Mass Cytometry: A High-Throughput Platform to Visualize the Heterogeneity of Acute Myeloid Leukemia

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Summary: Behbehani and colleagues use mass cytometry to profile the heterogeneity of acute myeloid leukemia (AML) as compared with normal hematopoiesis. By using this approach and characterizing leukemic stem cells, important differences in response to chemotherapy between AML subsets can potentially be explained and new targeted approaches considered. Cancer Discov; 5(9); 912-4. ©2015 AACR.

See related article by Behbehani et al., p. 988 (3).

The ability to measure up to 18 different parameters—surface protein, intracellular protein, cell cycle, viability, and more—at a single-cell level has made traditional flow cytometry a vital tool in the study of multiple diseases. Although traditional flow cytometry maintains unique advantages, a new technology, mass cytometry, has the capacity to measure over 30 parameters simultaneously. Mass cytometry utilizes stable isotopes of nonbiologic metals conjugated to conventional antibodies, negating the issue of spectral overlap as the size of the panel increases (1). One breakthrough study in 2011 by Bendall and colleagues used mass cytometry to map the human immune system, demonstrating the impressive utility of this technique. They provide a holistic view of hematopoiesis, including immunophenotyping via surface antigen expression, intracellular signaling changes, and cell cycle, on one platform, at the level of a single cell (2). Building off of this work, mass cytometry can potentially be used to inform on the pathogenesis of disease as well as assess and guide therapeutic regimens that might not be apparent without analysis of subclones or small cell populations. A model disease where malignant stem cells are believed to be important in pathogenesis, acute myeloid leukemia (AML), is investigated in the current study by using a high-dimensional analysis via mass cytometry (3).

AML describes a group of heterogeneous myeloid malignancies, divided into several subsets based on cytogenetic, mutational, and morphologic distinctions. Response to chemotherapy as the current first-line treatment, reported by Mrózek and colleagues ranged from 39% to 96% complete remission (CR; ref. 4). These response rates are hypothesized to vary widely as a result of the disease heterogeneity, from which several risk factors have been identified to divide patients into prognostic groups based on favorable, intermediate, and unfavorable risk. The favorable-risk group generally responds well to chemotherapy alone, the unfavorable-risk group may be advised for allogeneic stem cell transplantation, given eligibility, and the best course of treatment for the intermediate-risk group is currently unclear. Furthermore, why some favorable-risk group patients respond so well to cell cycle–directed therapy (i.e., cytarabine) is also poorly understood. In the present study, Behbehani and colleagues demonstrate in vivo phenotypic differences between patients primarily with AML, other myeloid malignancies, and healthy donor bone marrow. Of interest, core binding factor AML (CBF-AML) of the favorable-risk group and normal karyotype AML (NK-AML) with internal tandem duplications within the fms-related tyrosine kinase 3 gene (FLT3-ITD) from the intermediate-risk group are directly compared. The authors propose a mechanism explaining their differential response to chemotherapy as an example of the exploratory inference that this technique can provide, aiding in our understanding of observed clinical biology.

The authors use a single platform, mass cytometry, to elegantly unveil several stages of phenotyping: cell subsets present, their relative quantities, 28 individual markers of abnormal protein expression, cell cycle, intracellular signaling, and in vivo response to therapy in a high-dimensional analysis of patients with myeloid malignancy of different subtypes compared with healthy donors. Samples of bone marrow aspirates from 35 AML, 4 acute promyelocytic leukemia (APL), 2 myelodysplastic syndrome, and 5 healthy donors were immediately collected and minimally processed prior to freezing the samples until the time of mass cytometric analysis. Using two panels covering 39 parameters (25 in common between both), samples were bar-coded and run simultaneously. Barcoding provides the advantage of combining the samples into a single staining reaction, eliminating technical variability between samples (5). The results by mass cytometry were compared with clinical flow cytometry data, and the methods were highly consistent. Consistency between these techniques provides validation and suggests an eventual transition of this new technique to clinical diagnostic laboratories where direct decisions can be made based upon the results observed.

