Molecular pathology selected abstracts

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Assessing BCL11A gamma-globin repressor for sickle cell anemia, β-thalassemia

February 2021—Beta-thalassemia and sickle cell disease are common hereditary conditions that can have life-threatening complications. Both diseases are caused by genetic alterations affecting the beta subunit of hemoglobin. Mutations that reduce or prevent the synthesis of the beta-globin protein cause beta-thalassemia, a disease characterized by inadequate red blood cell production and, therefore, anemia. In contrast, sickle cell anemia results from a specific point mutation in the beta-globin gene that causes the resulting protein to polymerize. These protein polymers form rigid fibers that affect the stability of the red blood cell and cause its characteristic sickling deformity. Destruction of the aberrant red blood cells leads to anemia, and the sickled cells can also cause painful vaso-occlusive episodes and tissue damage. Long-term treatment for both diseases may involve blood transfusions with concomitant iron chelation therapy to prevent iron overload. Allogeneic hematopoietic stem cell transplantation is a curative treatment, but it is performed only in severe cases because of the risk of complications and graft-versus-host disease. An alternative therapy, hydroxyurea, elevates the level of fetal hemoglobin. The latter is formed from two alpha and two gamma subunits instead of the beta subunits that are mutated in beta-thalassemia and sickle cell patients. Elevated levels of fetal hemoglobin have been shown to be protective in sickle cell patients, improving morbidity and mortality. The production of fetal hemoglobin typically is tightly regulated and decreases in infancy to the extent that adults have only a small amount of it in circulating red blood cells. *BCL11A* is a transcription factor gene on chromosome 2 that normally represses the expression of gamma-globin. It was recently reported in a genomewide association study that polymorphisms affecting this gene are associated with increased expression of fetal hemoglobin. Therefore, reducing or eliminating *BCL11A* expression might increase gamma-globin expression in hematopoietic cells. This hypothesis was examined in two trials published simultaneously in *The New England Journal of Medicine*. A study by Frangoul, et al., involved two patients—one with beta-thalassemia and another with sickle cell disease—who were treated with autologous CD34+ stem cells that had been genetically edited with CRISPR-Cas9 to reactivate fetal hemoglobin production. Stem cells were mobilized and collected from the patients, after which the *BCL11A* erythroid-specific enhancer locus was edited with CRISPR-Cas9 using a single guide RNA molecule. The patients then underwent myeloablative conditioning and their modified stem cells were infused. Once the cells engrafted, about 30 days later, high levels of fetal hemoglobin were found in almost all red blood cells, and the patients' total hemoglobin and fetal hemoglobin levels increased rapidly. Most importantly, the patients no longer required transfusions. In the second trial, Esrick, et al., collected autologous CD34+ stem cells from six sickle cell disease patients and modified them using gene therapy with a lentiviral vector that encodes a short hairpin RNA that targets *BCL11A* mRNA. After myeloablative conditioning, the modified cells were infused, and the patients' total hemoglobin and fetal hemoglobin levels increased. Furthermore, clinical manifestations of sickle cell disease vastly declined, and none of the patients had vaso-occlusive crises after infusion of the modified cells. Both studies demonstrated the feasibility of infusing genetically modified autologous hematopoietic stem cells as a potential cure for beta-thalassemia, sickle cell disease, and other hemoglobinopathies.

Esrick EB, Lehmann LE, Biffi A, et al. Post-transcriptional genetic silencing of *BCL11A* to treat sickle cell disease [published online ahead of print December 5, 2020]. *N Engl J Med*. 2020;10. doi:10.1056/NEJMoa2029392

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Frangoul H, Altshuler D, Cappellini MD, et al. CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia [published online ahead of print December 5, 2020]. *N Engl J Med*. 2020;10. doi:10.1056/NEJMoa2031054

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Feasibility and efficacy of precision medicine to treat acute myeloid leukemia

Acute myeloid leukemia results in the uncontrollable growth of abnormal immature blood cells of myeloid lineage. There are many subtypes of the disease, and they are heterogeneous in clinical outcome and molecular pathogenesis. A wide variety of mutations affecting diverse cellular pathways contribute to acute myeloid leukemia (AML). Modern molecular methods have greatly advanced the medical community's understanding of AML leukemogenesis. However, the ability to use this knowledge to treat patients with targeted therapies has been limited. Acute myeloid leukemia has a high degree of morbidity and mortality, especially in older adults, and a diagnosis of AML is viewed with a sense of urgency. Therefore, the standard clinical practice is to begin induction chemotherapy as soon as possible, often before molecular testing has been completed. The Beat AML Master Clinical Trial is a prospective trial of AML patients 60 years and older that addresses whether it is feasible and effective to wait for full molecular results, which was a period of about seven days, before assigning targeted treatments. Of the 395 eligible patients, 224 elected to enroll in a targeted therapy substudy directly informed by molecular results, 103 in standard-of-care therapy, 40 in palliative therapy, and 28 in an alternative investigational study. There were no significant differences in clinical, demographic, laboratory, or molecular characteristics between the targeted therapy and standard-of-care groups, which were the main comparators of the study. Thirty-day mortality was 3.7 percent for patients electing targeted treatment compared with 20.4 percent for patients electing standard of care. Overall survival was also significantly longer for patients electing targeted treatment (median, 12.8 months) than for those electing standard-of-care (median, 3.9 months) or palliative (median, 0.6 months) treatment, but it was not significantly different from patients electing to enroll in an alternative investigational study (median survival not reached). This study demonstrates that delaying treatment for the vast majority of older AML patients for up to seven days, to allow for mutation results informing targeted therapy, appears to be safe and improves survival. Overall, the Beat AML trial provides evidence that the benefits of using precision medicine to directly inform AML therapy may outweigh the costs of delaying treatment to perform molecular characterization, even in older patients. It also emphasizes the importance of coordination and communication between clinicians and laboratorians to treat patients most effectively.

Burd A, Levine RL, Ruppert AS, et al. Precision medicine treatment in acute myeloid leukemia using prospective genomic profiling: feasibility and preliminary efficacy of the Beat AML Master Trial. *Nat Med*. 2020;26(12):1852–1858. doi:10.1038/s41591-020-1089-8

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