Downregulation of MCU led to features of the cancer-like mitochondrial-metabolic phenotype in PAH. In addition, miR-25 and miR-138 were increased in PAH PASMCs, whereas MCU was decreased in PAH arteries. Nebulized anti–miR-25 and anti–miR-138 restored MCU expression and reversed established monocrotaline-induced PAH in rats. Thus, miR-25 and miR-138 appear to be linked with metabolism in PASMC, an important mechanism in PAH that is attracting growing interest.

In an elegant review also published in the *Journal*, Chun and colleagues have outlined major miRNAs that are relevant in pulmonary hypertension, along with their targets and disease-relevant mechanisms (2). When they sorted miRNAs by molecular action, the authors created four main functional groups: metabolism and proliferation (miR-204, -223, -130/301, -17/92, -145, -193, -210, -424/503, -140, and -124), vasoconstriction (miR-130/301, -328, and -190), DNA damage (miR-204 and -223), and sex-specific signaling (miR-96 and miR-29) (2). However, in the metabolism and proliferation group, only miR-210 was directly related to metabolism (mitochondrial activity), and the other miRNAs were shown to regulate cell proliferation. Indeed, miR-210 was found to directly target and as such downregulate the iron-sulfur cluster assembly proteins ISCU1/2, which are essential for mitochondrial respiration (3). In addition to classification, Chun and colleagues (2) also performed computational network modeling to gain insight into miRNA activity in PAH, highlighting the central role of TGF/BMP signaling in PAH.

Given the impact of TGF/BMP signaling on PAH development, we would like to complete this overview by adding our recent findings regarding several important miRNAs that are regulated by TGFβ1 and the BMP2–peroxisome proliferator–activated receptor γ (PPARγ) axis in human PASMCs. The miR-130/301 family was recently demonstrated to be involved in PASMC proliferation (4), fibrosis, and vasoconstriction, and in our study we quantified an increased miR-130a/301b expression in laser-microdissected pulmonary arteries from patients with idiopathic PAH compared with control subjects (5). We also demonstrated in human PASMCs that the miR-130a/301b cluster is upregulated by TGFβ1. Indeed, TGFβ1 stimulation rapidly decreases PPARγ mRNA via miR-130a/301b, thus suppressing the vasoprotective BMP2/BMPR2–PPARγ axis. We identified PPARγ as the missing link that regulates the complex balance between mitogenic, glucose metabolism–promoting TGFβ1 signals and vasoprotective BMP2/BMPR2 (5).

Conversely, BMP2–PPARγ upregulates miR-148a and miR-331-5p expression in human PASMC (5). miR-148a is known to repress cell proliferation in bladder cancer cells (6). miR-331-5p downregulates the platelet isoform of phosphofructokinase messenger RNA (5), a rate-limiting enzyme of glycolysis and, as such, a proproliferative factor that we demonstrated for the first time to be overexpressed *in situ* in the pulmonary arteries of patients with idiopathic PAH (5). We propose that the BMP2/BMPR2–PPARγ axis upregulates miR-148a and miR-331-5p, thereby inhibiting SMC proliferation and glucose metabolism (5).

In conclusion, aside from the miRNAs related to cell proliferation, vasoconstriction, DNA damage, and sex-specific signaling reviewed by Chun and colleagues (2), we suggest that miR-25, miR-138 (1), and miR-331-5p (5) should be added to the group of miRNAs that are directly involved in PASMC metabolism. ■

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Reply

From the Authors:

Dr. Calvier and colleagues highlight valuable new information about additional microRNAs and their role in vascular metabolism in pulmonary hypertension. As evidenced by their elegant work and emphasized in our article (1), the list of microRNAs that are important in various aspects of pulmonary hypertension will undoubtedly continue to expand. In that context, an ongoing challenge in this field will be to discern regulatory hierarchies of how these molecules collaborate and interface with one another to elicit a final pathobiological effect. Strategies to achieve such insights will likely require a combination of more reliable *in vivo* or *ex vivo* models of pulmonary hypertension (2), more detailed molecular -omics profiling of those models, and more sophisticated computational approaches to analyze the

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resulting data (3). In regard to disease modeling, it will become increasingly important to develop models that can provide a platform to define the spatiotemporal relationships of these microRNAs within the vascular compartment, that is, to determine where and when the microRNAs are activated or repressed. To gain this level of insight, it likely will also be necessary to develop and/or standardize methods for quantifying and detecting alterations of microRNA expression and function, perhaps even in real time, from disease inception to end stage. As a scientific community, if we are able to achieve such levels of sophistication in our studies, we will have a much greater opportunity to leverage that information for diagnostic and therapeutic benefit in this otherwise exceedingly complicated pathobiology. ■

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What Should Be the Cutoff Value of Blood Eosinophilia as a Predictor of Inhaled Corticosteroid Responsiveness in Patients with Chronic Obstructive Pulmonary Disease?

