



# Commentary: Imaging Biomarkers and Pathobiological Profiling in a Rat Model of Drug-Induced Interstitial Lung Disease (DIILD) Induced by Bleomycin

Sotirios I. Sinis<sup>1,2</sup> and Sotirios G. Zarogiannis<sup>1,2\*</sup>

<sup>1</sup> Department of Physiology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece,

<sup>2</sup> Department of Respiratory Medicine, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece

**Keywords:** animal models, bleomycin, imaging biomarkers, translational research, drug induced interstitial lung disease

A Commentary on:

**Imaging Biomarkers and Pathobiological Profiling in a Rat Model of Drug-Induced Interstitial Lung Disease (DIILD) Induced by Bleomycin**

by Mahmutovic Persson, I., Falk Håkansson, H., Örbom, A., Liu, J., von Wachenfeldt, K., and Olsson, L. E. (2020). *Front. Physiol.* 11:584. doi: 10.3389/fphys.2020.00584

## OPEN ACCESS

### Edited by:

Andrew John Halayko,  
University of Manitoba, Canada

### Reviewed by:

Miranda Kirby,  
Ryerson University, Canada  
Asher Mendelson,  
University of Manitoba, Canada

### \*Correspondence:

Sotirios G. Zarogiannis  
szarog@med.uth.gr

### Specialty section:

This article was submitted to  
Respiratory Physiology,  
a section of the journal  
*Frontiers in Physiology*

**Received:** 06 April 2021

**Accepted:** 06 July 2021

**Published:** 28 July 2021

### Citation:

Sinis SI and Zarogiannis SG (2021)  
Commentary: Imaging Biomarkers  
and Pathobiological Profiling in a Rat  
Model of Drug-Induced Interstitial  
Lung Disease (DIILD) Induced by  
Bleomycin. *Front. Physiol.* 12:691650.  
doi: 10.3389/fphys.2021.691650

Drug-induced interstitial lung disease (DIILD) is a clinical entity bearing significant morbidity and mortality that presents with non-specific symptoms, more frequently cough, dyspnea, wheezing, hemoptysis, fever, and pleuritis (Matsuno, 2012; Carrington et al., 2018). Management heavily relies upon early suspicion, elimination of other causes and withdrawal of the offending agent. High-resolution computed tomography (HRCT) is an integral part of the diagnostic algorithm for DIILD albeit it does not provide pathognomonic evidence in most cases. Pulmonary insult from bleomycin, a chemotherapeutic used in Hodgkin lymphoma and germ cell tumors, is a commonly encountered DIILD paradigm characterized by specific radiological patterns (Skeoch et al., 2018). Series of case reports involving patients undergoing chemotherapy offer insight regarding the potential usefulness of HRCT with FDG-PET in the management of DIILD. During follow-up for assessment of treatment outcomes, new areas of FDG uptake in the lung parenchyma should alert the clinician as they likely represent early stages of an inflammatory process prior to the appearance of symptoms or notably HRCT abnormalities (Paschali et al., 2017; Beyhan Sagmen et al., 2019). On the other hand, pulmonary lesions showing signal attenuation could represent resolution of the disease or progression to fibrosis (Taywade et al., 2016; Paschali et al., 2017). There are many unanswered questions pertaining to the onset and temporal progression of DIILD that would be ethically, logistically and financially unsound to resolve in human studies. On these grounds, preclinical animal models that mimic the disease development of DIILD should be standardized in order to generate extrapolatable data on imaging biomarkers, a promising tool that will pave the way for advancing timely diagnosis and more effective therapy (Mahmutovic Persson et al., 2020b).

