Sarcoidosis and lymphoma, mainly NHL, may occur together, with sarcoidosis usually preceding lymphoma. Because many features of sarcoidosis and lymphoma are similar, histological confirmation of malignancy is necessary, especially if new nodal disease and splenomegaly are present. Sarcoidosis, although rare, may complicate the course of lymphoma. Prominent features of sarcoidosis were respiratory symptoms, high ACE levels, BHL in asymptomatic subjects and lung disease. Despite the limitations of this study, the above described features should alert clinicians to pursue a second diagnosis, in order either to properly diagnose malignancy in the sarcoidosis setting or to save cancer patient from a potentially toxic treatment escalation due to a presumed lymphoma recurrence.

I.C. Papanikolaou* and O.P. Sharma#
*Third Pulmonary Dept, Sismanoglio General Hospital, Athens, Greece. #Pulmonary and Critical Care Divison, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA.

Correspondence: I.C. Papanikolaou, Room 11-900, LAC–USC Medical Center, 1200 North State Street, Los Angeles, CA 90033, USA. E-mail: hliaspapa@hotmail.com

Statement of Interest: None declared.

REFERENCES

1 Brincker H, Wilbek E. The incidence of malignant tumours in patients with respiratory sarcoidosis. *Br J Cancer* 1974; 29: 247–251.
2 Rømer FK, Hommelgaard P, Schou G. Sarcoidosis and cancer revisited: a long-term follow-up study of 555 Danish sarcoidosis patients. *Eur Respir J* 1998; 12: 906–912.
3 Brincker H. The sarcoidosis–lymphoma syndrome. *Br J Cancer* 1986; 54: 467–473.
4 Hunninghake GW, Costabel U, Ando M, *et al.* ATS/ERS/WASOG statement on sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 1999; 16: 149–173.
5 Harris NL, Jaffe ES, Diebold J, *et al.* World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: Report of the Clinical Advisory Committee meeting-Airlie House, Virginia, November 1997. *J Clin Oncol* 1999; 17: 3835–3849.
6 Kim JH, Lee SH, Park J, *et al.* Primary pulmonary non-Hodgkin's lymphoma. *Jpn J Clin Oncol* 2004; 34: 510–514.
7 O'Reilly RA. Splenomegaly in 2,505 patients at a large university medical center from 1913 to 1995. 1963 to 1995: 449 patients. *West J Med* 1998; 169: 88–97.
8 Cetinkaya E, Yildiz P, Altin S, *et al.* Diagnostic value of transbronchial needle aspiration by Wang 22-gauge cytology needle in intrathoracic lymphadenopathy. *Chest* 2004; 125: 527–531.
9 Askling J, Grunewald J, Eklund A, *et al.* Increased risk for cancer following sarcoidosis. *Am J Respir Crit Care Med* 1999; 160: 1668–1672.
10 Boffetta P, Rabkin CS, Gridley G. A cohort study of cancer among sarcoidosis patients. *Int J Cancer* 2009; 124: 2697–2700.

DOI: 10.1183/09031936.00043010

Imatinib inhibits bone marrow-derived c-kit$^+$ cell mobilisation in hypoxic pulmonary hypertension

To the Editors:

We read with great interest the review by FADINI *et al.* [1] in the *European Respiratory Journal* discussing the role of bone marrow (BM)-derived stem cells and endothelial progenitors in pulmonary hypertension (PH). This topic is very relevant to pulmonary vascular medicine, indeed pioneering studies demonstrated that haematopoietic progenitor cells can be recruited in the pulmonary artery adventitia of neonatal animals with hypoxic PH [2]. In addition, BM-derived cells may express smooth muscle actin in hypoxia-remodelled pulmonary arteries, and selective depletion of circulating BM-derived precursors prevents pulmonary adventitial remodelling, suggesting that these cells may have functional relevance in the pathophysiology of hypoxia-induced PH [3]. CD117/c-kit, a transmembrane receptor tyrosine kinase for the progenitor cell factor (SCF), is a marker for BM-derived haematopoietic progenitor cells, and this receptor can be targeted by tyrosine kinase inhibitors which have been recently proposed as novel therapeutic agents to be tested in PH [4]. Indeed, a novel antiproliferative-based strategy using therapeutic agents such as imatinib mesylate inhibiting several tyrosine kinases associated with disease states, including BCR-ABL, c-kit, and platelet-derived growth factor (PDGF) receptors α and β, has been demonstrated to reverse pulmonary vascular remodelling in animal models of PH (chronic hypoxia- and monocrotaline-induced PH), through inhibition of proliferation and increased apoptosis rate of pulmonary arterial smooth muscle cells [5]. As imatinib has a broad spectrum of tyrosine kinase inhibition, we hypothesised that it may exert its beneficial effect on PH not only by decreasing smooth muscle cell proliferation through PDGF receptor inhibition, as initially suggested [5], but also by targeting c-kit. Hence, we wondered if administration of imatinib could attenuate experimental PH, at least in part, by reducing the pulmonary perivascular recruitment of BM-derived c-kit$^+$ progenitor cells.

