2. Gonzalez-Bermejo J, Morélot-Panzini C, Tanguy ML, Meininger V, Pradat PF, Lenglet T, *et al*. Early diaphragm pacing in patients with amyotrophic lateral sclerosis (RespiStimALS): a randomised controlled triple-blind trial. *Lancet Neurol* 2016;15:1217–1227.
3. Love FM, Son YJ, Thompson WJ. Activity alters muscle reinnervation and terminal sprouting by reducing the number of Schwann cell pathways that grow to link synaptic sites. *J Neurobiol* 2003;54:566–576.
4. Gigo-Benato D, Russo TL, Geuna S, Domingues NR, Salvini TF, Parizotto NA. Electrical stimulation impairs early functional recovery and accentuates skeletal muscle atrophy after sciatic nerve crush injury in rats. *Muscle Nerve* 2010;41:685–693.
5. Hansen S, Ballantyne JP. A quantitative electrophysiological study of motor neurone disease. *J Neurol Neurosurg Psychiatry* 1978;41: 773–783.
6. Guimarães-Costa R, Similowski T, Rivals I, Morélot-Panzini C, Nierat MC, Bui MT, *et al*.; on behalf of the RespiStimALS team (see Appendix). Human diaphragm atrophy in amyotrophic lateral sclerosis is not predicted by routine respiratory measures. *Eur Respir J* 2019; 53:1801749.
7. Jennekens FG, Tomlinson BE, Walton JN. Data on the distribution of fibre types in five human limb muscles: an autopsy study. *J Neurol Sci* 1971;14:245–257.
8. Bruneteau G, Simonet T, Bauché S, Mandjee N, Malfatti E, Girard E, *et al*. Muscle histone deacetylase 4 upregulation in amyotrophic lateral sclerosis: potential role in reinnervation ability and disease progression. *Brain* 2013;136:2359–2368.
9. Willand MP. Electrical stimulation enhances reinnervation after nerve injury. *Eur J Transl Myol* 2015;25:243–248.
10. Pinheiro-Dardis CM, Erbereli BT, Gigo-Benato D, Castro PATS, Russo TL. Electrical stimulation delays reinnervation in denervated rat muscle. *Muscle Nerve* 2017;56:E108–E118.
11. Martineau E, Di Polo A, Vande Velde C, Robitaille R. Dynamic neuromuscular remodeling precedes motor-unit loss in a mouse model of ALS. *eLife* 2018;7:e41973.
12. Le Pimpec-Barthes F, Legras A, Arame A, Pricopi C, Boucherie JC, Badia A, *et al*. Diaphragm pacing: the state of the art. *J Thorac Dis* 2016;8:S376–S386.

BET Bromodomain Inhibitors and Pulmonary Arterial Hypertension: Take Care of the Heart

To the Editor:

We read with great interest the article by Van der Feen and colleagues (1), who reported the promising results from a multicenter preclinical validation of BET (bromodomain and extraterminal motif) family inhibition for the treatment of pulmonary arterial hypertension (PAH). The BET family members are epigenetic readers that bind acetylated proteins, facilitating the localization of transcription factors and other coactivators to upregulate transcription (2). BET family proteins have been implicated in cardiac pathologic gene induction and heart failure pathogenesis. Indeed, pharmacological administration of several BET inhibitors had significant therapeutic effects in a diverse range of small animal models of heart failure via a mechanism that converges on suppression of profibrotic and proinflammatory gene expression programs in the heart (3). The role of BET proteins has also been studied in the frame of PAH and associated right ventricle (RV) hypertrophy and failure (4). In their study, Van der Feen and colleagues (1) demonstrated that RVX208, a clinically available BET inhibitor, modulates pro-proliferative, prosurvival, and proinflammatory pathways. This reversed the PAH phenotype in cultured pulmonary vascular cells and improved pulmonary hemodynamics and RV function in several animal models of PAH and isolated RV pressure load.