A great start toward this direction was achieved in a recently published study in *Frontiers in Physiology* by Mahmutovic-Persson et al. establishing a rat model of DIILD induced by bleomycin, with a very elaborate set of data comprising temporal evaluation of imaging biomarkers (MRI and PET/CT), lung tissue gene expression patterns, BALF cell counts and cytokine profiles in the time course of up to 28 days post bleomycin administration (Mahmutovic Persson et al., 2020a). This is one of the first comprehensive studies employing the conventional bleomycin-lung

injury model, which is praised for its clinical relevance, reproducibility and repeatability, in the context of preclinical model development for DIILD surveillance through imaging biomarkers. Prior to this work, literature involving intratracheal instillation of bleomycin mainly comprised preclinical fibrosis and IPF models which take advantage of the latent stage of the response starting from day 14 with maximal intensity at days 21–28 to recapitulate and study fibrosis in humans. Considerable heterogeneity in experimental conditions often permits qualitative comparison of datasets. Gadolinium and gallium based probes in conjunction with MRI or PET have been successfully tested in tracking fibrosis progression and resolution (Caravan et al., 2013; Chen et al., 2017; Desogere et al., 2017). On the other hand, a limited set of evidence describes employment of labeled polymorphonuclear cells and leukocytes that visualize the preceding inflammation that culminates at approximately day 7 post exposure (Das et al., 1988; Bondue et al., 2015). Another novel aspect of the study of Mahmutovic-Persson et al. is the use of MRI and PET post-bleomycin administration (1,000 iU once) to stratify rats based on lung volume expansion in High and Low responders (Mahmutovic Persson et al., 2020a). A quantitatively similar volume expansion assessed by MRI has been previously reported by Egger et al. in Sprague-Dawley rats receiving 2 and 3 mg/kg of bleomycin by oropharyngeal aspiration (Egger et al., 2014). MRI experiments showed that the increased lung volume in high-responders persisted and fibrotic lesions regressed at day 28, in contrast to the low responders whose MRI imaging biomarkers returned to baseline. Uptake pattern of FDG peaked in conjunction with inflammation at day 7 in exposed rats but remained elevated longer in the high responders (14 days) and was able to discriminate the relapse at day 28. This corroborates animal and patient derived data suggesting FDG could also be a biomarker of fibrosis (Bondue et al., 2015; Justet et al., 2017). Importantly these features were also reflected in the general health check of the animals. To validate the imaging biomarkers, apart from consistent histology patterns, the authors demonstrated that plasma exudation began at day 3 measured by BALF protein content accompanied by neutrophilic invasion

which was then followed by leukocytes, macrophages and eosinophils at day 7 according to BALF cytology. Concomitant studies relevant to gene expression patterns revealed differential lung gene expression of pro-inflammatory and pro-fibrotic genes in the bleomycin group while further analysis showed that there was differential expression of such genes between the Low and High bleomycin responders.

A very important issue in preclinical models is the efficient representation of the human disease in the murine model. Under realistic conditions, the lung is exposed through the systemic circulation in a continuous or intermittent fashion, which is in direct contrast with the bolus administration in the trachea that may (1) elicit pronounced acute inflammatory responses, (2) achieve inhomogeneous distribution throughout the lung, and (3) fail to properly simulate irreversible progressive fibrosis as seen in patients. Another significant limitation of the wide application of the model currently is the availability of the imaging modalities but progressively it is reasonable that core facilities using small animal MRI and PET/CT will increase since non-invasive translational approaches are encouraged.

Overall, the study of Mahmutovic-Persson et al. represents a very well-thought and executed study that provides multilevel information at the whole body, cellular, molecular, and imaging levels with important translational implications. The approach provides a solid foundation for advancement of: (1) preliminary screening protocols for: (i) pulmonary toxicity before sizeable funds are allocated to advance a drug to the clinic (ii) selection of appropriate agents to evaluate in more clinically translatable chronic *in vivo* models, and (2) the rational design of clinical trials aiming to: (i) promptly identify patients that will develop progressive DIILD and (ii) assess established and most importantly experimental therapeutic interventions in that population.

## AUTHOR CONTRIBUTIONS

SIS and SGZ wrote the manuscript and approved the submitted version.

