

with IPF. Importantly, in this non-IPF group, there was only one death over the period investigated, so it was not possible to investigate the relationship between CD71<sup>+</sup> AMs and mortality in this cohort.

We agree that further studies on the role of CD71 in the pathogenesis of fibrotic lung disease are needed to confirm our observations, and we thank the authors for adding to our findings. ■

**Author disclosures** are available with the text of this letter at [www.atsjournals.org](http://www.atsjournals.org).

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## Emphysema Is—at the Most—Only a Mild Phenotype in the Sugeng/Hypoxia Rat Model of Pulmonary Arterial Hypertension

To the Editor:

Translational research is essential to develop strategies for the treatment of pulmonary arterial hypertension (PAH) using animal models that reproduce the severity, the progressive nature, and the resistance to treatment of human PAH, including severe arterial remodeling and progressive right ventricular (RV) failure (1).

We read with interest the letter by Kojonazarov and colleagues, who propose to have found “severe emphysema in the SU5416/hypoxia (SuHx) rat model of pulmonary hypertension” (2). The authors report that Wistar-Kyoto rats exposed to the

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combination of vascular endothelial growth factor receptor 2 (VEGFR2) inhibition by SU5416 and chronic hypoxia had moderately increased right ventricular systolic pressure (RVSP) and RV mass compared with normoxic untreated animals (2). They applied *in vivo* micro-computed tomography (CT) to demonstrate an increase in lung volume and a decrease in lung density, as well as an unaltered amount of lung tissue, but an increased air-to-tissue ratio; they claim that these findings were confirmed by histologic analysis, including mean linear intercept as surrogate of emphysema (2). Indeed, SU5416 has been previously shown to induce emphysema in normoxia (3), but this required repetitive SU5416 dosing (three times per week over 3 wk) and occurred more predominantly in rats younger than 4 weeks of age (Norbert Voelkel, M.D., written communication, 2019). In addition, emphysema could be negated, at the cost of the development of severe angioproliferative hypertension, by concomitant exposure to hypoxia (4).

The SuHx model, which combines a one-time subcutaneous injection of Sugeng (SU5416), which blocks VEGFR2 but also other tyrosine kinases, with 2–4 weeks of hypoxia (5–7), is the most accepted rodent model for PAH. Although several modifications of the SuHx model exist, the most commonly used study design is the one-time injection of 20 mg/kg SU5416 subcutaneously in 6- to 8-week-old Sprague-Dawley rats (body weight 180–200 g), followed by 3 weeks hypoxia (10% oxygen), and subsequently a 1- to 10-week period in room air, until heart–lung function and morphology is assessed.

Experiments in male Sprague-Dawley rats demonstrated that RVSP increases over 90 mm Hg in response to SuHx after 3 (5, 6) or 4 weeks of hypoxia (7); it then decreases somewhat in some studies, but remains elevated at 73 mm Hg (7), 91 mm Hg (6), and 96 mm Hg upon return to normoxia for an additional 4 (7), 6, or 10 weeks (5, 6), respectively (Table 1). Importantly, at 6 (6) and 10 weeks (5) after the end of hypoxia, plexiform lesions that are very similar to such lesions in human idiopathic PAH were frequently found in SuHx rats (5, 6), in addition to concentric medial hypertrophy of small- and medium-sized pulmonary arteries (6). Legchenko and colleagues demonstrated that RV failure develops between 1 and 6 weeks after the end of 3 weeks of hypoxia in SuHx rats, together with progression of pulmonary vascular disease, loss of peripheral pulmonary arterioles, and a metabolic switch in the right ventricle (6).

The advantages of the SuHx rat versus most other rat models of PAH such as monocrotaline were highlighted by a group of experts, and include the intensification of the vascular remodeling process, leading to the appearance of human-like plexiform lesions, and the virtual unresponsiveness to current PAH treatments, correlating well with the common unresponsiveness of PAH to therapy in humans (1).

