine collagen fibers, Total length of the fiber skeleton and area ratio (orange to red only) of complex fibrosis architecture. Differences are observed at early or late timepoints.





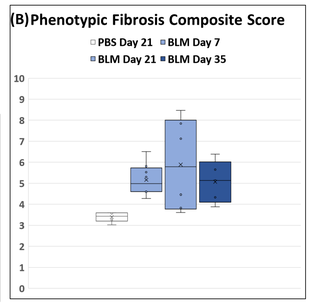
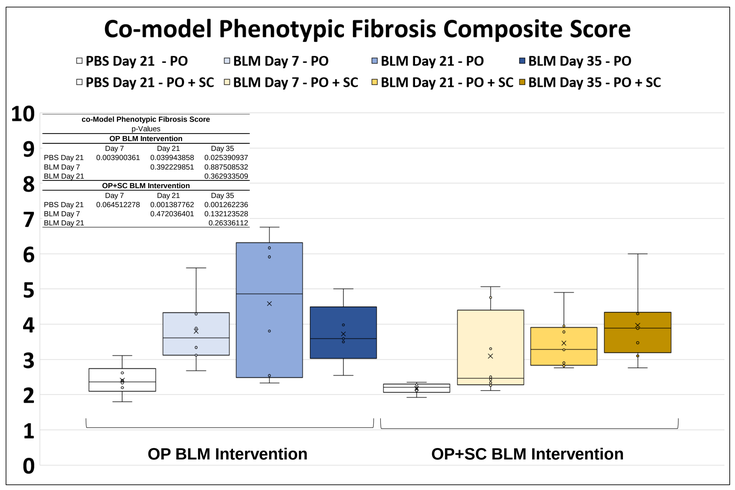


Fig. 2 (Top): Composite Scores: the principal qFTs identified in each study are combined into a Phenotypic Composite Score.

Fig. 3 (Left): Veen Diagram: for each study and intervention, the qFTs that account for the variance in the progression of fibrosis severity (principal qFTs) are selected (>25% change from control to d35, p<0.05). Principal qFTs are assembled in a Fibrosis Composite Score (Fig. 2). 106 principal qFTs are common to the two models, most (92) being related to the morphometric traits of the collagen fibers. These co-qFTs are used to generate a composite score (Co-model Composite Score, Fig. 4) that describe fibrosis progression in the two models.



5 Conclusions

Quantitative Digital Pathology (FibroNest) of stained lung sections was able to quantify and compare the histological phenotypes of fibrosis in OP+SC and OP administration methods of Bleomycin in mouse.

OP administration was shown to be more aggressive and rapid whereas OP+SC elicited a milder but more continuous response from the model.

Furthermore, FibroNest was able to identify differences in fibrosis phenotypes between the two models.

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