We used the murine model of PH induced by chronic normobaric hypoxia (10% inspiratory oxygen fraction).

6-week old male C57BL/6J mice (Janvier, Le Genest-St-Isle, France) were exposed to 10% normobaric hypoxia for 3 weeks. Imatinib (Novartis, Horsham, UK) was orally administrated during the period of hypoxia at 10 $mg \cdot kg^{-1} \cdot day^{-1}$. Immunofluorescent staining was performed on 7-μm acetone-fixed,

frozen lung sections. To unequivocally distinguish BM-derived progenitor c-kit$^+$ from mast cells, perivascular cells were characterised not only by c-kit expression but also by FcεRIα (immunoglobulin E high affinity receptor) and tryptase. Hence, c-kit$^+$/FcεRIα$^-$/tryptase$^-$ BM-derived progenitor cells are different from c-kit$^+$/FcεRIα$^+$/tryptase$^+$ mast cells. Thus, we combined biotinylated anti-c-kit (Abcam, Paris, France), anti-FcεRIα (immunoglobulin E high affinity receptor) (eBioscience, San Diego, CA, USA) and anti-mast cell tryptase antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Isotype-matched, irrelevant antibodies were used as negative controls. Medial thickness (defined as the ratio of the medial wall thickness to the external diameter of pulmonary arterioles with a diameter <30 μm, 30–50 μm, 50–75 μm or 75–100 μm) was measured on

