critically depends on standardized tracheal inflation pressure and volume, in the absence of a pleural leakage.

In summary, based on a search of the literature and on the analysis of our own SuHx rat studies (Table 1 and Figure 1), we cannot confirm the presence of moderate or severe emphysema in the established SuHx rat model of PAH. At most, there may be mild enlargement of intraalveolar spaces depending on rat strain, number of SU5146 doses, and timing of lung harvest. In contrast, there is ample evidence that repetitive SU5416 injections alone (i.e., blockade of VEGFR2 and other kinases), in the absence of hypoxia, can produce an emphysema-like lung phenotype, but the latter mainly occurs in younger rats in which postnatal lung development may still be ongoing (3). Our data provide evidence that the SuHx rat model, when yielding RVSP consistently >60 mm Hg, using adequate controls and standardized lung inflation, is currently one of the best rodent models for studying PAH and pulmonary vascular disease. The SuHx rat model allows the study of the mechanisms of cardiovascular remodeling, vessel loss and RV failure, and lacks a biologically relevant emphysema-like lung phenotype. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

Harm J. Bogaard, M.D., Ph.D.*
Amsterdam UMC
Amsterdam, the Netherlands

Ekaterina Legchenko, Ph.D.*
Hannover Medical School
Hannover, Germany

Ketul R. Chaudhary, Ph.D.
Ottawa Hospital Research Institute and the University of Ottawa
Ottawa, Ontario, Canada

Xiao-Qing Sun, M.D., Ph.D.
Amsterdam UMC
Amsterdam, the Netherlands

Duncan J. Stewart, M.D.
Ottawa Hospital Research Institute and the University of Ottawa
Ottawa, Ontario, Canada

Georg Hansmann, M.D., Ph.D.‡
Hannover Medical School
Hannover, Germany

ORCID IDs: 0000-0001-5371-0346 (H.J.B.); 0000-0001-7949-2973 (E.L.); 0000-0003-1725-7438 (K.R.C.); 0000-0002-9113-8691 (D.J.S.); 0000-0003-0709-3935 (G.H.).

*These authors contributed equally as first authors.
‡Corresponding author (e-mail: georg.hansmann@gmail.com).

References

1. Bonnet S, Provencher S, Guignabert C, Perros F, Boucherat O, Schermuly RT, *et al*. Translating research into improved patient care in pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2017;195: 583–595.
2. Kojonazarov B, Hadzic S, Ghofrani HA, Grimminger F, Seeger W, Weissmann N, *et al*. Severe emphysema in the SU5416/hypoxia rat model of pulmonary hypertension. *Am J Respir Crit Care Med* [online ahead of print] 29 Apr 2019; DOI: 10.1164/rccm.201902-0390LE.
3. Kasahara Y, Tuder RM, Taraseviciene-Stewart L, Le Cras TD, Abman S, Hirth PK, *et al*. Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. *J Clin Invest* 2000;106:1311–1319.
4. Taraseviciene-Stewart L, Kasahara Y, Alger L, Hirth P, Mc Mahon G, Waltenberger J, *et al*. Inhibition of the VEGF receptor 2 combined with chronic hypoxia causes cell death-dependent pulmonary endothelial cell proliferation and severe pulmonary hypertension. *FASEB J* 2001; 15:427–438.
5. Abe K, Toba M, Alzoubi A, Ito M, Fagan KA, Cool CD, *et al*. Formation of plexiform lesions in experimental severe pulmonary arterial hypertension. *Circulation* 2010;121:2747–2754.
6. Legchenko E, Chouvarine P, Borchert P, Fernandez-Gonzalez A, Snay E, Meier M, *et al*. PPARγ agonist pioglitazone reverses pulmonary hypertension and prevents right heart failure via fatty acid oxidation. *Sci Transl Med* 2018;10:eaao0303.
7. de Raaf MA, Schalij I, Gomez-Arroyo J, Rol N, Happé C, de Man FS, *et al*. SuHx rat model: partly reversible pulmonary hypertension and progressive intima obstruction. *Eur Respir J* 2014;44:160–168.
8. Jiang B, Deng Y, Suen C, Taha M, Chaudhary KR, Courtman DW, *et al*. Marked strain-specific differences in the SU5416 rat model of severe pulmonary arterial hypertension. *Am J Respir Cell Mol Biol* 2016;54: 461–468.
9. Dean A, Gregorc T, Docherty CK, Harvey KY, Nilsen M, Morrell NW, *et al*. Role of the aryl hydrocarbon receptor in Sugen 5416-induced experimental pulmonary hypertension. *Am J Respir Cell Mol Biol* 2018;58:320–330.
10. Dabral S, Tian X, Kojonazarov B, Savai R, Ghofrani HA, Weissmann N, *et al*. Notch1 signalling regulates endothelial proliferation and apoptosis in pulmonary arterial hypertension. *Eur Respir J* 2016;48:1137–1149.