Importantly, “severe emphysema” has not been observed previously in the SuHx rat model of PAH. Based on the report by Kojonazarov and colleagues (2), we have not only conducted a search of the literature but also analyzed lung histology from different SuHx rat studies in established laboratories (Table 1 and Figure 1). In contrast to Kojonazarov and colleagues, we did not find any severe or even moderate emphysema in any of the SuHx models analyzed (Figure 1 and Table 1). Mean linear intercept (MLI) as a surrogate for alveolar enlargement was not significantly different in SuHx versus untreated control lungs from Sprague-Dawley rats obtained from Charles River (Figures 1A and 1B). The Stewart group measured MLI in Sprague-Dawley rats obtained from Harlan, and found a mild (18%) increase in MLI in SuHx

Table 1. SU5416/Hypoxia Rat Models of Pulmonary Arterial Hypertension (2010–2019)

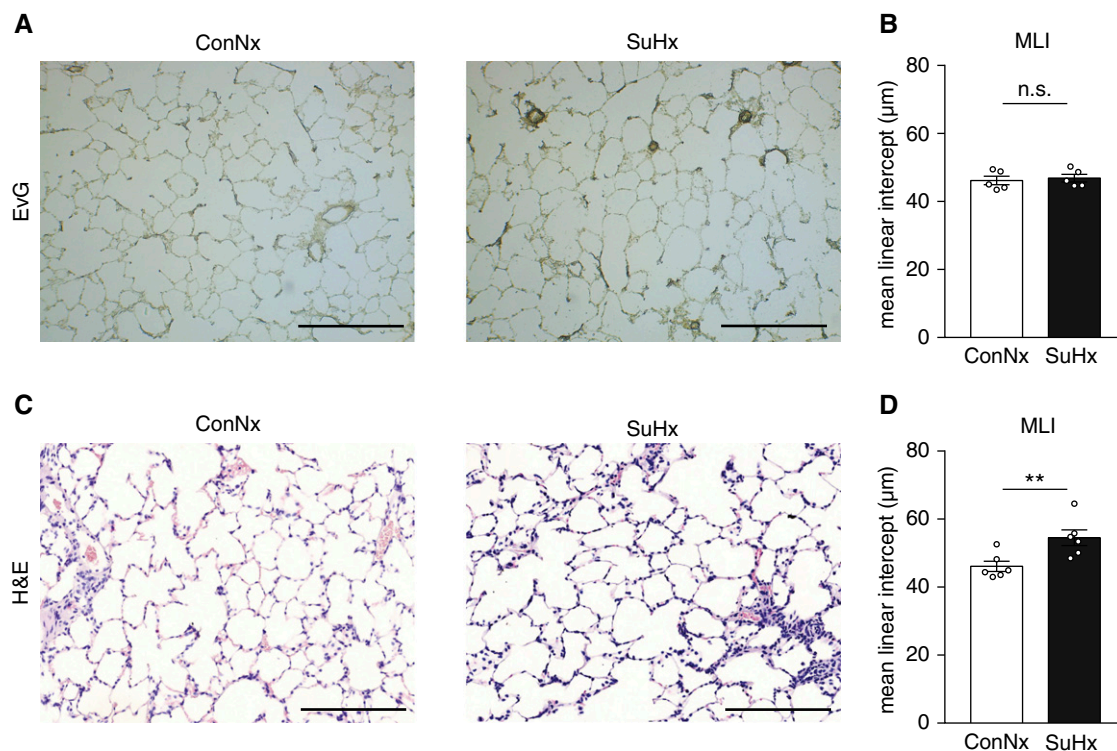
Reference	Rat Strain	Sex, Age, Weight at SU5416 Injection	SU5416 Brand, Single Subcutaneous Dose (Solvent)	Duration of Hypoxia + Normoxia (wk)	RVSP (mm Hg)	Emphysema Reported	Reanalysis/Comment
Kojonazarov <i>et al.</i> (2)	Wistar-Kyoto	Not reported	Brand not reported, 20 mg/kg (solvent?)	3 + 2	~55 ± 6	Yes, "severe"	SuHx vs. ConNx comparison: MLI (histology), ~45 vs. 35 μm; air-to-lung tissue volume ratio (CT), ~2.4 vs. 1.6; <i>P</i> < 0.001
