Molecular Therapy
Editorial

A pause in gene therapy: Reflecting on the unique challenges of sickle cell disease

Recently, the largest lentiviral vector (LV)-mediated β-globin replacement gene therapy trial in sickle cell disease (SCD) was suspended due to a reported suspected unexpected serious adverse reaction (SUSAR) of acute myeloid leukemia (AML).[1] Over the last 6 years, 47 patients with SCD have been treated with LV gene therapy in two related clinical trials (ClinicalTrials.gov: NCT02140554 and NCT04293185) that have evolved from the use of steady-state bone marrow (BM) for collection of hematopoietic stem cells (HSCs) along with standard manufacturing techniques and supportive care to now plerixafor mobilization with refined manufacturing techniques and obligatory transfusion supportive care. This evolution occurred across three consecutive cohorts: A, B, and C. One patient in group A was previously reported to have developed myelodysplastic syndrome (MDS) 3 years after treatment, which eventually transformed to AML. The absence of vector among the blasts along with complex cytogenetic abnormalities and driver gene mutations suggested that this complication arose from the busulfan (BU) conditioning and was unrelated to the LV.[2] Another patient in group A has just been reported to have developed AML 5.5 years after treatment. In this case, however, a similar analysis of the blasts demonstrated the presence of LV, raising the possibility of insertional mutagenesis. A SUSAR in a patient in group C is also being evaluated after trisomy 8 was found by fluorescence *in situ* hybridization at the 6-month marrow examination, yet no dysplasia or blasts were present in the marrow sample.[1,2] While the study is paused, investigations are actively ongoing to determine whether these events are LV related. For the SCD community at large, the question of the safety of any gene therapy method, LV-derived or otherwise, suddenly looms large for a population of patients in need of and hoping for curative options.

Attribution is difficult in these cases. Indeed, the use of the first US Food and Drug Administration approved drug for SCD, hydroxyurea (HU), remains limited in part due to concerns over a theoretical cancer risk, although such a risk attributable to HU has never been clearly established. Patients are at higher baseline risk of AML and other malignancies; however, rates were not higher after HU approval, suggesting the increased risk of leukemia is not related to HU.[3] Additionally, malignant transformation after HSC transplantation (HSCT) is unfortunately a universal, well-documented risk. After allogeneic HSCT for SCD, 1% of patients developed malignant neoplasms, including AML and MDS, in a cohort of nearly 1,000 patients transplanted from 2008–2017.[4] Often, the AML in SCD is described as "therapy related," with complex cytogenetic abnormalities, often with monosomy 7, and mutations in drivers such as RUNX1.[5] AML that appears therapy related has been reported in patients with SCD without prior HU therapy, with prior HU therapy, with prior allogeneic HSCT from a matched sibling or from a half-matched donor, and with an array of conditioning regimens, including BU and total body irradiation (TBI).

After the initial report and pause of the studies, further analyses of the AML revealed monosomy 7 by cytogenetics and mutations in RUNX1 and PTNP11 remarkably similar to those that had been reported in the first patient and to those reported in SCD patients in general. Integration site analysis demonstrated the presence of an integration in vesicle-associated membrane protein 4 or VAMP4, a gene without a known association with cancer, and no disruption of gene expression was found on nearby genes. These data led to the conclusion that the vector integration was unlikely to have played a role in the leukemia development.[6]

This leads to the yet unanswered question as to why there are now two cases of AML that, although vector appears exonerated, have nonetheless occurred. Chronic hypoxia, reactive oxygen species generation, endothelial and vascular damage, chronic systemic inflammation, constant erythropoietic stress with dysregulated hematopoiesis, and repeated bony infarction all likely lead to damage to the HSC compartment, reduced HSC number, and thus a predisposition to malignant transformation. In two patients with severe SCD who developed a myeloid malignancy 2–5 years after a failed allograft, the existence of clonal hematopoiesis of indeterminant potential (CHIP)-related mutations were detected by next-generation sequencing in pre-transplant samples.[7] There is thus an acute need to understand whether CHIP exists more broadly in patients with SCD and, if so, whether its presence contributes to the risk of AML development. Additionally, the ability of genotoxic agents, such as BU, to directly induce leukemogenic mutations and/or indirectly promote clonal selection of pre-existing, mutant HSCs needs to be better understood.[8] Autologous HSCT by itself carries a known risk of therapy-related myeloid neoplasms, for example, the presence of CHIP at the time of transplant in a lymphoma cohort raised the 10-year cumulative incidence from 4.3% to 14.1%.[9] For patients with SCD, autologous HSCT, combined with a BM likely at risk for CHIP, combined with poor cell doses achieved in the initial group A, combined with the risk of leukemogenic mutations after genotoxic conditioning, may have created the setup for evolution to malignant disease. The leap from a dysregulated BM environment to an increased risk for malignant transformation with or without transplantation, with or without HU, with or without chemotherapy, with or without TBI, with or without genetic modification, is scientifically plausible and, in some cases, likely. Understanding this pathophysiology will

ultimately better serve patients and individualize their risk to undergo experimental therapies. It may be that such therapies are best suited for younger patients prior to the development of severe end organ damage of the BM space.

The temporary suspension by bluebird bio of its two gene therapy trials in SCD and the temporary suspension of another LV-mediated trial in patients with SCD (ClinicalTrials.gov: NCT03282656) out of an abundance of caution is the ethically responsible decision to make, particularly given the consequences of insertional mutagenesis that plagued the early trials of gene therapy with oncoretroviral vectors in other disorders. Updated data point away from vector as causative in this new case. Additionally, there are no reports of leukemia to date in several hundred individuals who have undergone gene therapy with LVs for many indications other than SCD, including 63 β-thalassemia patients who received the identical vector (BB305) used for SCD in separate clinical trials being conducted by bluebird bio (ClinicalTrials.gov: NCT01745120, NCT02151526, NCT03207009, and NCT02906202). As curative options for SCD expand, data on overall survival (OS) and event-free survival (EFS) are important comparators among methods. To date, for those with SCD who have received BB305 utilizing the most up to date methods, OS and EFS are both 96% compared to historical allogeneic matched sibling HSCT OS and EFS of 95% and 92%, respectively.[10,11] Post-LV treatment, no acute chest syndrome or serious vaso-occlusive crises were observed, and participants reported clinically meaningful improvements in pain reduction at 12 months post-treatment. These data are encouraging; however, the trial suspensions in SCD were necessary to sort out whether a new risk from vector changed the risk-benefit assessment.

Ultimately, patients with SCD and their families deserve a medical community with a better understanding of their disease and the risks that any given therapy entails. At this early time, it is not clear if the reports of AML and MDS are related to BU conditioning, a poor cell dose, or pre-existing CHIP; however, it appears unrelated to LV mutagenesis. These reports, however, remind us of unknown risks associated with both newer and existing therapies, including non-LV-mediated therapies such as clusters of regularly interspaced short palindromic repeats (CRISPR) technology. While CRISPR technology avoids the theoretical risk of insertional mutagenesis poised by LV-mediated therapy, the questions surrounding toxicity to HSCs and their long-term engraftment potential persist. Early results in gene therapy for SCD remain promising and it is premature to disregard the potential benefit of LV-mediated therapy. This pause, however, provides time and perspective to once again acknowledge that the unique features of SCD must be understood so as to truly do no harm.

DECLARATION OF INTERESTS

J.F.T. is a principal investigator on ClinicalTrials.gov: NCT02140554 and A.L. is a co-investigator on ClinicalTrials.gov: NCT04293185.

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