To the Editor:

We read the article by Roche and colleagues (1) with great interest. The value of blood eosinophils as a predictor of responsiveness to an inhaled corticosteroid (ICS)/long-acting β_2-agonist combination versus a long-acting β_2-agonist/long-acting muscarinic antagonist combination for exacerbation prevention was investigated by Roche and colleagues. They compared treatment efficacy according to blood eosinophil percentage ($<$2% and $\geq$2%, $<$3% and $\geq$3%, and $<$5% and $\geq$5%) and absolute blood eosinophil count ($<$150 cells/μl, 150 to $<$300 cells/μl, and $\geq$300 cells/μl). The authors suggest that indacaterol/glycopyrronium is significantly superior to salmeterol/fluticasone for the prevention of exacerbations in the $<$2%, $\geq$2%, $<$3%, $<$5%, and $<$150 cells/μl subgroups, and at no cutoff was salmeterol/fluticasone superior to indacaterol/glycopyrronium. As a result, the authors commented that indacaterol/glycopyrronium provides superior or similar benefits over salmeterol/fluticasone regardless of blood eosinophil levels in patients with chronic obstructive pulmonary disease (COPD) (1).

The most notable point in this research was the cutoff values for eosinophilia. The eosinophilia cutoff value is described as $>$500 cells/μl, and 500–1,500 cells is considered mild, 1,500–5,000 moderate, and more than 5,000 severe eosinophilia. In addition, $>$1,500 cells/μl is accepted as eosinophilia with the potential for tissue infiltration and called hypereosinophilia (2). In eosinophilic severe asthma, studies with anti–IL-5 showed a better response to anti–IL-5 monoclonal therapies when the eosinophilia cutoff value was 300 cells/μl. There were no clinical or physiological responses with eosinophils less than 150 cells/μl in these studies. Although an eosinophil cutoff value higher than 300 is recommended even for anti–IL-5 therapies, we believe studies of ICS with eosinophil levels higher and lower than 300 may not lead to significant results that represent daily practice (3–5). Even though there is no consensus on eosinophilic asthma, the generally accepted opinion is that the eosinophil count should be $>$400 cells/μl (6). In addition, studies with patients with COPD indicate that patients with eosinophil counts between 300 and 400 cells/μl will also benefit from additional ICS treatment (7, 8 [this issue, pp. 1219–1221]).

We also use $\geq$300 as eosinophilia criteria whether it is asthma or COPD. We believe it reflects the real-life data better. Using an absolute value while evaluating the patients for peripheral eosinophilia will also lead to more consistent results in studies. In this study, baseline absolute cell count was also similar in the indacaterol/glycopyrronium and salmeterol/fluticasone treatment groups (median, 180 cells/μl; interquartile range, 110–280 cells/μl for both), both of which were far below eosinophilia criteria. This study had eosinophilia cutoff values such as 2%, 3%, and 5%; however, the absolute values that correspond to these values were not stated. Significant advantage was reported in patients with $<$150 cells/μl in prevention of exacerbations, but this superiority could not be demonstrated in absolute values $>$150 cells/μl. Moreover, in the blood eosinophil cutoff $\geq$500 cells/μl group, hazard ratios and estimated time to first moderate or severe COPD exacerbation was found to be better in the salmeterol/fluticasone group, although not significant.

Another point is the distribution of patient subgroups according to eosinophil levels: 375 (22.3%) patients had $\geq$300 cells/μl peripheral eosinophilia in the indacaterol/glycopyrronium group and 373 (22.2%) in the salmeterol/fluticasone group, forming 22.2% of the total study population, and 206 (12.3%) patients had $\geq$5% peripheral eosinophilia in the indacaterol/glycopyrronium group and 214 (12.7%) in the salmeterol/fluticasone group, making up 12.5% of total study population. The results will probably be different when a higher number of patients with $\geq$300 or 5% eosinophil levels are included in the study.

As a result, we believe $\geq$300 cells/μl will be a more appropriate cutoff value in studies on treatment efficacy in COPD according to

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