Dean <i>et al.</i> (9)	Wistar-Kyoto	F (no more information)	Brand not reported, 20 mg/kg (solvent?)	2 + 6	~50	No	
Legchenko <i>et al.</i> (6)	Sprague-Dawley*	M, 6–8 wk, 180–200 g	Sigma, 20 mg/kg (DMSO)	3 + 1, 3 + 3, 3 + 6	91 ± 7	No	Plexiform lesions at 3 + 6 wk very similar to human plexiform lesions. RV failure occurs between 3 + 1 and 3 + 6 wk (MRI, closed-chest right and left heart catheterization)
Dabral <i>et al.</i> (10)	Not reported	Not reported, 200–250 g	Brand not reported, 20 mg/kg (DMSO)	5 + 0	~52 ± 2	No	
Bogaard group (unpublished results)	Sprague-Dawley*	M, <200 g	Tocris, 25 mg/kg (CMC)	4 + 6	72 ± 2	No	SuHx vs. ConNx comparison: MLI (histology), 47 vs. 46 μm, n.s.; see Figures 1A and 1B
Stewart group (unpublished results)	Sprague-Dawley†	M, 6–7 wk, 175–200 g	Tocris, 20 mg/kg (CMC)	3 + 4	105 ± 10	Yes, "mild"	SuHx vs. ConNx comparison: MLI (histology), 55 vs. 46 μm, <i>P</i> < 0.01; see Figures 1C and 1D
Jiang <i>et al.</i> (8)	Sprague-Dawley†	M, 6–7 wk, 175–200 g	Tocris, 20 mg/kg (CMC)	3 + 5	104 ± 13	No	Assessed vascular remodeling
de Raaf <i>et al.</i> (7)	Sprague-Dawley*	M, <200 g	Tocris, 25 mg/kg (CMC)	4 + 3	73 ± 2	No	RVSP monitoring by telemetry
Abe <i>et al.</i> (5)	Sprague-Dawley†	M, 180–220 g	Brand not reported, 20 mg/kg (solvent?)	3 + 2, 3 + 5, 3 + 10.5	96 ± 11	No	Plexiform lesions at 3 + 10.5 wk indistinguishable from human plexiform lesions

