of PAL. However, the estimated completion date for VAST is not until December 2019. In the interim, our data add substantially to what is currently scarce literature, provide further encouragement to providers considering this intervention, and serve as a potential preview of what's to come. ■

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References

1. Criner GJ, Sue R, Wright S, Dransfield M, Rivas-Perez H, Wiese T, *et al*.; LIBERATE Study Group. A multicenter randomized controlled trial of Zephyr endobronchial valve treatment in heterogeneous emphysema (LIBERATE). *Am J Respir Crit Care Med* 2018;198: 1151–1164.
2. Chee CB, Abisheganaden J, Yeo JK, Lee P, Huan PY, Poh SC, *et al*. Persistent air-leak in spontaneous pneumothorax: clinical course and outcome. *Respir Med* 1998;92:757–761.
3. DeCamp MM, Blackstone EH, Naunheim KS, Krasna MJ, Wood DE, Meli YM, *et al*.; NETT Research Group. Patient and surgical factors influencing air leak after lung volume reduction surgery: lessons learned from the National Emphysema Treatment Trial. *Ann Thorac Surg* 2006;82:197–206. [Discussion pp. 206–207.]
4. Baumann MH, Strange C, Heffner JE, Light R, Kirby TJ, Klein J, *et al*.; AACP Pneumothorax Consensus Group. Management of spontaneous pneumothorax: an American College of Chest Physicians Delphi consensus statement. *Chest* 2001;119:590–602.
5. Havelock T, Teoh R, Laws D, Gleeson F; BTS Pleural Disease Guideline Group. Pleural procedures and thoracic ultrasound: British Thoracic Society Pleural Disease Guideline 2010. *Thorax* 2010;65:ii61–ii76.
6. Ding M, Gao Y-D, Zeng X-T, Guo Y, Yang J. Endobronchial one-way valves for treatment of persistent air leaks: a systematic review. *Respir Res* 2017;18:186.
7. Gilbert CR, Casal RF, Lee HJ, Feller-Kopman D, Frimpong B, Dincer HE, *et al*. Use of one-way intrabronchial valves in air leak management after tube thoracostomy drainage. *Ann Thorac Surg* 2016;101: 1891–1896.
8. Fiorelli A, D'Andrilli A, Cascone R, Occhiati L, Anile M, Diso D, *et al*. Unidirectional endobronchial valves for management of persistent air-leaks: results of a multicenter study. *J Thorac Dis* 2018;10: 6158–6167.
9. Mahajan AK, Verhoef P, Patel SB, Carr G, Kyle Hogarth D. Intrabronchial valves: a case series describing a minimally invasive approach to bronchopleural fistulas in medical intensive care unit patients. *J Bronchology Interv Pulmonol* 2012;19:137–141.
10. Bermea R, Miller J, Dugan K, Frye L, Murgu S, Hogarth D. Intrabronchial valves successfully treat iatrogenic, primary spontaneous, and secondary spontaneous persistent air leaks: a case series of 49 patients [abstract]. *Chest* 2018;154:856A–858A.
11. Mahajan AK, Doeing DC, Hogarth DK. Isolation of persistent air leaks and placement of intrabronchial valves. *J Thorac Cardiovasc Surg* 2013;145:626–630.

Transcriptomic Analysis of Alveolar Immune Cells in Acute Respiratory Distress Syndrome: To Lump or to Split?

To the Editor:

We read with interest the article recently published in the *Journal* by Morrell and colleagues entitled "Alveolar Macrophage Transcriptional Programs Are Associated with Outcomes in Acute Respiratory Distress Syndrome" (1). The authors sought to identify alveolar macrophage (AM) transcriptional signatures associated with poor outcomes in a single-center cohort of patients with acute respiratory distress syndrome. We applaud the authors for their work. Novel methods to improve phenotyping of critically ill patients, including those with acute respiratory distress syndrome, are urgently needed to aid both prognostic and predictive enrichment of clinical trials (2).

The study methodology used by the authors highlights an important question in research that aims to leverage omics technologies to identify clinically informative immune cell signatures in acutely ill patients: should we "lump" or should we "split"? In their study, Morrell and colleagues lump potentially heterogeneous cells together by isolating a broadly defined population of alveolar macrophages through negative selection (1). Marker expression of CD163 and CD71 is shown to support that the isolated cells are truly AMs. Genome-wide transcriptional profiling using microarray was then performed on these cells. Investigating AMs as a single cell type has advantages, including simplifying both sample processing and downstream analysis. In addition, it is plausible that clinically informative transcriptional changes can be identified across a broadly defined cell population irrespective of the complexities of the underlying biology. Indeed, a genomic classifier for lung cancer has been developed using a similar lumping approach with airway epithelial cells (3).

