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Perspective

Leukemia after gene therapy for sickle cell disease: insertional mutagenesis, busulfan, both, or neither

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Recently, encouraging data provided long-awaited hope for gene therapy as a cure for sickle cell disease (SCD). Nevertheless, the transient suspension of the bluebird bio gene therapy trial (clinicaltrials.gov: NCT02140554) after participants developed acute myeloid leukemia/myelodysplastic syndrome (AML/MDS) raised concerns. Potential possibilities for these cases include busulfan, insertional mutagenesis, both, or neither. Busulfan was considered the cause in the first reported case because the transgene was not present in the AML/MDS. However, busulfan is unlikely to have contributed to the most recent case. The transgene was present in the patient's malignant cells, indicating they were infused after busulfan treatment. Several lines of evidence suggest an alternative explanation for events in the bluebird bio trial, including that SCD population studies show an increased relative, but a low absolute, risk of AML/MDS. We propose a new hypothesis: after gene therapy for SCD, the stress of switching from homeostatic to regenerative hematopoiesis by transplanted cells drives clonal expansion and leukemogenic transformation of preexisting premalignant clones, eventually resulting in AML/MDS. Evidence validating our hypothesis will support prescreening individuals with SCD for preleukemic progenitors before gene therapy. While presumed viable, safe strategy has been implemented to resume gene therapy in adults with severe SCD, reasonable alternative curative therapy should be considered for children and adults with severe SCD. Currently, open multicenter clinical trials are incorporating nonmyeloablative conditioning, related haploidentical donors, and posttransplantation cyclophosphamide. Preliminary results from these trials appear promising, and National Institutes of Health–sponsored trials are ongoing in individuals with SCD using this platform.

Background

Sickle cell disease (SCD) is the most common inherited blood disease in the United States, affecting approximately 1 in 360 African American newborns and about 100 000 individuals.[1] SCD is caused by a point mutation in codon 6 of the β-globin chain that results in an amino acid substitution of valine for glutamic acid. Red blood cells from individuals with SCD assume sickled forms under hypoxic conditions, leading to chronic hemolysis, inflammation, ischemia-reperfusion injury, and immune system activation, resulting in progressive organ disease.[1] Common complications include acute vaso-occlusive pain events, acute chest syndrome, and stroke.

Survival of children with SCD has improved in the United States and Europe because of newborn screening, penicillin prophylaxis, improvement in conjugated vaccines, preventive therapy for strokes, hydroxyurea, and evidence-based supportive medical care. For children, SCD can no longer be referred to as a life-threatening disease but rather a chronic disease that has life-threatening events.[2] However, for adults with SCD, the median survival of 48 years has not changed significantly in the last 25 years.[3,4]

Curative therapy for SCD

SCD has been the prototype monogenic disease for the promise of curative gene therapy. Given that the germline mutations in SCD only affect red blood cells and hematopoietic stem cells (HSCs) can be and frequently are transplanted, an ideal curative approach would remove affected HSCs, genetically repair them, and reinfuse them into the patient. Accordingly, transplantation of normal allogeneic HSCs cures SCD. However, relatively few (about 2000 worldwide) have undergone the procedure because of historical obstacles of limited donor availability and toxicity related to myeloablative conditioning and graft-versus-host disease.[5]

With the onset of recent gene therapy trials for SCD in the last 5 years, curative therapy has become a ray of hope for many families with SCD and has energized the research field.[6] At least 10 different gene therapy or gene editing trials are currently registered in clinicaltrials.gov (Table 1). Gene therapy for SCD involves removing (or harvesting) HSCs from the patient, ex vivo transduction using a vector carrying a γ-globin or β-globin transgene, and reinfusion of transduced HSCs after myeloablative chemotherapy,

which is needed to make space for the corrected HSCs to expand.[5] Genetically corrected HSCs are patient derived, thus overcoming the obstacles of limited donor availability and graft-versus-host disease associated with allogeneic blood or marrow transplantation (alloBMT). However, gene therapy trials have not overcome the requirement for myeloablative conditioning. Most adults with SCD and comorbidities, such as strokes or significant heart, lung, or kidney disease, are not eligible for curative therapy trials because of the perceived risk of therapy-related mortality or morbidity.[5] Approximately 60% of adults with SCD have at least one of the following: heart disease defined as tricuspid regurgitant jet velocity greater than 2.5 m/s; lung disease characterized as forced expiratory volume in 1 second predicted ≤70%, or significant renal disease associated with earlier death.[7]