*Definition of abbreviations:* CMC = carbonylmethylcellulose; ConNx = naive (no vehicle) normoxic control rats; CT = computed tomography of the chest; MLI = mean linear intercept, a surrogate parameter for intraalveolar space and emphysema; MRI = magnetic resonance imaging; n.s. = not significant; RV = right ventricle; RVSP = right ventricular systolic pressure; SuHx = Sugen/hypoxia rats.

The rat model consists of a one-time subcutaneous injection of SU5416 (Sugen), followed by a period of 3–5 weeks of hypoxia (10% oxygen). Subsequently, the rats were returned to room air (normoxia) for 0–11 weeks (for example, 3 + 6 wk means 3 wk hypoxia followed by 6 wk normoxia). The reported RVSP and quantitative tissue analysis was obtained at the last time point, labeled in bold in the fifth column. In the study by Kojonazarov and colleagues, Wistar-Kyoto rats were obtained from the vendor Janvier Labs.

\*In these studies, male Sprague-Dawley rats were obtained from the vendor Charles River.

†In these studies, male Sprague-Dawley rats were obtained from the vendor Harlan Laboratories.



**Figure 1.** Emphysema is at most only a mild phenotype in the SU5416/hypoxia (SuHx) rat model of pulmonary arterial hypertension. (A) Representative pictures of Elastica van Gieson staining of the lungs from control normoxia (ConNx) and SuHx rats. Scale bar: 200  $\mu\text{m}$ . (B) Quantification of mean linear intercept (MLI) shows that there is no significant difference between the ConNx group and the SuHx group. (C) Representative pictures of hematoxylin and eosin staining of the lungs from ConNx and SuHx rats from the Stewart group. Scale bar: 200  $\mu\text{m}$ . (D) Quantification of MLI shows that MLI has a significant but mild increase in SuHx rats compared with ConNx rats. The MLI (also called air space chord length, Lm), as a surrogate of airspace diameter, was determined as follows. (A and B) The left lung was tracheally filled by a 1:1 mix of saline and cryofixative (Tissue-Tek OCT; Sakura Finetek), and snap-frozen in liquid nitrogen. Lung cryosections (5  $\mu\text{m}$ ) were stained with Elastica van Gieson for morphometric analysis. Six to eight random fields ( $\times 100$  magnification) for each rat were analyzed for mean linear intercept. Five to ten lines of 800- $\mu\text{m}$  length were drawn per image, alveolar intercepts per line were counted, and MLI was calculated. (C and D) The left lobe of the lung was inflated via the trachea with 1:1 OCT/saline solution (Tissue-Tek OCT) and then removed. The left lobe was then cut into thick cross-sections and fixed in 4% paraformaldehyde for 24 hours, rinsed, and washed in PBS for 8 hours, then stored in 70% ethanol until the day of paraffin embedding. Eight random high-power fields ( $\times 100$  magnification) for each rat were analyzed for MLI. Three lines of 1,000- $\mu\text{m}$  length were drawn per image, alveolar intercepts per line were counted, and MLI was calculated. SuHx group: rats were injected once with SU5416 (20–25 mg/kg/dose, subcutaneously) and subsequently exposed to chronic hypoxia (i.e., 10% oxygen). (A and B) Four weeks of hypoxia, followed by 6 weeks in room air and lung harvest. (C and D) Three weeks of hypoxia followed by lung harvest. Rat supplier for A and B, Charles River; rat supplier for C and D, Harlan. For additional experimental details, see Table 1. Columns and error bars represent mean  $\pm$  SEM ( $n = 5\text{--}6$  per group). Nonparametric Mann-Whitney test.  $**P < 0.01$ ; EvG = Elastica van Gieson; H&E = hematoxylin and eosin; n.s. = not significant; OCT = optimal cutting temperature compound. Scale bar = 200  $\mu\text{m}$ .

versus untreated control lungs rats (Table 1 and Figures 1C and 1D) (8).

Several reasons may explain the differences in the above findings and the recent report by Kojonazarov and colleagues (2): This group studied Wistar-Kyoto (2) rather than the Sprague-Dawley rats that have been used by most other groups, and observed a much weaker PAH hemodynamic phenotype (RVSP 55 mm Hg at 3 + 2 wk SuHx) when compared with results described in many other publications (e.g., RVSP 91–107 mm Hg upon return to normoxia for 4–10 wk; Table 1) (5–8). It is possible that the Wistar rat strain may be more prone to emphysema after SuHx; however, Dean and colleagues did not report any emphysema-like lung phenotype in female Wistar rats exposed to SuHx (RVSP 55 mm Hg) (9). The Stewart group compared the response to SuHx in several different strains of rats: whereas

Fischer and Sprague-Dawley rats developed similar increases in RVSP to  $\sim 100$  mm Hg (8), Lewis rats exhibited no significant increase in RV pressure, highlighting the importance of background in determining the phenotype in the SuHx model (D.J. Stewart, unpublished results).

In addition to the above strain differences, technical factors may also contribute to the differences observed between research groups. While we congratulate Kojonazarov and colleagues (2) on performing *in vivo* micro-CT to quantify the air-to-lung tissue volume ratio as a surrogate of airspace disease (emphysema), it remains unclear whether and—if so—how the images were gated to the respiratory cycle. From the clinical experience, expiratory chest CT lung images must be interpreted with caution, and of course yield lower such ratio numbers than do end-inspiratory CT images. In addition, valid measurement of MLI in *ex vivo* histologic studies

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 SU5146 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](http://www.atsjournals.org).

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## 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 SU5146/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 ( $\mu$ CT)-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  $\mu$ CT. We further verified the results in histology, suggesting that high-resolution  $\mu$ CT 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

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