ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA
DIPARTIMENTO DI SCIENZE MEDICHE VETERINARIE

Dottorato di ricerca in Scienze Veterinarie
35° Ciclo- A.A. 2021-2022
Anno di attività: 3°

Dott: Zahra KhalajZeyqami
Tutor: Prof.ssa Luciana Giardino

Development of a new and long-lasting murine model of lung fibrosis for preclinical studies

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease with no curative pharmacological therapy, characterized by progressively impaired pulmonary function and a median survival of 2-4 years after diagnosis.

The available animal models, can not fully replicate human IPF. Although bleomycin (BLM)-induced fibrosis is the most used and best-characterized model either to investigate lung fibrotic mechanisms or to drug screening , but a comprehensive guideline reporting optimal doses, routes and frequency of BLM is still lacking.

OUR MAIN OBJECTIVE

was to identify the ideal BLM dose regimen to reach an appropriate balance between sustained lung fibrosis up to 28 days and animal welfare in C57Bl/6 male mice, to extended therapeutic window to 3 weeks, which allows to reveal the drugs side effects due to longer drug exposition, as well as lighting up more in-depth the anti-fibrotic efficacy in primary screening.

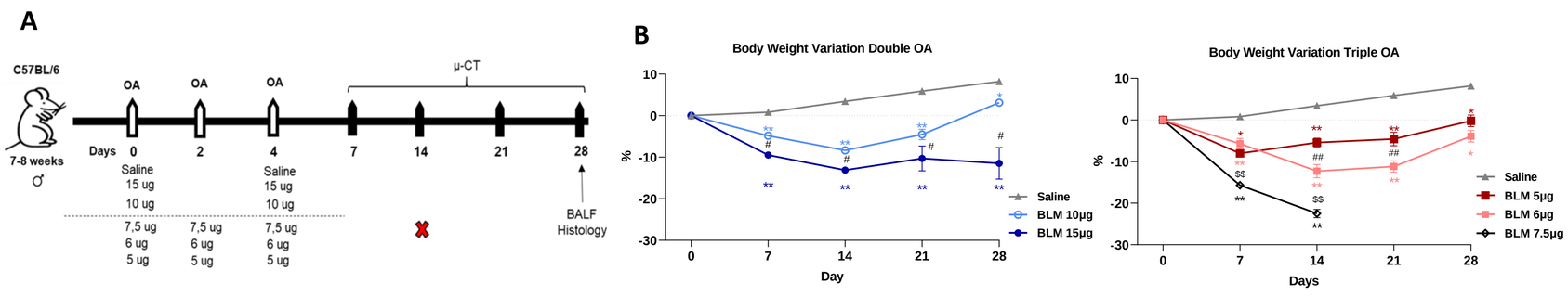
Micro-CT has been used as a noninvasive imaging technique to quantify lung fibrosis progression. Micro-CT application could decrease intra-experimental variability, and, as a result, significantly reducing the number of animals used per experiment, in agreement with the 3R's principles.

METHODS

7-8-week-old male C57Bl/6 mice(~26 gr) were used to induce IPF by bleomycin hydrochloride (Baxter) via a double oropharyngeal administration (OA) under 2.5% isoflurane anaesthesia (Fig.1.A). The fibrosis progression was longitudinally assessed by micro-CT every 7 days till day 28. Quantitative micro-CT parameters; Lung aeration degrees normalized on total lung volume and Air/Tissue ratio (A/T) were validated then at day 28 with lung histology and Bronchoalveolar Lavage Fluid (BALF) cells. All data are presented as mean ± SEM. One or two-way (ANOVA) was performed, followed by Dunnett or Tukey's multiple comparison post-hoc tests to compare different experimental groups.

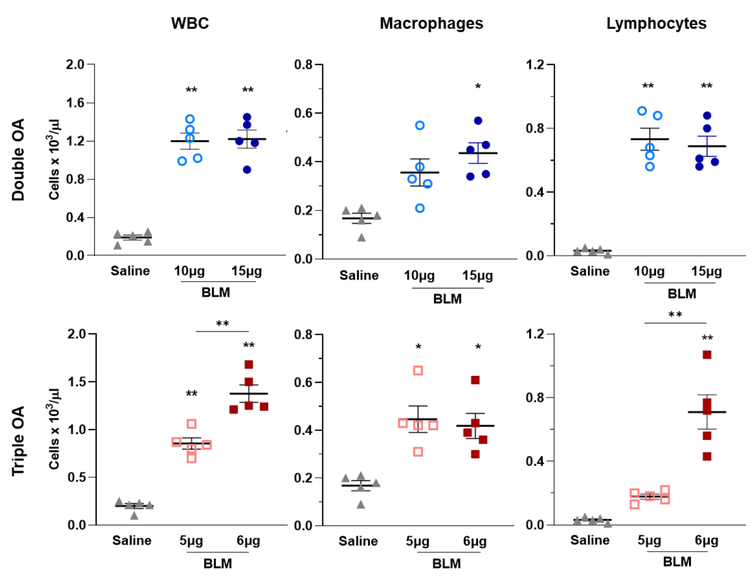
P-value <0.05 (*), n= 5 each group.