Therapy-related myeloid neoplasms (t-MN), previously called therapy-related acute myeloid leukemia (AML)/myelodysplastic syndrome (MDS), were also theoretical concerns associated with SCD-related gene therapy from either insertional mutagenesis, busulfan myeloablation, or both. Self-inactivating lentiviral vectors are believed to eliminate the risk for vector-mediated insertional mutagenesis. Despite the expectation that busulfan will not affect the transplanted HSCs, the chemotherapy agent may not be completely myeloablative. Thus, busulfan could induce mutations in residual HSCs.

Suspension of myeloablative gene therapy trial

The SCD gene therapy trial HGB-206 (clinicaltrials.gov: NCT02140554) was suspended between February and June 2021 after 2 participants developed t-MN.[8] The first participant developed MDS 3 years after treatment, followed by transformation to AML with monosomy 7; the second participant developed AML 5.5 years after therapy.[8] A total of 47 individuals have been treated in bluebird bio SCD gene therapy trials using LentiGlobin: a gene therapy product containing autologous $CD34^+$ cells transduced ex vivo with the BB305 lentiviral vector encoding β-globin, $\beta^{A\text{-}T87Q}$ (clinicaltrials.gov: NCT02140554 and NCT04293185).[9]

In the first participant, vector-mediated insertional oncogenesis was excluded as the cause of AML/MDS because the transgene was not present in the AML blasts.[10] As no alternative explanation could be made regarding the basis for AML/MDS, the investigators concluded that busulfan was likely the cause of the t-MN by inducing DNA damage in hematopoietic cells it failed to eliminate.[10] The finding of monosomy 7 and its known association with alkylating agent-induced t-MN was reported to support busulfan as the etiology of the AML.

In the second patient in whom the investigative team reported the development of AML in the bluebird bio clinical trial, the transgene was found in the t-MN cells; this excludes busulfan as the

Table 1. Genome editing and gene therapy clinical trials in SCD as of March 2021

Goal	Nuclease/target	Sponsor, collaborator	Clinical trial ID	Estimated participants
Elevate HbF	CTX001/BCL11A	Vertex Pharmaceuticals Incorporated, CRISPR Therapeutics	NCT03745287	45
Elevate HbF	Plerixafor/BCL11A	Bioverativ, a Sanofi company	NCT03653247	8
Elevate HbF	OTQ923 or HIX763/BCL11A	Novartis Pharmaceuticals	NCT04443907	30
Goal	**Viral vector**	**Sponsor, collaborator**	**Clinical trial ID**	**Estimated participants**
Repair HbS mutation	Lenti/G-βAS3-FB lentiviral Vector	California Institute for Regenerative Medicine	NCT02247843	6
Elevate HbF	ARU-1801	Aruvant Sciences GmbH	NCT02186418	10
Repair HbS mutation	GLOBE1 lentiviral vector expressing the βAS3 globin gene	Assistance Publique–Hôpitaux de Paris	NCT03964792	10
Repair HbS mutation	LentiGlobin BB305 lentiviral vector	bluebird bio	NCT04293185	35
Repair HbS mutation	Lentiviral vector encoding the normal β-globin gene	Memorial Sloan Kettering Cancer Center, Sanofi	NCT02193191	39
Repair HbS mutation	LentiGlobin BB305 lentiviral vector	bluebird bio	NCT02140554	50
Elevate HbF	Lentiviral vector containing a short hairpin RNA targeting BCL11A	Boston Children's Hospital	NCT03282656	15

cause because the cells were infused into the patient after the drug was given. Subsequently, bluebird bio announced that the vector's integration site was within *VAMP4*, a gene with no established association with AML/MDS.[11] The patient did have chromosomal abnormalities and mutations in the *RUNX1* and *PTPN11* genes in leukemia cells, 2 well-established AML-associated genes. A third participant developed anemia and trisomy 8 in the bone marrow 6 months after gene therapy.[8] Thus, 2 cases of AML and 1 case of trisomy 8 in a patient with anemia resulted in a transient moratorium on enrollment in the bluebird bio SCD trial and the National Institutes of Health–sponsored gene-editing trial (clinicaltrials.gov: NCT03282656).

