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Spotlight

Cellular hierarchies predict drug response in acute myeloid leukemia

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In a recent *Nature Medicine* study, Zeng and colleagues integrate both genomic and stem cell models of acute myeloid leukemia (AML) by deconvoluting cellular hierarchies of more than 1,000 AML samples. This work introduces a framework capable of predicting drug responses to targeted therapies in future clinical trials.

A major driver of resistance to cancer therapy is heterogeneity within the tumor. Genetic or non-genetic complexity, such as sub-clonality or the tumor-microenvironment, causes the outgrowth of a subset of cancer cells after therapy, impeding the success of precision oncology that aims to target oncogenic lesions in individualized treatments (Tannock and Hickman, 2016). Especially with novel agents entering the clinic, advanced biomarkers are required for a better understanding of tumor heterogeneity and to predict therapy responses more accurately.

In acute myeloid leukemia (AML), an aggressive hematologic malignancy with poor clinical outcome, inter- and intrapatient heterogeneity is explained by two complementary models: a genomic model that is based on cytogenetic and mutational drivers present in leukemic cells (Papaemmanuil et al., 2016) and a stem cell model that reflects the non-genetic hierarchical organization of AML similar to that of healthy hematopoiesis (Dick, 2008). Leukemia stem cells (LSCs), residing at the top of this hierarchy, harbor biological properties that differ from the remaining leukemic cells despite typically sharing identical genomic alterations. In current clinical practice, therapy decisions are almost exclusively influenced by the genomic model, although LSCs are known to drive chemotherapy resistance and/or disease relapse due to their capacity of long-term self-renewal and a transient status of cell-cycle quiescence or even dormancy. Thus, approaches that integrate both genomic and hierarchical models are needed for more accurate therapy decisions.

Single-cell sequencing is a powerful technology to delineate cellular hierarchies that has re-shaped our understanding of healthy and malignant hematopoiesis (van Galen et al., 2019; Velten et al., 2017). However, cost and analytical complexity prohibit the near-future implementation of such technologies into clinical routine diagnostics.

In their recent publication in *Nature Medicine*, Zeng and colleagues from the group of John Dick provide a framework for understanding heterogeneity and leveraging cellular hierarchies to predict drug responses in AML (Zeng et al., 2022). Instead of analyzing several hundred AML patient samples by single-cell technologies, the authors applied a two-step-process. First, authors re-analyzed existing single-cell transcriptomic data of 13,653 cells from 12 AML patients (van Galen et al., 2019) to define 7 AML subpopulations ranging from immature (“quiescent LSPCs,” “primed LSPCs,” “cycling LSPCs”) to more differentiated cell states (“GMP-like,” “Pro-Mono-like,” “Mono-like,” or “cDC-like”). In a second step, the abundance of each of these cell types was estimated in more than 1,000 AML patient samples using CIBERSORTx (Newman et al., 2019), a deconvolution algorithm that employs single-cell reference data to estimate the cellular composition of bulk gene expression profiles. As a result, for each patient a characteristic “hierarchy composition” was calculated (Figure 1). The relevance of this novel operationalization of the stem cell model of AML was comprehensively studied throughout the paper.

Depending on the relative abundance of each cell type, Zeng et al. identified four distinct groups of cellular hierarchies from 854 AML samples at initial diagnosis: samples that were enriched in primitive stem-like leukemia cells (primitive), were enriched in granulocyte-monocyte progenitor-like leukemia cells (GMP), were enriched in leukemia cells of mature phenotype (Mature), or were of intermediate composition (Intermediate). In a principal component analysis, samples were separated along two main axes: first, a continuum of primitive-to-GMP-enriched and second, a continuum of primitive-to-mature-enriched leukemic cellular hierarchies.

Interestingly, cytogenetic aberrations separated primarily along the primitive-versus-GMP axis with adverse cytogenetic alterations, e.g., inv(3) being composed of