Reply to Bogaard *et al.*

From the Authors:

We thank Bogaard and coworkers for opening such an important discussion on the role of emphysema in the SU5416/hypoxia (SuHx) rat model of pulmonary hypertension. In our study (1), we subcutaneously injected male Wistar-Kyoto (WKY) rats at the age of 8–10 weeks (Janvier Labs) with Sugen 5416 (Su5416, 20 mg/kg body weight; Tocris) dissolved in DMSO, followed by chronic hypoxia (10% oxygen) exposure for 21 days and normoxia reexposure for an additional 14 days (SuHx). Microscopic computed tomography (μCT)-derived end-expiratory lung volume was used to estimate lung density, FRC, and air-to-tissue ratio. We demonstrated the presence of pulmonary emphysema in WKY rats subjected to SuHx in comparison with normoxic control by *in vivo* high-resolution μCT. We further verified the results in histology, suggesting that high-resolution μCT is a powerful tool in monitoring the disease progression in SuHx rats.

We fully agree with the authors that the histological airspace assessment critically depends on the fixation protocol and degree of the lung inflation. In our study, we used an established

Supported by the Deutsche Forschungsgemeinschaft, Projektnummer 268555672, SFB 1213, Project CP02, A07, and A08.

Originally Published in Press as DOI: 10.1164/rccm.201907-1367LE on August 22, 2019

standardized fixation protocol that is commonly used in our laboratory for lungs that will undergo emphysema quantification. After vascular perfusion with saline solution, fixation was performed under simultaneous vascular perfusion (22 cm H_2O) and inflation (12 cm H_2O) with formalin (Otto Fischar GmbH). After 10 minutes of fixation, the lung has been removed and kept in formalin solution for 24 hours, followed by dehydration and paraffin embedding. This method prevents atelectasis formation and ensures constant airway pressure during fixation of the lung structure for further quantification by standard morphometric assessment of the mean linear intercept (MLI) that is established in our group (2–4).

The authors demonstrated that MLI remained unchanged in SuHx rat lungs compared with the corresponding control lungs in Sprague Dawley (SD) rats obtained from Charles River. However, our results are confirmed by the group of Duncan Stewart, in which investigators revealed the increased MLI in the SuHx model in SD rats obtained from Harlan. Similarly, Spiekerkoetter and coworkers reported increased right ventricular systolic pressure (RVSP) (~55–60 mm Hg) and emphysema in male SD rats aged 8 weeks, weighing between 180 and 220 g, subjected to Sugen (Cayman Chemicals), and exposed to chronic hypoxia for 3 weeks, followed by a period of 5 weeks of normoxia (5).