MDS/AML after transplantation for diseases other than SCD

Therapy-related myeloid malignancy (t-MN) also occurs after autologous transplantation for lymphomas, with cumulative risks as high as 15%.[12,13] Most of the data find premalignant clones that eventually generate the t-MN are present before the autologous transplant, presumably from the conventional-dose cytotoxic chemotherapy patients received as well.[14-16] t-MN after autologous transplantation for lymphomas could arise from damaged residual host hematopoietic progenitors that survived the transplant conditioning. Alternatively, the t-MN could arise from premalignant progenitors transplanted with the reinfused autologous cells.

Donor cell leukemia (DCL) is a well-established risk after alloBMT. DCL and AML/MDS that develop from transplanted donor cells are increasingly being reported.[17] Several reports now show that donor cell AML/MDS usually arises from clonal hematopoiesis of indeterminate potential (CHIP) present in the donor.[18-20] CHIP is a common age-related condition that is unusual before 50 years of age, but increases dramatically in prevalence after 60 years of age.[21,22] Although the somatic mutations associated with CHIP frequently affect genes previously reported in hematologic malignancies, the risk of progression to a clinically apparent disease is estimated to be low (<1%/y).[23] This risk of malignant progression is similar to another age-related premalignant condition: monoclonal gammopathy of unknown significance. This increase in DCL appears to be mostly in patients undergoing alloBMT using older donors, with an incidence that may be as high as 5% to 10% when the donors are older than age 60.[18]

None of the donors in any reported DCL cases have developed AML/MDS, consistent with the low rate of AML/MDS transformation associated with CHIP.[18-20] Moreover, the variant allele frequency of the CHIP in the donors was often below the standard level (5%) of detection.[18,20] Thus, the process of alloBMT may hasten the natural history of CHIP. Clinically silent premalignant clones that remain dormant under homeostatic conditions may undergo an accelerated malignant transformation from the stress associated with 2% to 3% of the transplanted donor's marrow dramatically expanding to fully restore lymphohematopoiesis in the recipient.

We postulate the same process occurring with DCL is also taking place in the cases of t-MN after autologous transplantation for lymphoma. Chemotherapy increases CHIP incidence, with a prevalence after chemotherapy estimated to be 25% to 30%.[24,25] Receiving chemotherapy is one of the few circumstances in which CHIP is seen in individuals younger than 40 years of age.[24,25] Moreover, about one-half of the patients who develop t-MN after autologous transplantation for lymphoma had CHIP present in their pretransplant marrows,[25] and many even had detectable premalignant clones.[14-16] Premalignant clones will be present in both the patient and the transplanted cells. However, the use of myeloablation and the higher t-MN frequency seen with autologous transplantation compared with conventional-dose therapy[12,13] suggests that most t-MN after autologous transplantation arises from transplanted premalignant progenitors.

Increased relative risk of myeloid malignancy in SCD

Two extensive population studies have documented a higher than expected relative, but low absolute, risk of AML in individuals with SCD compared with the general population.[26,27] In the most robust study to date, Brunson et al[26] used a standardized incidence ratio (SIR) to compare California residents' cancer incidence in individuals with SCD to the general population without SCD. A total of 6423 individuals with SCD were identified over 24 years (1991 to 2014), and 115 were diagnosed with cancer, with a total of 6 AML cases. AML had the highest SIR of all statistically significant cancers (SIR, 3.59; 95% confidence interval, 1.32-7.82). In the second population study based on all hospital admissions in England over 12 years, the rate ratios of cancer in individuals with SCD were compared to individuals without SCD.[27] In this study a total of 8 cases of AML occurred, and the rate ratio for AML was statistically significant (rate ratio, 11.0; 95% confidence interval, 3.86-30.17). As is the case with most administrative data sets, results are primarily used for hypothesis generation because of the limitation in the accuracy of the diagnoses, which would typically require central adjudication of the medical records. Despite the intrinsic limitation of both administrative data analyses, together the 2 studies provide at least preliminary evidence to support a prospective registry of malignancy in children and adults with SCD pre-and postcurative therapy.

