Linking Clinical Outcomes in AML with the Cellular Origins of Mutations

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As a heterogeneous group of blood cancers, acute myeloid leukemia (AML) is subdivided into risk groups according to chromosomal translocations and gene mutations. Precisely which cell originally acquires these molecular alterations is unclear, but could explain why some patients with low-risk mutations respond poorly to treatment or why some patients with high-risk mutations do well. To address this question, the laboratory of Dr. Roland Walter in the Clinical Research Division developed new methods to culture the small numbers of immature cells from AML patients. Their study, published in the journal *Leukemia*, confirmed that the cell of mutation origin varies between individual AML patients and influences clinical outcomes.

Several decades ago, researchers at the University of Washington and the Fred Hutchinson Cancer Research Center suggested the cell of mutation origin varies between individual AML patients (Fialkow *et al*., 1987). The researchers examined X chromosome inactivation patterns across different types of mature blood cells in female AML patients. In healthy individuals, X chromosome inactivation is polyclonal among red and white blood cells. In contrast, in some AML patients, both red and white blood cells had clonal X inactivation, suggesting that leukemic mutations originated in a hematopoietic stem cell that gives rise to both cell types. Other AML patients presented with clonal expansion only in myeloid cells, a subset of white blood cells, suggesting the genetic change driving leukemogenesis occurred in a more committed precursor cell.

In the current study, Walter and colleagues developed methods to specifically isolate and study the rare immature stem/progenitor cells from AML patient specimens. The researchers mimicked conditions found in the bone marrow niche where stem cells reside, including low oxygen conditions and co-culture with engineered endothelial cells (Butler JM, *et al*., 2010). The AML cells were also cultured with an inhibitor to the aryl hydrocarbon receptor, which maintained the cells in an immature state. These cells were then studied at the functional and phenotypic level to detail the acquisition of known genetic changes in cells, and link them to clinical outcomes.

Specifically, the researchers separated immature and more mature bone marrow cells by staining for cell surface markers and then using fluorescence-activated cell sorting. Less mature cells were

defined as those that expressed CD34 but lacked expression of CD33, whereas slightly more mature progenitors were defined as those that expressed both CD34 and CD33. Sorted cells were examined for the ability to give rise to myeloid colonies *in vitro* either immediately, 2, 4, 6, or 8 weeks after sorting. The resulting colonies were then subject to molecular analysis, assessing the clonality of the cells by examining X-inactivation of the *HUMARA* gene. As was seen in the classic Fialkow experiment, Walter observed that some patients exhibited polyclonality from long-term CD34+/CD33- culture indicating that the immature cells were predominantly normal, while other patients had clonality, indicating the leukemia originated in more immature cells. By performing these experiments on 50 AML patient samples with known clinical outcomes, the researchers found that patients with predominantly leukemic cells deriving from the most immature CD34+/CD33- population had significantly lower overall and relapse-free survival than those with predominantly normal growth (P=0.045 and P=0.03 respectively, see figure).

In one subset of AML patient specimens, the researchers were able to use their methods to study the sequence of mutational acquisition for the first time in human specimens. These patients had a *CBF* translocation and other acquired mutations important for leukemogenesis. The researchers detected only *CBF* translocations in the most immature cells after long-term culture, and not the other acquired mutations. This result suggests that the *CBF* translocation arose first, priming cells to a pre-leukemic state, followed by additional mutations in more committed progenitor cells to develop full-blown AML.

According to Dr. Walter, "combining functional assays with clinical information together suggests that not only is there heterogeneity with respect to the cell type in which AML mutations are acquired, but that the maturity of mutation acquiring cells may dictate patient outcome." Furthermore, Dr. Walter states that the data supports "the idea that the context in which the mutation occurs may be more important than the mutation per se." Dr. Walter cautions that newer targeted therapies aimed at eradicating AML stem cells cannot be a "one size fits all" approach, but may need to be tailored to the type of AML. Specifically, their results suggest that treatments which target cells with the more differentiated marker CD33 may fail for patients that acquire mutations in more immature stem cells.

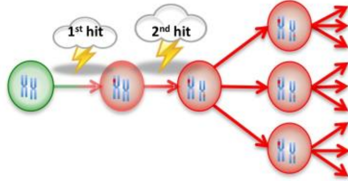
Walter, RB, Laszlo, GS, Lionberger, JM, Pollard, JA, Harrington, KH, Gudgeon, CJ, Othus, M, Rafii, S, Meshinchi, S, Appelbaum, F, Bernstein, I. 2014. Heterogeneity of clonal expansion and maturation-linked mutation acquisition in hematopoietic progenitors in human acute myeloid leukemia. *Leukemia*. Epub ahead of print, doi: 10.1038/leu.2014.107.

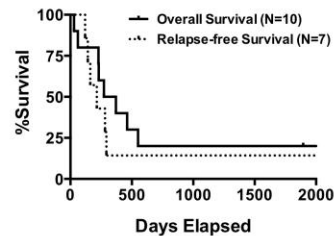
See also: Butler JM, Nolan DJ, Vertes EL, Varnum-Finney B, Kobayashi H, Hooper AT, Seandel M, Shido K, White IA, Kobayashi M, Witte L, May C, Shawber C, Kimura Y, Kitajewski J, Rosenwaks Z, Bernstein ID, Rafii S. 2010. Endothelial cells are essential for the self-renewal and repopulation of Notch-dependent hematopoietic stem cells. *Cell Stem Cell*. 6:251-264.

Fialkow PJ, Singer JW, Raskind WH, Adamson JW, Jacobson RJ, Bernstein ID, Dow LW, Najfeld V, Veith R. 1987. Clonal development, stem-cell differentiation, and clinical remissions in acute nonlymphocytic leukemia. *N Engl J Med*. 317:468-473.

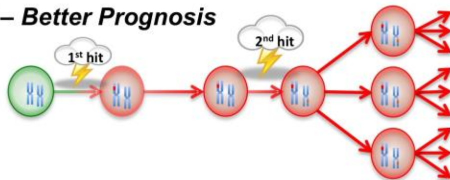
Multipotent Progenitors (CD34+/CD33-) -----------> **Committed Precursors (CD34+/CD33+)**

"Immature AML" – Worse Prognosis





"Mature AML" – Better Prognosis



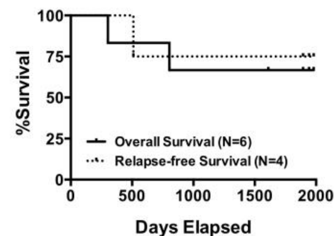


Image provided by Dr. George Laszlo

If DNA mutations occur in more immature stem/progenitor cells in acute myeloid leukemia (AML) patients, overall and relapse-free survival rates are decreased. AML patients with more mature cells of mutation origin have a better prognosis.