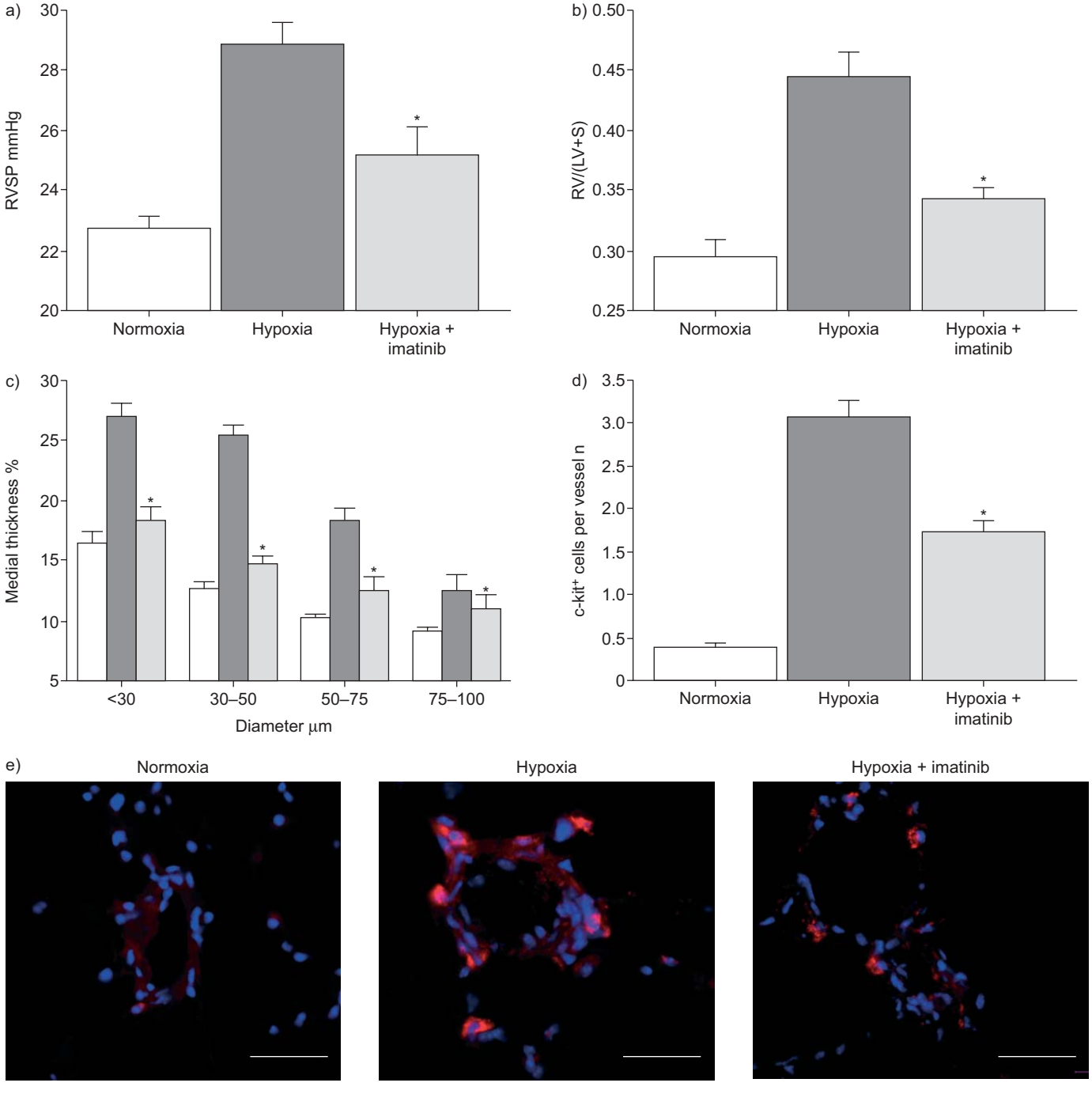

FIGURE 1. Imatinib treatment significantly decreases a) the right ventricular systolic pressure (RVSP), b) the right ventricular hypertrophy defined by the ratio right ventricle/(left ventricle+septum) (RV/(LV+S)), c) the medial wall thickness of small pulmonary arteries, and d) the pulmonary perivascular c-kit$^+$ bone marrow-derived progenitor cell recruitment induced by chronic normobaric hypoxia. □: normoxia; ■: hypoxia; ■: imatinib. *: $p<0.05$ in imatinib-treated mice as compared to untreated hypoxic control. e) Immunostaining of lung section for c-kit (red) and DAPI (4′,6-diamidino-2-phenylindole, nuclear staining; blue) revealed the accumulation of c-kit$^+$ cells around small pulmonary arteries in hypoxic lung, which was reduced by imatinib. Data are presented as mean±SEM. n=8–10 animals per group. Scale bars=50 μm.

30 randomly selected arterioles stained for smooth muscle cell α-actin. The mean number of c-kit$^+$ cells per pulmonary artery (cross-sectional diameter 30–100 μm) was calculated by counting c-kit$^+$ cells on 90 randomly selected arterioles per group. Statistical analysis was performed with StatEl software (Ad Science, Paris, France) using parametric (ANOVA) or nonparametric tests (Kruskall–Wallis, Mann–Whitney).

Right ventricular systolic pressure (RVSP) was recorded by right ventricle catheterisation in mice anaesthetised with intraperitoneal injection of ketamine (60 mg·kg^{-1}) and xylazine (8 mg·kg^{-1}). Right ventricular hypertrophy was assessed by calculating the weight ratio of the right ventricle (RV) to the left ventricle (LV) plus septum (S): RV/(LV+S).

After 3 weeks of hypoxia, the RVSP was significantly elevated, as compared to normoxia. RVSP was significantly lower in imatinib-treated mice, as compared to hypoxic mice, but still higher than in normoxic mice (fig. 1a). Hypoxia-induced PH was associated with a significant hypertrophy of the right ventricle, as compared to normoxic controls. In accordance with the beneficial effect of imatinib on RVSP, the RV/(LV+S) ratio was significantly decreased in imatinib-treated hypoxic mice, as compared to hypoxic controls (fig. 1b). Medial thickness of pulmonary arterioles <30 μm, 30–50 μm, 50–75 μm and 75–100 μm of hypoxic mice was significantly increased, as compared to normoxic mice. Imatinib-treated mice showed significant decrease of medial thickness compared with hypoxic mice (fig. 1c). Hypoxia-induced pulmonary vascular remodelling was associated with a significant accumulation of c-kit$^+$/FcεRIα$^-$/tryptase$^-$ cells in the adventitia of remodelled pulmonary arteries, as compared to normoxia. The recruitment of c-kit$^+$/FcεRIα$^-$/tryptase$^-$ cells in the perivascular area of imatinib-treated mice was significantly decreased as compared to hypoxic mice (fig. 1d).

