Molecular pathology selected abstracts

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Evaluation of the safety and efficacy of a sickle cell disease gene therapy

June 2022—Sickle cell disease is caused by a point mutation in the gene encoding β-globin and is characterized by chronic hemolytic anemia, painful vaso-occlusive events, and increased risk of early death. The disease can be managed using supportive therapy, but this does not halt its progression. HLA-matched allogeneic hematopoietic stem cell transplantation is a potential curative treatment option. However, it is not without risk, including the possibility of such complications as graft-versus-host disease, graft rejection, and transplantation-associated death. Furthermore, stem cell transplantation is recommended primarily for younger patients and is limited because only 14 to 20 percent of patients have an HLA-matched donor. Gene therapies that use autologous hematopoietic stem and progenitor cells (HSPCs) have the potential to overcome many of the potential therapeutic obstacles. Lenti-Globin gene therapy (Bluebird Bio, Cambridge, Mass.) utilizes HSPCs transduced with a lentiviral vector encoding a modified β-globin gene, which produces an anti-sickling hemoglobin (HbA^{T87Q}). The latter inhibits polymerization of sickle hemoglobin. In an ongoing phase one/two study, the authors evaluated the safety and efficacy of LentiGlobin in treating sickle cell disease. The patients in study group C (35 patients) used updated LentiGlobin therapy protocols. (Groups A and B were studied using prior LentiGlobin protocols.) The stringent criteria for participating in group C included a minimum of four severe vaso-occlusive events in the 24 months prior to enrollment. The median follow-up for this group was 17.3 months (range, 3.7–37.6 months), and engraftment occurred in all 35 patients. Without additional infusions of packed red cells, the median total hemoglobin increased from 8.5 to 11 g/dL or more. The one-time infusion led to stable, durable production of the anti-sickling hemoglobin. HbA^{T87Q} contributed to at least 40 percent of total measured hemoglobin and was expressed in approximately 85 percent of red cells. Furthermore, LentiGlobin infusion reduced sickle hemoglobin levels and markers of hemolysis. The median rate of severe vaso-occlusive events in group C 24 months prior to enrollment was 3.5 per year (range, 2–13.5 events). In comparison, no severe vaso-occlusive events were reported in the 25 patients who had at least six months of follow-up after LentiGlobin infusion. Three patients had adverse events, including leukopenia, decreased diastolic blood pressure, and febrile neutropenia, that were probably or definitely related to LentiGlobin infusion. The adverse events resolved within one week of their onset. While these early study results are promising, additional studies with long-term follow-up are needed to further evaluate the safety and efficacy of LentiGlobin, including the long-term durability of the treatment, changes to sickle cell physiology, and effects on hemolysis, vaso-occlusive events, and risk of death.

Kanter J, Walters MC, Krishnamurti L, et al. Biologic and clinical efficacy of LentiGlobin for sickle cell disease. *N Engl J Med*. 2022;386(7):617–628.

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Insights into mechanisms of resistance to noncovalent Bruton’s tyrosine kinase inhibitors

The introduction of covalent (irreversible) Bruton’s tyrosine kinase inhibitors has significantly impacted the treatment of chronic lymphocytic leukemia and other B-cell cancers. However, resistance mechanisms, including acquired Bruton’s tyrosine kinase (BTK) mutations at residue C481, have been well documented. Noncovalent (reversible) inhibitors of BTK, such as pirtobrutinib, are meant to overcome this mechanism of resistance, inhibiting

both wild-type BTK and C481-mutant BTK. PLCγ2 (phospholipase C gamma 2) mutations have also been reported as a mechanism of resistance to ibrutinib therapy. Unlike covalent inhibitors, however, mechanisms of resistance to noncovalent inhibitors have not been well elucidated. Consequently, the authors sought to identify the genetic characteristics of chronic lymphocytic leukemia (CLL) with clinical resistance to noncovalent BTK inhibitors. They obtained peripheral blood samples, and bone marrow and lymph node biopsy samples when indicated, from patients who had relapsed or refractory CLL treated with pirtobrutinib and who participated in the BRUIN phase one/two multicenter study of oral pirtobrutinib monotherapy (NCT03740529). The specimens were pretreatment samples and samples obtained at the time of disease progression. Nine patients, whose pretreatment and post-treatment specimens were available for analysis, demonstrated resistance to pirtobrutinib. The authors performed genomic analyses using targeted next-generation sequencing. Seven of the nine patients with clinical resistance demonstrated newly acquired mutations, including V416L, M437R, T474I, and L528W (alone or in combination), in the BTK kinase domain outside the C481 residue. The remaining two patients had persistence of the PLCγ2 mutations that had been identified after ibrutinib but before pirtobrutinib therapy. None of the sequenced patients had a new BTK C481 mutation arise during pirtobrutinib treatment. However, all of the seven patients with newly acquired non-C481 BTK kinase domain mutations had undergone previous ibrutinib treatment, which was discontinued for progressive CLL. Notably, in four patients with pre-existing C418 BTK mutations, the C481 clone was suppressed during pirtobrutinib therapy. However, those patients acquired new non-C481 mutations, which conferred clinical resistance to pirtobrutinib at the time of disease progression. The authors performed structural remodeling, BTK-binding assays, and cell-based assays to study mutations conferring resistance to noncovalent BTK inhibitors. They characterized the functions of the non-C481 mutations using a BTK-dependent human B-cell lymphoma cell line by measuring the half-maximal inhibitory concentration upon exposure to noncovalent BTK inhibitors, including pirtobrutinib, fenebrutinib, vecabrutinib, and ARQ-531, as well as ibrutinib. Cells with the non-C481 BTK mutations were much less sensitive to noncovalent BTK inhibitors than cells expressing wild-type BTK or C481-mutant BTK. Some of the non-C481 mutations, as well as the PLCγ2 mutations, conferred resistance to noncovalent and covalent BTK inhibitors. The non-C481 BTK mutations identified by the authors were associated with a reduction in BTK catalytic activity and interfered with the impact of BTK inhibitors on BTK autophosphorylation. While these newly acquired mutations paradoxically hinder BTK catalytic activity, downstream pathway activation and B-cell receptor and AKT signaling were still effective, even in the presence of covalent and noncovalent BTK inhibitors. The study findings provide insight into potential genetic mechanisms of resistance to noncovalent BTK inhibitors and how these mutations may impact BTK and downstream pathways. However, additional analyses and larger sample sizes are needed to confirm these findings and expand on them.

Wang E, Mi X, Thompson MC, et al. Mechanisms of resistance to noncovalent Bruton's tyrosine kinase inhibitors. *N Engl J Med*. 2022;386(8):735–743.

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