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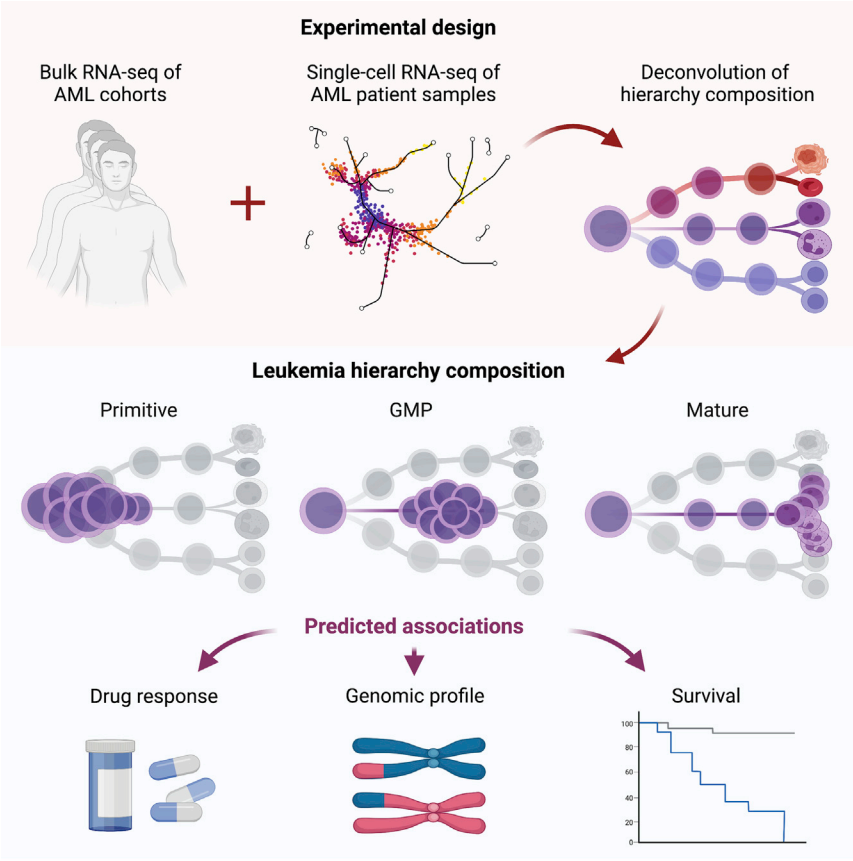

Figure 1. Graphical overview of the study
The experimental design (top row) and the major findings (bottom two rows) are illustrated.

more primitive hierarchies compared to favorable cytogenetic events. The primitive-versus-mature axis instead was more associated with genetic driver mutations and their combinations. For example, *RAS* mutations rather favored differentiation, whereas *IDH* mutations led to early differentiation blocks. By applying hierarchy deconvolution on well-annotated AML samples at large scale, Zeng and colleagues thus pioneered uniting both genomic and stem cell models of AML into a single framework.

How can this framework and its two axes be leveraged in clinical settings? First, analysis of paired diagnostic and relapse samples revealed that the hierarchy composition is altered during the course of the disease. In almost 90% of cases with longitudinal sampling, the primitive compartment had expanded significantly at the time of relapse, being the dominant cell type in most cases. This change in cellular hierarchy should be considered when interpreting early-phase clinical trials with novel compounds, which are usually evaluated in relapse or refractory settings. Compounds that might benefit patients at first diagnosis might therefore be overlooked.

Second, the primitive-versus-GMP-axis was associated with patient prognosis. Patients with GMP-dominant hierarchies showed a significantly better outcome compared to the other groups. This effect seems mostly driven by the fact that acute promyelocytic leukemia (APL) cases are classified into GMP-dominant hierarchies and thus constitute a substantial weight within this group. APL is a highly curable disease with overall survival (OS) rates of over 95%. Current gene expression prognostic scores, such as the LSC17 score introduced earlier by the group, associate with this axis and seem to perform superior as prognostic tools (Ng et al., 2016).

Third, the primitive-versus-mature axis was capable of predicting *ex vivo* drug responses to more than 100 investigational drugs, thus potentially providing guidance for informed personalized therapy decisions. To make this insight applicable for broader clinical use, Zeng and colleagues derived a sub-score of the LSC17 score that can be measured in a CAP/CLIA-certified clinical assay within 48 h (Ng et al., 2022). This 7-gene lineage classification sub-score (LinClass-7) performed comparably to the primitive-versus-mature axis in the prediction of *ex vivo* drug responses. In a retrospective analysis of the gemtuzumab-ozogamicin (GO) clinical trial, the LinClass-7 score was able to predict the effect of GO treatment for event-free and relapse-free survival. It remains to be shown whether LinClass-7 can serve as a useful predictive biomarker for other therapies, including approved but still novel drug combinations, such as venetoclax and azacytidine.

The authors also evaluated the hierarchy framework in the context of drug development, where candidates are usually selected based on their potential to kill bulk leukemia or cell lines. Compounds with a predominant effect on disease-propagating immature cells, but not the leukemic bulk, may be discarded erroneously in this process. Indeed, cellular deconvolution of RNA-sequencing data before and after drug treatment from public datasets revealed that the cell type composition changed in more than 80% cases. While ATRA, as expected, induced differentiation in mostly GMP-like blasts, the DHODH inhibitor Brequinar reduced the number of quiescent LSPCs. In preclinical xenotransplantation studies presented by the same group earlier, application of the cellular hierarchy framework helped to predict the response to the JAK2-inhibitor fedratinib and a novel GSPT1-degrader.

In summary, deconvolution of cellular hierarchies presented by Zeng and colleagues can serve as a framework for predictive biomarkers in future clinical trials. The connection between cell type composition and drug response in AML was also confirmed in a recent update of the BEAT AML cohort (Bottomly et al., 2022). Interestingly, no association was found between prognostic factors and predictors of drug response. A possible explanation for this discrepancy is that while a predicted drug is able to kill the majority of leukemic cells of a cellular hierarchy, the rare and disease propagating LSCs survive and drive deadly relapses.

Hence, LSCs remain a prime target to improve clinical outcomes of patients suffering from AML. Future single-cell multi-omics studies of AML will likely provide deeper insights into these issues.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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