Bogaard and coworkers stated, "Dean and colleagues did not report any emphysema-like lung phenotype in female Wistar rats exposed to SuHx" (6). To our knowledge, in this particular study and in other studies listed as references to underline the notion that emphysema is not a feature of the SuHx model, the authors did not perform lung alveolar morphometry and focused predominantly on pulmonary vascular remodeling analysis (5, 7–11). In addition, there are substantial differences between Wistar and WKY rats. As reported previously, WKY rats are more prone to inflammation in comparison to Wistar and SD rat strains when exposed to hypoxia (12). It could at least partially contribute to the more prominent emphysema phenotype in WKY in comparison with SD rats in response to SuHx. The pronounced differences in the vascular (and maybe alveolar) response to SU5416/hypoxia in different rat strains and colonies has recently been demonstrated (10).

Bogaard and coworkers raised an important issue regarding the severity of emphysema. Although there is no clear definition with regard to the severity of emphysema in animal models, more than 25% of MLI and a 30% increase of μCT-derived lung aeration can be considered severe. Nevertheless, to address this issue, invasive physiological measurements of lung function are required (such as lung compliance).

In a mouse model of cigarette smoke–induced emphysema that is established in our laboratory, we observed a similar degree of emphysema. A long-term exposure of 8 months leads to development of emphysema, seen as a 15–30% increase in MLI (2, 4). It appears that in rodent models, because of the significantly shorter life span, symptoms of chronic diseases such as emphysema do not reach severity as observed in human patients. For that reason, we consider a 25% increase in MLI in such a short time span as severe. The SuHx rat model represents one of the most commonly used animal models of pulmonary hypertension, which closely mimics the vascular changes observed in human specimens. However, we disagree with the statement "that the Sugen-hypoxia (SuHx) rat model, when yielding RVSP consistently >60 mm Hg using adequate controls and standardized lung inflation, is currently one of the best rodent models to study PAH." This statement does not consider strain differences in the vascular and alveolar changes in response to SuHx or variations in hemodynamics (right ventricular systolic pressure, $\dot{Q}$). Several studies conducted in SD rats report mean RVSP values <60 mm Hg (5, 13–15), partly dependent on the time range of normoxic reexposure, which can lead to a partial decrease in pulmonary artery pressure (9). Finally, other factors such as stroke volume, $\dot{Q}$, heart rate, and hematocrit contribute to RVSP values in rodents as well.

We initiated a longitudinal study in different rat strains including lung μCT imaging and extensive histological analysis to describe the alveolar and vascular changes in the SuHx model dependent on the strain and different times of normoxic reexposure to identify the right strain and time for pharmacologic testing at our center. As we have stated previously, the potential presence of emphysema in the SuHx model of pulmonary hypertension should be taken into account when drawing conclusions and interpreting results.

In essence, the comments by Bogaard and colleagues highlight the need for international harmonization of protocols (including disease induction and thorough methodological workup) in the field of preclinical disease models. The same rigor should be applied as has been done for decades now for the harmonization of human clinical trial protocols. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

Baktybek Kojonazarov, M.D., Ph.D.*‡
Stefan Hadzic, M.Sc.*‡
Hossein Ardeschir Ghofrani, M.D.
Friedrich Grimminger, M.D., Ph.D.
Justus-Liebig University of Giessen
Giessen, Germany

Werner Seeger, M.D.
Justus-Liebig University of Giessen
Giessen, Germany
and
Max-Planck Institute for Heart and Lung Research
Bad Nauheim, Germany

Norbert Weissmann, Ph.D.
Ralph Theo Schermuly, Ph.D.
Justus-Liebig University of Giessen
Giessen, Germany

*Corresponding authors (e-mail: ralph.schermuly@innere.med.uni-giessen.de).
‡These authors contributed equally to this work.

References

1. Kojonazarov B, Hadzic S, Ghofrani HA, Grimminger F, Seeger W, Weissmann N, *et al*. Severe emphysema in the SU5416/hypoxia rat model of pulmonary hypertension. *Am J Respir Crit Care Med* [online ahead of print] 29 Apr 2019; DOI: 10.1164/rccm.201902-0390LE.
2. Seimetz M, Parajuli N, Pichl A, Veit F, Kwapiszewska G, Weisel FC, *et al*. Inducible NOS inhibition reverses tobacco-smoke-induced emphysema and pulmonary hypertension in mice. *Cell* 2011;147:293–305.