## REFERENCES

- Beyhan Sagmen, S., Comert, S., Turan Erkek, E., Küçüköz Uzun, A., Dogan, C., Yilmaz, G., et al. (2019). Can we predict bleomycin toxicity with PET-CT? *Acta Haematol.* 142, 171–175. doi: 10.1159/000502374
- Bondue, B., Sherer, F., Van Simaey, G., Doumont, G., Egrise, D., Yakoub, Y., et al. (2015). PET/CT with 18F-FDG- and 18F-FBEM-labeled leukocytes for metabolic activity and leukocyte recruitment monitoring in a mouse model of pulmonary fibrosis. *J. Nucl. Med.* 56, 127–132. doi: 10.2967/jnumed.114.147421
- Caravan, P., Yang, Y., Zachariah, R., Schmitt, A., Mino-Kenudson, M., Chen, H. H., et al. (2013). Molecular magnetic resonance imaging of pulmonary fibrosis in mice. *Am. J. Respirat. Cell Mol. Biol.* 49, 1120–1126. doi: 10.1165/rcmb.2013-0039OC
- Carrington, R., Jordan, S., Pitchford, S. C., and Page, C. P. (2018). Use of animal models in IPF research. *Pulm. Pharmacol. Ther.* 51, 73–78. doi: 10.1016/j.pupt.2018.07.002
- Chen, H. H., Waghorn, P. A., Wei, L., Tapias, L. F., Schu Hle, D. T., Rotile, N. J., et al. (2017). Molecular imaging of oxidized collagen quantifies pulmonary and hepatic fibrogenesis. *JCI Insight* 2:91506. doi: 10.1172/jci.insight.91506
- Das, D. K., Steinberg, H., Bandyopadhyay, D., Hoory, S. (1988). Potential use of indium-111-labeled polymorphonuclear leukocytes for the detection of lung microvascular injury. *J Nucl Med.* 29, 657–662.
- Desogere, P., Tapias, L. F., Hariri, L. P., Rotile, N. J., Rietz, T. A., Probst, C. K., et al. (2017). Type I collagen-targeted PET probe for pulmonary fibrosis detection and staging in preclinical models. *Sci. Transl. Med.* 9:aaf4696. doi: 10.1126/scitranslmed.aaf4696
- Egger, C., Gerard, C., Vidotto, N., Accart, N., Cannet, C., Dunbar, A., et al. (2014). Lung volume quantified by MRI reflects extracellular-matrix deposition and altered pulmonary function in bleomycin models of fibrosis: effects of SOM230. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 306, L1064–L1077. doi: 10.1152/ajplung.00027.2014
- Justet, A., Laurent-Bellue, A., Thabut, G., Dieudonne, A., Debray, M. P., Borie, R., et al. (2017). [(18)F]FDG PET/CT predicts progression-free survival in patients with idiopathic pulmonary fibrosis. *Respir. Res.* 18:74. doi: 10.1186/s12931-017-0556-3
- Mahmutovic Persson, I., Falk Håkansson, H., Örbom, A., Liu, J., von Wachenfeldt, K., and Olsson, L. E. (2020a). Imaging biomarkers and pathological profiling

- in a rat model of drug-induced interstitial lung disease induced by bleomycin. *Front. Physiol.* 11:584. doi: 10.3389/fphys.2020.00584
- Mahmutovic Persson, I., von Wachenfeldt, K., Waterton, J., and Olsson, L. (2020b). Imaging biomarkers in animal models of drug-induced lung injury: a systematic review. *J. Clin. Med.* 10:107. doi: 10.3390/jcm10010107
- Matsuno, O. (2012). Drug-induced interstitial lung disease: mechanisms and best diagnostic approaches. *Respir. Res.* 13:39. doi: 10.1186/1465-9921-13-39
- Paschali, A., Gnanasegaran, G., and Cook, G. (2017). FDG PET-CT: need for vigilance in patients treated with bleomycin. *Indian J. Nucl. Med.* 32:122. doi: 10.4103/0972-3919.202242
- Skeoch, S., Weatherley, N., Swift, A., Oldroyd, A., Johns, C., Hayton, C., et al. (2018). Drug-induced interstitial lung disease: a systematic review. *J. Clin. Med.* 7:356. doi: 10.3390/jcm7100356
- Taywade, S. K., Kumar, R., Bhethanabhotla, S., Bal, C. (2016). Role of 18F-FDG PET-CT in monitoring the cyclophosphamide induced pulmonary toxicity in patients with breast cancer — 2 case reports. *Nucl. Med. Mol. Imaging* 50, 261–265. doi: 10.1007/s13139-015-0388-3

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Sinis and Zarogiannis. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.