Paradoxically, we recently demonstrated that *in vivo* administration of BET inhibitor I-BET-151 induces structural and functional alterations of the heart mitochondria in healthy male mice and rats (5). We showed the occurrence of specific ultrastructural alterations and progressive destruction of cardiomyocyte mitochondria after I-BET-151 exposure, whereas the skeletal muscles of exposed animals were unaffected. In rats, I-BET-151 decreased the oxidative capacities of cardiac mitochondria in a dose-dependent manner, and at high dose (10 mg/kg/d during 3 wk), it also decreased mitochondrial mass. I-BET-151 reduced the right and left ventricular fractional shortening and decreased the velocity–time integral in the aorta and the pulmonary artery. Both functional reductions are suggestive of impaired heart function under I-BET-151 exposure. Although a 3-week washout period allowed a partial restoration of mitochondrial structure in the RV and an improvement of left ventricle function, full normalization of both mitochondrial morphology and ventricular function was not observed in either the RV nor the left ventricle. The ultrastructural alterations of heart mitochondria we observed in healthy mice and rat exposed to I-BET-151 may be partly a result of c-Myc inhibition (5, 6).

Several BET inhibitors are currently being evaluated in phase 1 clinical trials, and promising results have been obtained in several lymphomas, as well as some solid tumors. One of these trials was conducted to evaluate for the first time the toxicity and efficacy of BET inhibitor BAY1238097 in humans (2). Although this study showed that the drug did inhibit its target, several patients reported unexpected pain (headache and back pain) occurring shortly after taking the drug. These toxicities were not observed in the preclinical tests and differed from the reported toxicities with the other BET inhibitors, suggesting they were related to an off-target effect. Further study of the clinical and preclinical data revealed that BAY1238097 also binds the adenosine receptor, potentially increasing the concentration of mediators involved in the pain. The occurrence of these pains at doses lower than doses that would have been effective in clinics led to the cessation of the development of this drug. However, this negative test has been reported because it could inform the development of other BET inhibitors.

In conclusion, BET inhibitors hold promising potential as a new therapy for left and right heart failure and PAH. However, the BET family of proteins regulates a vast network of transcriptional pathways, raising concerns regarding their safety. Attention must be paid about possible off-target effects of BET inhibitors, as well as potential cardiotoxicity of this new therapeutic class. ■

F.P. was supported by the French National Research Agency (ANR-13-JSV1-0011-01).

Originally Published in Press as DOI: 10.1164/rccm.201905-0993LE on August 16, 2019

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

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References

1. Van der Feen DE, Kurakula K, Tremblay E, Boucherat O, Bossers GP, Szulcek R, *et al*. Multicenter preclinical validation of BET inhibition for the treatment of pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2019;200:910–920.
2. Postel-Vinay S, Herbschleb K, Massard C, Woodcock V, Soria J-C, Walter AO, *et al*. First-in-human phase I study of the bromodomain and extraterminal motif inhibitor BAY 1238097: emerging pharmacokinetic/pharmacodynamic relationship and early termination due to unexpected toxicity. *Eur J Cancer* 2019;109:103–110.
3. Padmanabhan A, Haldar SM. Drugging transcription in heart failure. *J Physiol* [online ahead of print] 30 Mar 2019; DOI: 10.1113/JP276745.
4. Mumby S, Gambaryan N, Meng C, Perros F, Humbert M, Wort SJ, *et al*. Bromodomain and extra-terminal protein mimic JQ1 decreases inflammation in human vascular endothelial cells: implications for pulmonary arterial hypertension. *Respirology* 2017;22:157–164.
5. Piquereau J, Boet A, Péchoux C, Antigny F, Lambert M, Gressette M, *et al*. The BET bromodomain inhibitor I-BET-151 induces structural and functional alterations of the heart mitochondria in healthy male mice and rats. *Int J Mol Sci* 2019;20:E1527.
6. Sarosiek KA, Fraser C, Muthalagu N, Bhola PD, Chang W, McBrayer SK, *et al*. Developmental regulation of mitochondrial apoptosis by c-Myc governs age- and tissue-specific sensitivity to cancer therapeutics. *Cancer Cell* 2017;31:142–156.