A working hypothesis to explain the AML/MDS after gene therapy in SCD

We postulate the increased background incidence of AML/MDS in SCD,[26,27] and the cases of t-MN following SCD gene therapy are not directly related to prior hydroxyurea therapy,[28-30] myeloablative busulfan therapy, or insertional mutagenesis after ex vivo manipulation. Instead, we suggest another possibility, the presence of underlying CHIP in SCD bone marrow.[31] We believe the biological basis for why early-onset CHIP would be present is the unique SCD bone marrow microenvironment includes chronic inflammation,[32] hypoxemia, and expanded hematopoiesis, which together contribute to mutations. Moreover, the incidence rate of MDS/AML in SCD is relatively low and to that seen with age-related CHIP.[23] However, analogous to what we propose is the pathogenesis of DCL[18-20] and t-MN after autologous transplantation for lymphomas, the rapid hematopoietic expansion associated with transplantation of genetically corrected progenitors may transform CHIP into AML/MDS.

We propose a working hypothesis that after gene therapy or gene editing for SCD, the stress of switching from homeostatic to regenerative hematopoiesis of transplanted progenitor cells

drives clonal expansion and eventually leukemogenic transformation of preexisting premalignant CHIP. If further work supports our working hypothesis, finding a nongenotoxic alternative to busulfan or a better strategy for repairing the hemoglobin genes in HSCs for gene therapy or gene editing of SCD is unlikely to lessen the risk of t-MN associated with reinfusing premalignant progenitors.

Potential strategies to reduce the incidence rate of AML/MDS in SCD after curative therapy

Perhaps the most obvious approach to reducing the t-MN risk after gene therapy and autologous HSC reinfusion is prescreening individuals with SCD for preleukemic progenitors using next-genome sequencing before considering gene therapy. However, many of the CHIP mutations associated with AML/MDS after transplantation are at levels below the threshold for standard clinical next-genome sequencing testing.[18,20] The presence of specific leukemia-associated mutations defines CHIP and its oncogenic potential. Potentially, a targeted panel of approximately 50 genes associated with leukemia would allow an increased depth of coverage in the range of 1000 to 10 000 times, rather than 40 times as in the case of whole genomic sequencing. Moreover, even if CHIP is pervasive in individuals with SCD, not all CHIP mutations appear to pose an increased risk for AML/MDS after transplantation.[33] Unfortunately, this strategy would not eliminate the MDS/AML risk for those individuals with high-risk CHIP mutations. Furthermore, the threshold for variant allele frequency of CHIP mutations that confer a risk of AML/MDS after transplantation is unknown and will likely be challenging to determine because of the stochastic evolution of CHIP transformation to AML/MDS.

A consideration that we do not endorse for decreasing the incidence rate of t-MN following gene therapy or gene editing is to enroll young children who would be expected to have a lower incidence of CHIP. The risk-benefit ratio of curative therapy in children with SCD is not the same as for adults with SCD. The survival for children with SCD approaches 99% in high-income countries.[34,35] In the United States, the institutional review boards and the Data Safety Monitoring Board must consider the specific code of regulation for conducting research greater than the minimum risk in children. In children, the relationship between the anticipated benefit to the risk of curative therapy must be at least as favorable to children as presented by available alternative approaches: 45 Code of Regulation §46.405(b). Children with SCD living in a high-income setting have less than a 1% anticipated mortality before 18 years of age. Even children with severe SCD living in a high-income setting have a lower mortality than the currently expected mortality with bluebird bio gene therapy. With an estimated 47 individuals with SCD who have participated in the SCD bluebird bio gene therapy, there is a wide 95% confidence interval for the prevalence of t-MN: 4.2% (2 of 47 estimated total number of individuals), with a 95% confidence interval of 1.1% to 14.3%. Moreover, with ongoing follow-up of the cured participants in the bluebird bio SCD trial, there may be an increased prevalence of AML/MDS.