3. Weissmann N, Lobo B, Pichl A, Parajuli N, Seimetz M, Puig-Pey R, *et al*. Stimulation of soluble guanylate cyclase prevents cigarette smoke-induced pulmonary hypertension and emphysema. *Am J Respir Crit Care Med* 2014;189:1359–1373.
4. Pichl A, Sommer N, Bednorz M, Seimetz M, Hadzic S, Kuhnert S, *et al*. Riociguat for treatment of pulmonary hypertension in COPD: a translational study. *Eur Respir J* 2019;53:1802445.
5. Spiekerkoetter E, Tian X, Cai J, Hopper RK, Sudheendra D, Li CG, *et al*. FK506 activates BMPR2, rescues endothelial dysfunction, and reverses pulmonary hypertension. *J Clin Invest* 2013;123:3600–3613.
6. Dean A, Gregorc T, Docherty CK, Harvey KY, Nilsen M, Morrell NW, *et al*. Role of the aryl hydrocarbon receptor in sugen 5416-induced experimental pulmonary hypertension. *Am J Respir Cell Mol Biol* 2018;58:320–330.
7. Abe K, Toba M, Alzoubi A, Ito M, Fagan KA, Cool CD, *et al*. Formation of plexiform lesions in experimental severe pulmonary arterial hypertension. *Circulation* 2010;121:2747–2754.
8. Legchenko E, Chouvarine P, Borchert P, Fernandez-Gonzalez A, Snay E, Meier M, *et al*. PPARγ agonist pioglitazone reverses pulmonary hypertension and prevents right heart failure via fatty acid oxidation. *Sci Transl Med* 2018;10:eaao0303
9. de Raaf MA, Schalij I, Gomez-Arroyo J, Rol N, Happé C, de Man FS, *et al*. SuHx rat model: partly reversible pulmonary hypertension and progressive intima obstruction. *Eur Respir J* 2014;44:160–168.
10. Jiang B, Deng Y, Suen C, Taha M, Chaudhary KR, Courtman DW, *et al*. Marked strain-specific differences in the SU5416 rat model of severe pulmonary arterial hypertension. *Am J Respir Cell Mol Biol* 2016;54:461–468.
11. Dabral S, Tian X, Kojonazarov B, Savai R, Ghofrani HA, Weissmann N, *et al*. Notch1 signalling regulates endothelial proliferation and apoptosis in pulmonary arterial hypertension. *Eur Respir J* 2016;48:1137–1149.
12. Li M, Horn T, McKeon BA, Brown RD, Frid M, Nelson N, *et al*. Rat genetic strain differences in right ventricular remodeling in response to hypoxia-induced pulmonary hypertension [abstract]. *Am J Respir Crit Care Med* 2012;185:A4756.
13. Caruso P, Dunmore BJ, Schlosser K, Schoors S, Dos Santos C, Perez-Iratxeta C, *et al*. Identification of MicroRNA-124 as a major regulator of enhanced endothelial cell glycolysis in pulmonary arterial hypertension via PTBP1 (polypyrimidine tract binding protein) and pyruvate kinase M2. *Circulation* 2017;136:2451–2467.
14. Drozd K, Ahmadi A, Deng Y, Jiang B, Petryk J, Thorn S, *et al*. Effects of an endothelin receptor antagonist, Macitentan, on right ventricular substrate utilization and function in a Sugen 5416/hypoxia rat model of severe pulmonary arterial hypertension. *J Nucl Cardiol* 2017;24:1979–1989.
15. Hansen MS, Andersen A, Holmboe S, Schultz JG, Ringgaard S, Simonsen U, *et al*. Levosimendan prevents and reverts right ventricular failure in experimental pulmonary arterial hypertension. *J Cardiovasc Pharmacol* 2017;70:232–238.