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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

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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 Sugden/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:

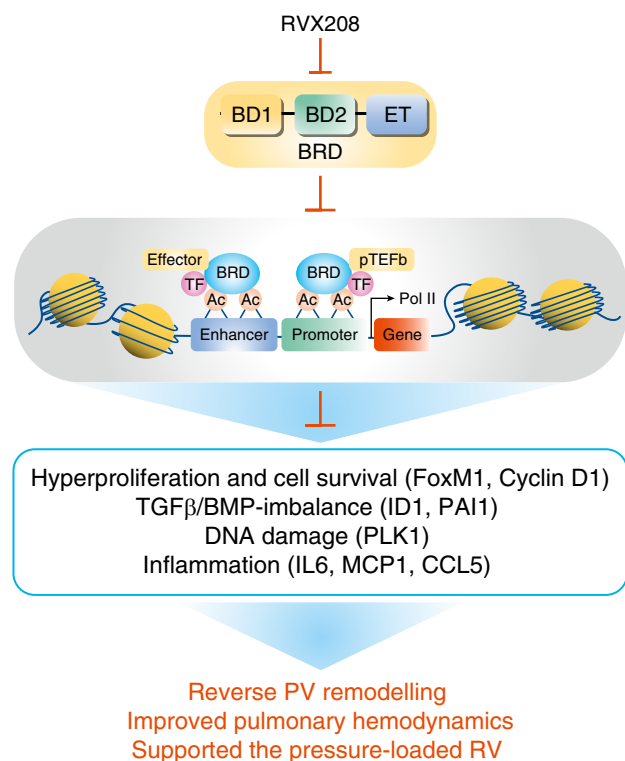


Figure 1. Schematic model of a specific BET inhibitor (RVX-208) on reversal of pulmonary arterial hypertension. Specific BRD inhibitors, such as RVX-208, bind to the second bromodomain of BRDs and compete with the BRDs for binding to acetylated histones. By binding to acetylated histones, the BRDs contribute to the assembly of histone acetyltransferase-dependent chromatin complexes at gene promoters and to the recruitment of TF and p-TEFb. This leads to the phosphorylation of RNA Pol II. BRD4 also binds acetylated histones in enhancers and assists transcriptional elongation at enhancers and gene bodies. Thus, RVX-208 prevents the above-mentioned histone modification and transcription of their target genes. This leads to decreased proliferation, inflammation and suppressed DNA damage response and balance of TGF β /BMP signaling, cell differentiation, and maturation and altered gene expression. This subsequently leads to reversal of pulmonary vascular remodeling, improved pulmonary hemodynamics, and support for the pressure-loaded right ventricle. Ac = acetylated; BD1 = bromodomain 1; BD2 = bromodomain 2; BET = bromodomain and extraterminal motif; BRD = bromodomain; ET = extraterminal domain; p-TEFb = positive transcription elongation factor B; PV = pulmonary vascular; RNA Pol II = RNA polymerase II; RV = right ventricle; TF = transcription factor; TGF β = transforming growth factor β .

NCT03655704) is set to start in Canada that will look at changes in pulmonary vascular resistance as the primary endpoint, as well as safety and tolerability.

Unlike JQ1 and I-BET-151, apabetalone acts as a selective BET inhibitor by targeting the second bromodomain (BD2) in BRDs (BRD2–4, BRDP) (15). This specific inhibition of BD2 raised interest in terms of therapeutic responses, as selective inhibition of either BD1 or BD2 can result in distinct transcriptional outcomes (16, 17). Importantly, phase 1 and 2 studies that were conducted in healthy volunteers and patients with diabetes and low levels of high-density lipoprotein showed a satisfactory safety and tolerability profile for apabetalone (18). Although the safety data for apabetalone are encouraging, its ultimate success will rely

on whether it shows disease-modifying effects in combination with U.S. Food and Drug Administration-approved therapies. It should be stressed that no BRD inhibitor has been granted Food and Drug Administration approval thus far, but several drugs have been or are currently in clinical trials. The attractive therapeutic potential of BET inhibition continues to draw the interest of academic researchers and drug companies. The major challenge in developing new BET inhibitors will be to maximize therapeutic efficacy while reducing toxicity. Cancer studies have demonstrated that BET inhibitors work better in combination with other antiproliferative compounds such as PD1 inhibitors and tyrosine kinase inhibitors, another drug class that has been investigated in PAH in recent years (19). Furthermore, new strategies, such as protein-targeting chimeric molecules for BRDs, have already been developed and have shown promising activity on cancer cell lines (20). Independently of the results of the ongoing apabetalone study, BET inhibition remains an attractive mechanism for drug development, and it is hoped that in the coming years this approach will prove to be successful for treatment of PAH. Moreover, it is important to emphasize that studies to assess the role of domain- and isoform-selective inhibitors, as well as each BET protein, in regulating specific biological roles, gene transcription, transcription factor interactions, and the epigenome on a genome-wide scale using RNA sequencing, chromatin immunoprecipitation sequencing, and proteomic approaches are warranted. ■

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hypertension (PAH) (1). In their letter to the editor, Piquereau and Perros raised concerns about the potential cardiotoxic effects of BET inhibitors in patients with PAH based on published data demonstrating structural and functional alterations of the heart in healthy rodents treated with the pan-BET inhibitor I-BET-151 (2). A contrast must be drawn between highly potent, nonselective BET inhibitors such as I-BET-151, which are under development for oncology indications, and RVX208, which is a BD2-selective BET inhibitor with lower potency that has an excellent cardiovascular (CV) safety profile to date. A series of phase 2 studies evaluating RVX208 in a total of 789 patients with CV disease recently demonstrated a >40% reduction in major CV events (3). RVX208 is currently being evaluated in a phase 3 clinical trial involving 2,425 patients with high-risk type 2 diabetes and coronary artery disease (ClinicalTrials.gov identifier: NCT02586155). This study is being monitored by an independent data and safety monitoring board, which recommended that the study should continue as per protocol. To date, the total exposure in patients with high-risk CV disease is approaching 2,000 subjects, with no heart failure signal identified. To ensure the safety of the drug in the context of right ventricular dysfunction, we conducted experiments in a rat model of increased ventricular afterload induced by pulmonary artery banding. Treatment with RVX208 was not accompanied by deleterious effects, and, on the contrary, promoted the ventricular response to increased afterload (1). Combined, these results affirm a favorable safety profile of RVX208 for use in patients with PAH and PAH-associated right ventricular dysfunction and support the establishment of a clinical trial. Finally, as pointed out by Pullamsetti and de Jesus Perez, a comprehensive understanding of how domain- and isoform-selective BET inhibition can regulate specific transcriptional and biological responses will further refine its applications in the clinical arena. ■

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Reply to Piquereau and Perros and to Pullamsetti and de Jesus Perez

From the Authors:

In a recent issue of the *Journal*, we reported results from the first multicenter, preclinical study to show the therapeutic benefits of the BET (bromodomain and extraterminal motif) inhibitor RVX208 in multiple animal models of pulmonary arterial

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