EpiHope for the Treatment of Pulmonary Arterial Hypertension: Selective versus Nonselective BET Inhibition

To the Editor:

Epigenetic regulation of chromatin structure is fundamental to establish and maintain cell type–specific gene expression during development and disease states (1, 2). Importantly, acetylation and methylation of histone tails and methylation of DNA by a specific group of enzymes are the most common epigenetic modifications that occur at specific sites and residues (3). For example, histone acetyltransferases acetylate histone tails, histone deacetylases remove acetyl groups from histone tails, and BRD (bromodomain) proteins are chromatin readers that recognize and bind acetylated histones. The latter also play a key role in the transmission of epigenetic memory across cell divisions and transcription regulation, and thus have emerged as an attractive drug class for the treatment of cancer and other nonmalignant disorders (4–6).

Pulmonary arterial hypertension (PAH; group 1 pulmonary hypertension [PH]) is a progressive pulmonary vascular disease with a poor prognosis that culminates in right heart failure. Despite our progressive understanding of the pathogenesis of PAH and recent therapeutic advances, PAH remains a fatal disease (7). Several lines of evidence suggest the contribution of epigenetic mechanisms to vascular remodeling in PH/PAH. First, both the initiation and progression of PH/PAH are influenced by environmental factors, and thus it has been speculated that exposure to viruses, drugs, toxins, hypoxia, and inflammation drives epigenetic mechanisms underlying PH/PAH pathogenesis. Second, PAH is also a genetic disease, and genetic factors also influence epigenetic mechanisms. Third, epigenetic modifications are also crucial for the persistent activation of PAH vascular cells when cultured *ex vivo*, a hallmark feature of PAH. Finally, emerging evidence suggests that the pathogenesis of PH is influenced by aberrant expression and activity of DNA and histone-modifying enzymes (8, 9), including upregulation of BRD4. Accordingly, earlier studies demonstrated that pan-BRD (JQ1 and I-BET-151) and selective BRD4 knockdown inhibited pulmonary arterial smooth muscle cell proliferation and restored mitochondrial membrane potential in patients with PAH (10), and prevented the production of proinflammatory cytokines by pulmonary microvascular endothelial cells (11). Importantly, studies showed that pan-BRD inhibitors reversed established PAH in the Sugen/hypoxia and hypoxia/pulmonary inflammation rat models (10, 12). However, in contrast to these promising studies, Piquereau and colleagues found that Wistar rats and C57Bl/6J mice treated with I-BET-151 for 3 weeks developed cardiomyopathy as demonstrated by progressive mitochondrial damage and a global reduction in cardiac function (13). The conflicting data from these studies can be understood in light of the wide-ranging effects that BETs have in reprogramming the epigenome and off-target effects. Thus, domain- and isoform-specific BET (bromodomain and extraterminal motif) inhibitors are highly needed to avoid the adverse effects of prolonged pan-BET inhibition.

In the setting of a multicenter preclinical trial, Van der Feen and colleagues report that apabetalone (RVX-208), a clinically available domain-selective BET inhibitor, reversed vascular remodeling and improved pulmonary hemodynamics in several experimental models of PAH (14). This study provides convincing data, obtained both *in vitro* and *in vivo*, indicating that apabetalone normalized the hyperproliferative, apoptosis-resistant and proinflammatory phenotype of microvascular endothelial cells and pulmonary arterial smooth muscle cells isolated from patients with PAH, as well as in animal models of PAH (Figure 1). Importantly, at a clinically relevant dose, RVX-208 reversed vascular remodeling in multiple complementary preclinical models of PAH and could be combined safely with current PAH therapy. Finally, apabetalone was shown to support the pressure-loaded right ventricle in rats, indicating a beneficial, dual mode of action for patients with PAH-associated right-ventricle pressure overload. Based on these exciting preclinical results, a 16-week phase 2 pilot study (ClinicalTrials.gov identifier:

Originally Published in Press as DOI: 10.1164/rccm.201906-1235LE on August 16, 2019