Our study provides evidence that imatinib, a BCR-ABL/c-kit/PDGF receptor tyrosine kinase inhibitor, may prevent pulmonary vascular remodelling, PH and RV hypertrophy induced by chronic hypoxia. These effects were associated with a significant reduction of perivascular BM-derived c-kit$^+$ progenitor cell accumulation. Imatinib has been demonstrated to reverse pulmonary vascular remodelling in animal models of PH and in a small number of human cases of pulmonary arterial hypertension [6, 7]. It is widely accepted that PDGF and its receptors are overexpressed within the pulmonary artery wall of patients displaying pulmonary arterial hypertension, and that PDGF promotes pulmonary arterial remodelling, which can be, at least in part, treated by PDGF inhibition with imatinib. Moreover, this molecule has been shown to inhibit expansion of haematopoietic progenitor cells *in vitro* [8], and in patients with chronic myeloid leukemia [9]. We demonstrated that imatinib decreases the perivascular accumulation of BM-derived c-kit$^+$ progenitor cells in mice exposed to chronic hypoxia. We therefore hypothesise that c-kit blockade by imatinib may exert its beneficial effect in hypoxic PH, not only by decreasing pulmonary artery smooth muscle cell proliferation through PDGF receptor inhibition, but also through reduced recruitment of BM-derived progenitor c-kit$^+$ cells by directly blocking their expansion in the BM and/or *in situ* in the lungs [5, 10].

N. Gambaryan[*,#,¶], F. Perros[*,#,¶], D. Montani[*,#,¶], S. Cohen-Kaminsky[*,#,¶], G.M. Mazmanian[*,¶,+], and M. Humbert[*,#,¶]

*Université Paris-Sud, Faculté de Médecine, #Centre National de Référence de l'Hypertension Pulmonaire Sévère, Service de Pneumologie et Réanimation Respiratoire, Hôpital Antoine Béclère, Clamart, ¶INSERM U999, Hypertension Artérielle Pulmonaire, Physiopathologie et Innovation Thérapeutique, Centre Chirurgical Marie Lannelongue, and +Laboratoire de Chirurgie Expérimentale, Centre Chirurgical Marie Lannelongue, Le Plessis-Robinson, France.

Correspondence: M. Humbert, Service de Pneumologie et Réanimation, Respiratoire, Hôpital Antoine-Béclère, 157 rue de la Porte de Trivaux, 92140 Clamart, France. E-mail: marc.humbert@abc.aphp.fr

Support Statement: This study has been supported, in part, by grants from Ministère de l'Enseignement Supérieur et de la Recherche and the Université Paris-Sud.

Statement of Interest: Statements of interest for M. Humbert and D. Montani can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

REFERENCES

1 Fadini GP, Avogaro A, Ferraccioli G, *et al.* Endothelial progenitors in pulmonary hypertension: new pathophysiology and therapeutic implications. *Eur Respir J* 2010; 35: 418–425.
2 Davie NJ, Crossno JT Jr, Frid MG, *et al.* Hypoxia-induced pulmonary artery adventitial remodeling and neovascularization: contribution of progenitor cells. *Am J Physiol Lung Cell Mol Physiol* 2004; 286: L668–L678.
3 Frid MG, Brunetti JA, Burke DL, *et al.* Hypoxia-induced pulmonary vascular remodeling requires recruitment of circulating mesenchymal precursors of a monocyte/macrophage lineage. *Am J Pathol* 2006; 168: 659–669.
4 Ogawa M, Matsuzaki Y, Nishikawa S, *et al.* Expression and function of c-kit in hemopoietic progenitor cells. *J Exp Med* 1991; 174: 63–71.
5 Schermuly RT, Dony E, Ghofrani HA, *et al.* Reversal of experimental pulmonary hypertension by PDGF inhibition. *J Clin Invest* 2005; 115: 2811–2821.
6 Souza R, Sitbon O, Parent F, *et al.* Long term imatinib treatment in pulmonary arterial hypertension. *Thorax* 2006; 61: 736.
7 Ghofrani HA, Seeger W, Grimminger F. Imatinib for the treatment of pulmonary arterial hypertension. *N Engl J Med* 2005; 353: 1412–1413.
8 Bartolovic K, Balabanov S, Hartmann U, *et al.* Inhibitory effect of imatinib on normal progenitor cells *in vitro*. *Blood* 2004; 103: 523–529.
9 Agis H, Jaeger E, Doninger B, *et al. In vivo* effects of imatinib mesylate on human haematopoietic progenitor cells. *Eur J Clin Invest* 2006; 36: 402–408.
10 Perros F, Montani D, Dorfmuller P, *et al.* Platelet-derived growth factor expression and function in idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2008; 178: 81–88.

DOI: 10.1183/09031936.00052210