1.Experimental Design and Body Weight Variation



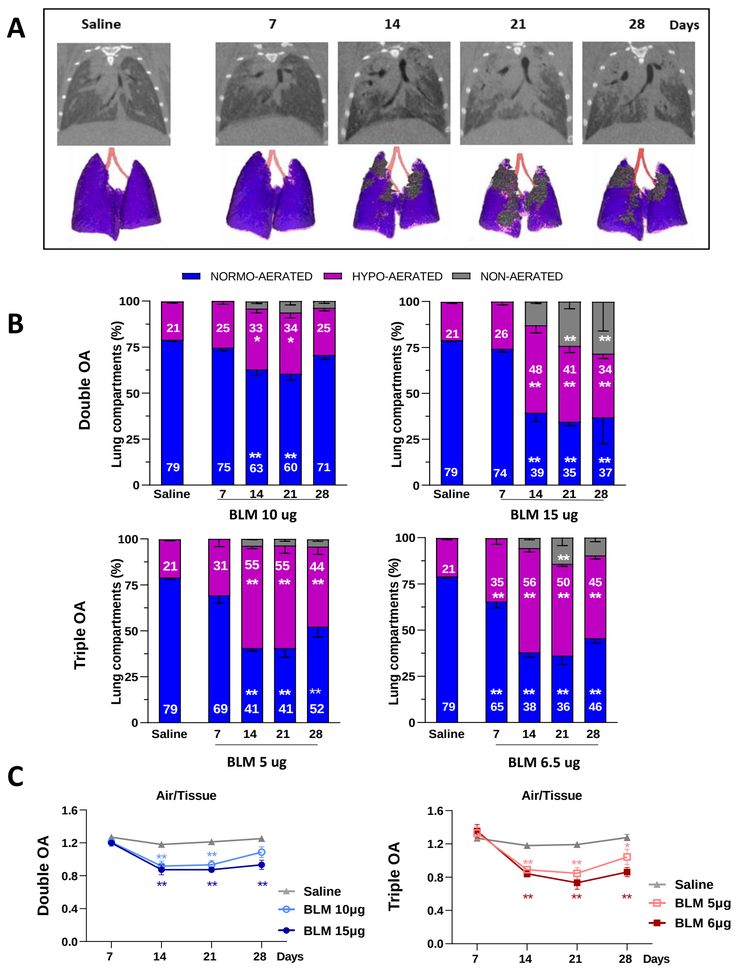
B: Body weight variation expressed as % variation respect to day 0, Animal received triple dose of 7 ug BLM, were euthanized at day 14 due to >20% body weight loss. Other groups except double OA of 15ug BLM recovered body weight after day 21.

4. Bronchoalveolar Lavage Fluid Cells



Total WBC and and leukocyte subpopulations indicate higher cell numbers in BLM-dosed groups.

