An atlas of an aggressive leukemia
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"Now, we have a very quantitative way of determining the different cell types present within these tumors."

In the new study, which will be published online on February 28 in the journal *Cell*, Bernstein and his colleagues, including Ludwig Harvard investigators Jon Aster and Andrew Lane, combined cutting-edge technologies—single-cell RNA (scRNA) sequencing, long-read nanopore DNA sequencing and machine learning—to distinguish normal cells from cancerous ones and organize them based on their DNA sequence and gene expression similarities.

"This project was an interdisciplinary effort that connected engineers at MIT and the Broad Institute to cancer researchers and oncologists at the Massachusetts General Hospital, Dana-Farber Cancer Institute and Brigham and Women's Hospital," said Bernstein, of the Ludwig Center at Harvard, Harvard Medical School and Massachusetts General Hospital.

"We couldn't have done this without the vision that the Ludwig directors had in funding an effort to begin banking leukemia samples," added Bernstein.

scRNA sequencing captures a snapshot of a cell's entire transcriptome—that is, all the mRNA molecules present in a cell at a given moment. This allows researchers to break down a population of cells into distinct types and to track the evolution of cellular lineages. The team employed a new scRNA sequencing method to capture the full transcriptomes of nearly 40,000 bone marrow cells gathered from 16 AML patients and five healthy donors.

"This is the first time this has been done in a high-throughput manner," said study co-first author Volker Hovestadt, a postdoctoral research fellow in Bernstein's lab. "Instead of profiling only a couple hundred single cells, we were able to profile tens of thousands."

Bradley Bernstein of the Ludwig Center at Harvard, Harvard Medical School and Massachusetts General Hospital. Credit: Bradley Bernstein

A team of researchers led by Bradley Bernstein at the Ludwig Center at Harvard has used single-cell technologies and machine learning to create a detailed "atlas of cell states" for acute myeloid leukemia (AML) that could help improve treatment of the aggressive cancer.

AML is characterized by the accumulation of white blood cells in the bone marrow and blood. The disease is notoriously difficult to study because the bone marrow of AML patients contains a variety of normal and malignant cell types. This includes undifferentiated primitive cancer cells with stem cell-like properties and differentiated mature cancer cells that affect the tumor environment in different ways. AML cells also constantly acquire DNA mutations, branching into lineages of related "subclones."

"We've known in a qualitative way since the 1960s that AML tumors are heterogenous mixtures of cells," said Peter van Galen, a lead coauthor of the paper and a postdoctoral research fellow in Bernstein's lab at Massachusetts General Hospital.
But the gene expression profile of AML cells can resemble that of normal ones. To address this challenge, the team performed single-cell genotyping on the bone marrow cells, screening them for a set of known AML genetic markers to pick out those that were cancerous. "To really nail this, we had to incorporate a third-generation DNA sequencing technique called long-read nanopore sequencing, which enhanced detection of mutations across the genome," van Galen said.

Long-read sequencing scans much longer DNA fragments, boosting the odds of capturing all mutations accumulated in a gene. It allowed the researchers to more efficiently identify descendants of differently mutated cells, called subclones, that can variably affect the cancer's growth rate, metastatic potential and response to therapy.

Lastly, the researchers employed a machine learning algorithm to bring the scRNA sequencing and genotyping data together. The end result was a map or "atlas" of different AML cell types and the normal blood cell types with which they co-exist in the bone marrow environment.

The atlas allowed the researchers to see at a glance not only which cells were cancerous, but also their states—primitive, differentiated or in the process of differentiating. Since tumor samples were gathered from patients at different timepoints, the researchers could also compare the developmental hierarchies of AML tumors between individuals and track the evolution of those hierarchies from first diagnosis through treatment and outcome.

The researchers show that the transcriptional programs of AML cells are aberrantly regulated. "In normal cells, development progresses in stages. You go from cell type X to cell type Y," Bernstein said. "These leukemia cells co-express a mish-mash of genes from different stages."

The researchers also discovered multiple subclonal populations within a single AML patient. "In at least one case, the two subclones behaved completely differently and had different gene expression profiles," Bernstein said. "One subclone was mostly differentiating while the other was arrested in a highly aggressive state, and the aggressive subclone had a mutation that was consistent with a poor patient prognosis."

The findings may also explain why therapies that harness T cells of the immune system to target tumors have been relatively less successful against AML. The researchers identified a class of cells that behaved like white blood cells known to suppress anticancer immune responses.

"You might assume that these are normal cells, but when you look at their genotype, you see that they harbor leukemic mutations," Bernstein said. "These are cells that the tumor is producing to suppress the immune system. It's an adaptive survival mechanism for the tumor."

Their findings, the researchers note, should aid in the development of novel strategies for immunotherapy and precision medicine for the treatment of AML.

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