Clonal heterogeneity is believed to be a cancer hallmark. This is best exemplified by acute myeloid leukemia (AML), an aggressive hematopoietic malignancy in which myeloid progenitors accumulate in the bone marrow. Primary AML tumors contain multiple subclones, which display distinct sets of cytogenetic abnormalities, somatic mutations, epigenetic features, and functional properties. This multifaceted heterogeneity, moreover, is dynamic as the clonal composition of the tumor evolves during disease progression and relapse.

Although single-cell genomic technologies have greatly improved the characterization of AML biology, they present several shortcomings. Standard single-cell RNA sequencing (scRNAseq) methods are able to read full-length transcripts but they lack sufficient throughput to discern malignant from normal cells. Conversely, digital technologies, such as nanowell-based scRNAseq, which provide higher-resolution data, are not able to fully capture the mutational status of malignant cells as they present a 3’ bias in the read coverage.

In a recent issue of Cell, Peter van Galen and colleagues moved the technology one step forward and investigated AML hierarchies performing both transcriptional and mutational analysis at the single-cell level (Fig. 1). The authors profiled 38,410 single cells from 16 AML patients and 5 normal bone marrow aspirates using a high-throughput nanowell-based scRNAseq (seq-well), which they adapted to sequence frequently mutated AML genes. To do so, the researchers took advantage of an amplification step in the transcriptome protocol, which generated full-length cDNAs bearing cell-specific barcodes appended to the 3’ ends. Using primers adjacent to the mutational sites previously detected by targeted DNA sequencing, they next generated amplicons

**Figure 1. Clonal heterogeneity of AML.** Single-cell transcriptomic and mutational analysis revealed that AML samples have a variable cell-type composition, which correlates with genetics, surface markers, cellular morphology, and patient outcome. Less differentiated cells had stem cell characteristics, while more differentiated myeloid cells were shown to have an immunosuppressive function negatively affecting normal T-cells.
containing mutational sites and barcodes. Sequencing these amplicons by short- and long-read sequencing provided comprehensive genotyping of individual cells (ie, insertions, deletions, fusions, and point mutations of recurrently mutated AML genes). Transcriptomic and genotyping data were then integrated using a machine learning algorithm to distinguish malignant from normal cells.

The massive amount of data thereby generated was next interrogated to elucidate the composition of cellular hierarchies. To this end, the researchers first classified the leukemic cells based on their similarity to their normal bone marrow counterparts. This analysis identified 6 malignant AML cell types (hematopoietic stem cell (HSC)-like, progenitor-like, granulocyte-macrophage progenitor (GMP)-like, promonocyte-like, monocyte-like and conventional dendritic cell-like), whose relative abundance markedly varied between patient samples and correlated with the cellular morphology and surface phenotypes of the tumor bulk as well as patient outcome. In a second step, the authors obtained gene signatures for each of these AML cell types and used them to hierarchically cluster the bulk expression profiles of 179 diagnostic AML samples from the cancer genome Atlas. This strategy led the researchers to identify 7 different cellular clusters. Most of them comprised leukemias characterized by the predominance of 1 specific cell type (eg, progenitor-like), while another cluster included leukemias containing several malignant cell types along the differentiation spectrum (ie, from the hematopoietic stem cell-like to the myeloid-like type). Interestingly, each of these cell composition-based clusters closely correlated with prototypic genetic lesions, thus suggesting that genetics is an important force shaping the cellular composition in AML.

Lastly, the authors investigated in more depth 2 cell types at the opposite ends of the differentiation spectrum, namely the HSC-like AML cells and the monocyte-like cells. Confirming previous studies,5 HSC-like cells were found to co-express stemness-related and myeloid-priming genes. Monocyte-like cells, instead, expressed immunomodulatory factors and immunosuppressive myeloid markers, and strongly inhibited T-cell activation in vitro (Fig. 1). Albeit variable in abundance, myeloid-like cells were found in most of the AML samples analyzed, thus suggesting that they may play important roles in shaping an immunosuppressive microenvironment in the bone marrow. Functional studies will be necessary to extend these observations and dissect the mechanisms by which myeloid-like AML cells contribute to the development of the disease. Although it remains under debate whether T-cells can interact with and eliminate leukemia stem cells (LSCs), it will be intriguing to explore this scenario and verify whether the myeloid-like AML cells protect LSCs from immune-mediated elimination. Along this line and supporting previous findings,6 T-regulatory cells, a subset of T-cells endowed with immunosuppressive properties, were decreased in bone marrow aspirates from AML patients. In light of these findings, it is worth dissecting how myeloid-like AML cells affect the leukemic bone marrow microenvironment and LSC niches. Last, but not least, it will be important to exploit the technological advances developed by van Galen and colleagues to characterize preleukemic clones as well as the heterogeneity at the LSC level.

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