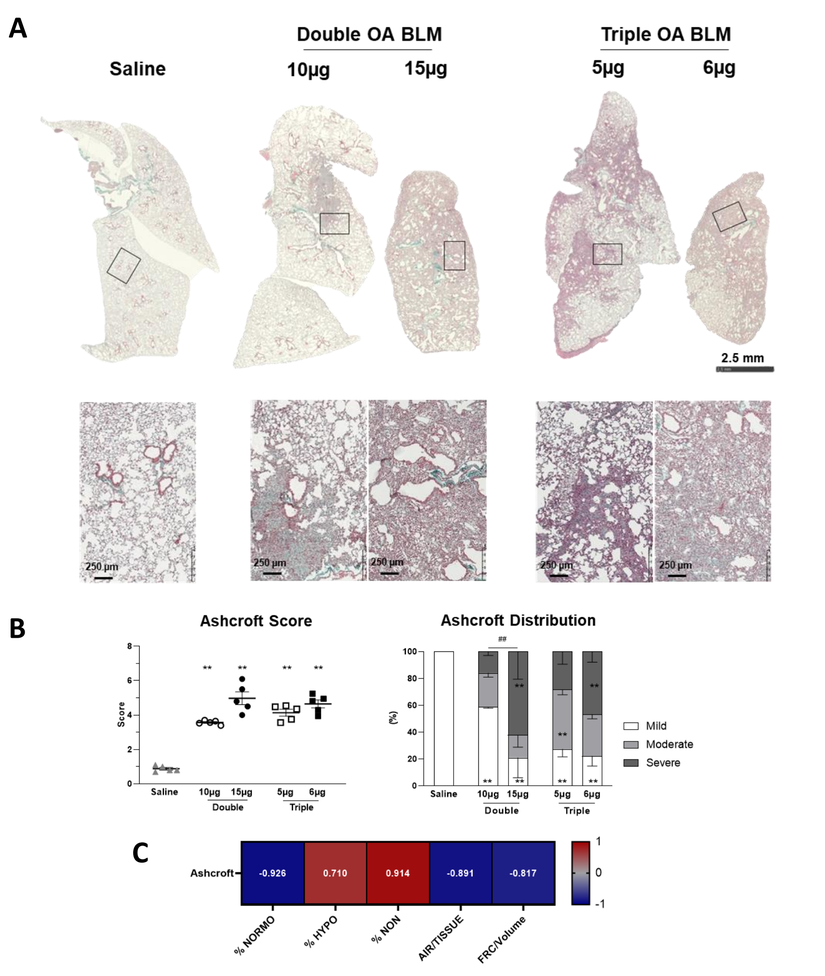
2. Longitudinal Micro-CT assessment of lungs



A: Representative Sagittal and spatial reconstruction images at the end of expiration phase of saline- and BLM- mice at 7, 14, 21 and 28 days . B: Lung aeration degrees expressed as percentage of normo-, hypo-, and non-aerated tissues at 7, 14, 21, 28 days for the saline group and BLM groups at different dose regimens show a significant increase of non-aerated tissue in higher dose of BLM.

C: Longitudinal quantification of air/tissue show a high sensibility to evaluate the lung fibrosis from day 14 to 28. its decline remained significantly lower compared to saline in higher dose of BLM.

3. Histological Analysis and its correlation with µ-CT



A: Representative microphotographs of saline, BLM- treated groups. stained with Masson's trichrome (MT) at day 28 show the collagen deposition (Blue) in lung parenchyma. The squares indicate the selected zone with higher magnification.

B: The Ashcroft indicate the significant collagen deposition in the lungs. Ashcroft frequency distribution in saline and BLM groups highlight the significant severe-injured parenchyma in groups treated with higher dose of BLM.

C: Spearman's correlation confirmed well- correlation between Micro-CT parameters and Ashcroft Score.

Conclusion and future perspective

In this study Micro-CT was used in development of an optimized mouse model of lung fibrosis. Longitudinal evaluations, allowing a drastic reduction in the number of animals used to reach statistically significant results and/or variability. This will permit a valuable decrease in costs and time to develop disease models. Moreover, the assessment of air and tissue volumes, coupled with the quantification of lung aeration degrees provides a wide and detailed overview of the alterations affecting disease progression, allowing selection of the optimal protocol for BLM studies. Notably, these readouts could be harnessed to evaluate the therapeutic efficacy of a drug candidate as well, facilitating the development of new therapies for IPF patients. we identified a triple dose of 6 µg BLM as the optimal regimen to induce a sustained lung fibrosis that doesn't spontaneously revert after 21 days, thus extending the time window for pharmacological treatment from 2 to 3 weeks.

Next Step… (ongoing)

Histological characterization of selected dose (triple 6ug) at day 7, 14,21,28 and 35 and Evaluate the anti-fibrotic effect of Nintedanib on this murine model for 3 weeks.

Period Abroad

Visiting PhD student ; Department of Pulmonary medicine of Erasmus Medical Center. Rotterdam- Netherlands (Feb 2022-now)

Project: *Development of a new murine model of pulmonary fibrosis via administration of adenoviral vector expressing transforming growth factor beta 1. (Ad.TGF-β1) virus.*

Tashiro, J. et al. Exploring Animal Models That Resemble Idiopathic Pulmonary Fibrosis. Front. Med. (2017) doi:10.3389/FMED.2017.00118.
Ruscitti, F. et al. Quantification of Lung Fibrosis in IPF-Like Mouse Model and Pharmacological Response to Treatment by Micro-Computed Tomography. Front. Pharmacol. ***11****, 1 (2020).*