Immunophenotypic analysis was completed with 19 surface markers and Spanning-tree Progression Analysis of Density-normalized Events (SPADE) analysis. SPADE analysis enables a system-wide view of cell phenotypes, the relative
relationships between populations, and relative abundance in a hierarchical diagram of high-dimensional data (6). There was a consistent phenotype across healthy donors and a large (50-fold) increase in immature cells [hematopoietic stem cells (HSC); lin CD34+CD38−CD45RA CD90+CD33−, and multipotent progenitor cells (MPP); lin CD34+CD38−CD45RA CD90+CD33−] in CBF–AML and adverse karyotype (ARK) AML and a smaller increase (3–10-fold) in NK–AML and APL. Next, surface marker expression (28 parameters) of AML samples compared with healthy donors revealed aberrations in over 35 immunophenotypic populations. All AML samples had aberrations in every stage of myeloid lineage cells, and for several patients, aberrations were also detected in nonmyeloid cells, including B, T, and natural killer cells. There were distinctions made between subsets of AML in the hematopoietic stem and progenitor cell populations (HSPC; lin’ CD34+CD38lo). This finding is of particular interest to the hypothesized pathogenesis of AML. Until now, only indirect evidence in vitro and using murine transgenic and xenograft models has been able to support the presence of HSC-like leukemia stem cells (LSC; lin’CD34+CD38-) that are thought to be responsible for relapsed AML (7). In this study, additional in vitro evidence of an aberrant lin’ CD34+CD38lo population distinct between AML and normal counterparts is provided. The potential to monitor the effects of therapy on this particular population could be a critical step forward in the treatment of AML and for following patients post-therapy for the elimination of these cells. In addition, for differential surface antigen expression, this study identified FLT3-ITD+ NK–AML HSPCs to have more CD33 and CD123 expression, providing justification for targeted antibody or antibody-toxin–directed treatment toward this subset.

The authors further characterize the differences among HSPCs using viSNE to represent high-dimensional data. viSNE has the power to visualize several parameters simultaneously in 2 to 3 dimensions. With viSNE analysis, cells can be clustered by phenotype even without the addition of canonical markers. This tool enables one to robustly visualize changes in the phenotypic landscape across samples (8). Distinctions were observed with 19 surface markers across canonical markers. This tool enables one to robustly visualize changes in the phenotypic landscape across samples (8). Distinctions were observed with 19 surface markers across canonical markers. This tool enables one to robustly visualize changes in the phenotypic landscape across samples (8).

With regard to cell cycle, healthy donor bone marrow had the highest percentage of S-phase cells in early committed progenitor populations (myelomonoblasts, promonocytes, and pro- myelocytes) with the HSPC compartment having a relatively lower percentage of S-phase cells. AML samples on average had a lower percentage of S-phase cells compared with their normal counterparts. Comparing AML subsets, CBF–AML had the highest S-phase fraction of HSPCs, whereas FLT3-ITD+ NK–AML HSPCs had a lower percentage of S-phase cells than other AML samples. These data correlate with clinical response to chemotherapy, where CBF–AML tends to have higher CR rates after initial therapy than FLT3-ITD+ NK–AML (4). Next, Behbehani and colleagues looked at the aberrant CD34+CD38lo populations for intracellular signaling differences compared with their normal counterparts and found that almost all AML subtypes exhibited higher MAPKAPK2 phosphorylation, and, furthermore, the FLT3-ITD+ AML had higher pSTAT5A, a known downstream target of FLT3-ITD. In vitro evidence for phenotypic abnormalities in specific populations of cells can guide identification of potential therapies to target malignant populations of cells, while potentially sparing normal cells. Finally, the in vitro response to therapy (oral hydroxyurea; HU) was assessed in 10 treated patients compared with 23 untreated patients. Myeloid lineage populations were mostly unaffected by HU treatment as measured by percentage of S-phase cells. CD34+CD38lo populations were least affected by HU, and actively cycling cells were unchanged or increased in treated patients. This result is in contrast with the effectiveness against proliferative cells determined using in vitro studies, but in line with the lack of efficacy observed as a long-term effect of this agent in treating AML (9, 10).

Behbehani and colleagues have opened doors with the current study. An astonishing amount of information was afforded from one set of bone marrow aspirates. The combination of analyzing a sample directly from the patient and immediate determination of cell subsets present, their specific aberrations in protein expression, signaling, or cell cycle via mass cytometry could potentially be a new method of guiding clinical decisions and will certainly deepen our understanding of disease pathogenesis and response to therapy.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**REFERENCES**


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