mission bio

tapestri
RESEARCHER SPOTLIGHT

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Dr. Ravi Majeti's clinical focus is in hematology and his lab focuses on the molecular and genomic characterization and therapeutic targeting of leukemia stem cells in human hematologic malignancies, particularly acute myeloid leukemia (AML). In parallel, his lab also investigates normal human hematopoiesis and hematopoietic stem cells. An important part of his research is finding better tools to measure residual disease, including being able to differentiate between clonal hematopoiesis and true leukemic cells.

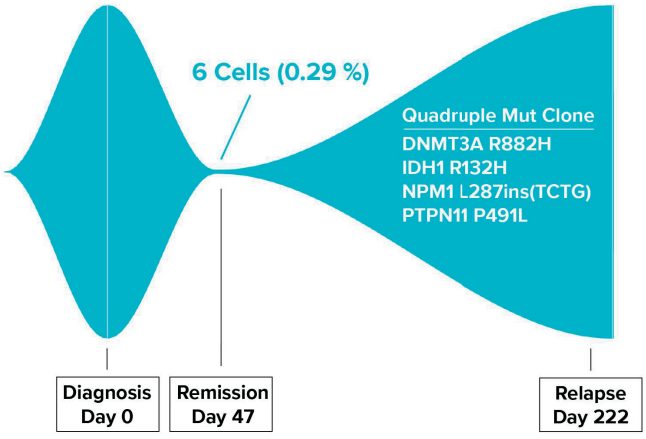
Transforming the Detection of Minimal Residual Disease (MRD) with Single-Cell DNA Analysis

Dr. Ravi Majeti shares his thoughts on using the Tapestri™ Platform to measure residual disease in acute myeloid leukemia (AML).

Q: What is unique about AML as a disease that makes it amenable to genomic and single-cell analysis?
Ravi Majeti (RM): AML is relatively unique in that it is easy to sample the disease tissue, that being the bone marrow or the blood, at serial time points during the course of the disease, as well as during the course of therapy. Moreover, the genetics of this disease have been very well described, making it possible to design relatively comprehensive genomic panels.

Q: How is genomics currently being used to test and treat AML?
RM: The current paradigm for treating AML patients involves upfront genotyping with next generation sequencing to identify potential variants that are relevant to prognosis and therapy selection. This is important as a number of targeted therapies have been recently FDA-approved, with others likely to be approved in the near future. These therapies will need to be utilized in patients with the corresponding mutations. For example, recently approved IDH-mutant specifi ɑnhibitors are only relevant to patients whose disease is characterized by IDH mutations. Fundamentally, this is how we think about using mutational analysis for the stratification of AML patients and therapy selection.

MRD Detection at Remission



Longitudinal single-cell DNA sequencing was performed on AML samples at diagnosis, remission and relapse with Tapestri. Rare cells harboring all four mutations found at relapse were detected during remission.

Q: What challenges do clinicians face in measuring MRD today?
RM: Multiple methods are traditionally used for MRD detection. They include cytogenetics, that's been done for decades, and patients with complex cytogenetics have worse prognosis with a higher risk of relapse. Flow cytometry can also be used. And molecular genotyping can be used, with PCR assays to look for specific mutations that are in expressed genes. More recently, NGS panels of genes can cover the spectrum of mutations in AML. All of the MRD methodologies have limitations. And that's where the ability to do deep interrogation at the single-cell level has real potential value in the care and monitoring of AML patients.

Q. How do you think single-cell analysis can improve MRD detection?
RM: Single-cell measurements of residual disease have the advantages of addressing a couple of key issues. First of all, in AML there is often a condition preceding the disease known as clonal hematopoiesis, and we have identified pre-leukemic stem cells that have acquired some of the mutations that will ultimately give rise to the leukemic clones. One challenge is that patients in remission can still have a significant burden of these pre-leukemic mutations present even though they are not reflective of actual leukemia. Single-cell analysis will allow us to determine if the cells carrying these mutations are actually these pre-leukemic/clonal hematopoiesis cells. The second issue is that patients with AML often have multiple mutations co-occurring in different subclones, leading to a complex clonal architecture. With bulk sequencing information, we often cannot resolve the actual clonal composition. That's the real power of the single-cell approach, because it can determine whether the variants in question are occurring in the same cell or not. And that can be very important in determining therapy response, drug sensitivity, and therapy resistance. I think that a lot of these questions are open, but certainly the single-cell approach will allow us to start to get some answers.

Q. What kind of single-cell data have you generated?
RM: We have done a few pilot studies, looking at paired specimens obtained from the same individual patient, and at different time points: diagnosis, post-chemotherapy remission, and relapse. We used the Tapestri™ Platform to determine the clonal composition of these samples, including the detection of all of the variants that were found by classic NGS. We determined their co-occurrence in individual cells, thereby determining clone size and clone frequency at these different time points. This is really powerful because this information could not always be resolved from bulk sequencing information. With Tapestri, we were actually able to identify a very low frequency residual leukemic cell population, at less than 0.3% of the total remission cells, that clearly gave rise to relapse (see figure). So, even in a small set of cases, we can see that there's power to using the single-cell approach for the detection of rare cells at remission that can give rise to relapse. And that's really what minimal, or measurable, residual disease is all about.

Q. Where do you see the field of MRD in the next 5 years?
RM: The whole field is very actively focused on this question. In AML, MRD assessments conducted at the time of initial clinical remission may potentially help select consolidation therapies. It may be that patients with a higher burden of residual disease, or any detectable residual disease above a certain threshold, might actually be at poor risk and therefore recommended for transplant early in their disease course. It could be that patients during their consolidation therapy, or even post-transplantation, need to be monitored, and that the presence of clones detected by single-cell methods could dictate whether maintenance therapy is appropriate, and how long to use maintenance therapy. These are the types of clinical questions currently under investigation, and it may be that this single-cell approach will be the most valuable to making those clinical decisions.