Similar to many phase 1 oncology clinical trials, we firmly believe young children should be the last age group, not among the first age groups, offered experimental autologous gene therapy. Based on the recent findings of AML/MDS in the bluebird bio gene therapy trial, after the trial resumes, we believe that the safest approach is to offer the therapy only to adults with SCD because the risk-benefit ratio strongly favors adults compared with children with SCD. Subsequently, after a sufficient follow-up duration of the adult-only stratum, the SCD scientific community and families of children with SCD will have better estimates of the incidence rates of AML/MDS and mortality. These data can then inform if and when children with SCD should be offered the same therapy. Possibly, the severe adverse event rates in adults with SCD will be significantly higher than in children with SCD. Nevertheless, these data are required for informed decision making to initiate or forgo gene therapy or gene editing in the most vulnerable population, children with SCD. We recognize that parents of children with severe SCD and adults with severe SCD may not want to wait an indeterminate period or incur the perceived high risk of AML/MDS after gene therapy or gene editing. However, there are other current curative clinical trial options available for most children and adults with SCD.

alloBMT offers an alternative curative option to autologous gene therapy for SCD that avoids genetic manipulation, myeloablation, or reinfusion of premalignant progenitors. Although alloBMT is often dismissed as a curative option,[36,37] especially in adults, significant advances such as related haploidentical alloBMT with posttransplant cyclophosphamide (PTCy) now allow most individiuals in need access to safe and effective alloBMT.[38] The ability to use related haploidentical-related donors is of particular benefit to individuals with SCD because most individuals with SCD (>80%) will lack matched sibling or unrelated donor options.[39,40] Accordingly, recent studies demonstrate that nonmyeloablative related haploidentical BMT with PTCy can cure more than 90% of individuals with SCD.[41,42] The limited toxicity of nonmyeloablative conditioning can eliminate the barrier associated with toxicity in adults with significant heart, lung, and kidney disease that precludes myeloablation and current gene therapy and gene editing trials.

Haploidentical alloBMT with posttransplantation PTCy is not without risks of AML/MDS. In a small single-center trial for individuals with SCD treated at the National Institutes of Health, 2 of 21 individuals developed high-grade MDS 2 and 5 years after related haploidentical BMT with PTCy and a goal of 100% engraftment, and 1 of 55 individuals developed AML 2.5 years after matched related alloBMT. In all 3 of these cases, participants had concomitant graft rejection, and the AML/MDS arose from host cells.[43] Fortunately, newer haploidentical transplant platforms with PTCy have not shown high graft failure or high AML/MDS incidence rates.[41,42] If the ongoing multicenter Bone Marrow Transplant-Clinical Trial Network trial of related nonmyeloablative haploidentical alloBMT with PTCy for individuals with SCD (BMT-CTN 1507, clinicaltrials.gov: NCT03263559) confirms the preliminary results seen in recent phase 2 trials,[41,42] coupled with the anticipated improved clinical outcomes with autologous gene therapy or gene editing therapy, the long-awaited hope of a cure for most children and adults with SCD may still be within our grasp.

Authorship

Contribution: R.J.J. and M.R.D. wrote the paper.

Conflict-of-interest disclosure: M.R.D. was a medical advisor for the development of the CTX001 Early Economic Model; provided medical input on the economic model as part of an expert reference group for the Vertex/CRISPR CTX001 Early Economic Model in 2020; and is the current chair of the steering committee for a Novartis-supported phase 2 trial to prevent priapism in men. He is also the principal investigator for 2 investigator-initiated observation studies supported by Global Blood Therapeutics, and is a consultant for Forma. R.J.J. declares no competing financial interests.

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Footnotes

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