However, in recent years, a growing body of work has demonstrated the power of splitting, or teasing out functionally different subpopulations within broader cell types. We now know that the injured lung contains two distinct populations of AMs: embryonically derived resident AMs and recruited macrophages derived from circulating monocytes (4). Work using RNA sequencing supports that these subsets are transcriptionally and metabolically unique and play distinct roles in lung inflammation, lung fibrosis, and injury resolution

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(5, 6). With increasing use of single-cell profiling, the breadth and potential clinical importance of AM heterogeneity have become even more apparent. Our group has used single-cell RNA sequencing to identify novel populations of alveolar macrophages expressing profibrotic genes in patients with pulmonary fibrosis (7). In addition, a single-cell approach was recently used to identify five transcriptionally distinct clusters of AMs within the inflamed lung (8).

High-resolution studies aimed at splitting apart relevant populations of AMs in the injured lung can be limited by high cost and complex downstream analysis. In addition, it remains to be seen whether these novel approaches can support discovery of uniquely informative biomarkers or clinical phenotypes. However, it is important to consider how these splitting approaches may complement or improve on studies that analyze an immune cell population in the broadest terms. As an example, in the work by Morrell and colleagues, there were no differences in genome-wide expression profiles between patients with good versus poor clinical outcomes after adjustment for multiple testing (1). This may be because of the noise present in genomic datasets derived from critically ill patients, but it may also be driven in part by the limited resolution of the analysis; an overly broad view can make even complex systems look uniform.

Should we lump or split immune cells when studying the injured lung? If the goal is to advance our understanding of the pathobiologic mechanisms of disease, then splitting using high-resolution approaches offers particular promise. If the aim is to identify clinically informative disease phenotypes, then both approaches may prove useful and synergistic. However, when we choose the 10,000-foot view, we should remember and be informed by the complexity that lies below. ■

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References

1. Morrell ED, Bhatraju PK, Mikacenic CR, Radella Ii F, Manicone AM, Stapleton RD, *et al*. Alveolar macrophage transcriptional programs are associated with outcomes in acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2019;200:732–741.
2. Prescott HC, Calfee CS, Thompson BT, Angus DC, Liu VX. Toward smarter lumping and smarter splitting: rethinking strategies for sepsis and acute respiratory distress syndrome clinical trial design. *Am J Respir Crit Care Med* 2016;194:147–155.
3. Silvestri GA, Vachani A, Whitney D, Elashoff M, Porta Smith K, Ferguson JS, *et al*.; AEGIS Study Team. A bronchial genomic classifier for the diagnostic evaluation of lung cancer. *N Engl J Med* 2015;373:243–251.
4. Joshi N, Walter JM, Misharin AV. Alveolar macrophages. *Cell Immunol* 2018;330:86–90.
5. Misharin AV, Morales-Nebreda L, Reyfman PA, Cuda CM, Walter JM, McQuattie-Pimentel AC, *et al*. Monocyte-derived alveolar macrophages drive lung fibrosis and persist in the lung over the life span. *J Exp Med* 2017;214:2387–2404.
6. Mould KJ, Barthel L, Mohning MP, Thomas SM, McCubbrey AL, Danhorn T, *et al*. Cell origin dictates programming of resident versus recruited macrophages during acute lung injury. *Am J Respir Cell Mol Biol* 2017;57:294–306.
7. Reyfman PA, Walter JM, Joshi N, Anekalla KR, McQuattie-Pimentel AC, Chiu S, *et al*. Single-cell transcriptomic analysis of human lung provides insights into the pathobiology of pulmonary fibrosis. *Am J Respir Crit Care Med* 2019;199:1571–1536.
8. Mould KJ, Jackson ND, Henson PM, Seibold M, Janssen WJ. Single cell RNA sequencing identifies unique inflammatory airspace macrophage subsets. *JCI Insight* 2019;4:126556.

Reply to Walter and Reyfman

From the Authors:

We appreciate Drs. Walter and Reyfman's correspondence regarding our study (1). We agree that immune cell heterogeneity—particularly alveolar macrophage (AM) diversity—likely plays a key role in the disease pathogenesis of acute respiratory distress syndrome (ARDS). Recent studies by our group (2) and others (3, 4) have used single-cell approaches to better characterize alveolar immune subtypes in ARDS and animal models of acute lung injury. However, we caution against solely relying on "splitting" approaches such as single-cell RNA sequencing to understand the pathobiology of complex human syndromes. Highly granular approaches performed on limited numbers of subjects may not capture the diversity of clinical phenotypes that exist in critical illness, and there remain significant technical and computational limitations (5) regarding single-cell approaches.

Critical care translational studies rely on analyzing data from relatively large patient cohorts to overcome external confounders that can bias results such as variation in clinical interventions, timing in the onset of risk factors, and baseline genetic diversity. The complexity and cost of single-cell approaches currently limit the number of samples that can be practically analyzed. In addition, many important genes are not captured with commonly used single-cell RNA sequencing platforms because of the limited depth of sequencing coverage and amplification bias (6). For example, *Myd88* (myeloid differentiation primary response 88) and *Tlr9* (toll-like receptor 9) are two important macrophage effector genes that were not detected in a recent single-cell RNA sequencing experiment identifying AM subtypes in an animal model of acute lung injury (3).

Our bulk microarray approach was inclusive of 18,415 unique genes; however, we did not identify any differentially expressed genes in AMs from subjects with good versus poor clinical outcomes after adjustment for multiple hypothesis testing. We concur with

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