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Review

Gene therapy for hemoglobinopathies: Clinical trial results and biology of hematopoietic stem cell and the bone marrow niche

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SUMMARY

Hemoglobinopathies, including beta-thalassemia (Bthal) and sickle cell disease (SCD), are among the most prevalent inherited blood disorders. Genetic mutations affecting hemoglobin synthesis result in severe anemia and multi-organ complications. The development of gene therapy (GT) aimed at correcting or modifying the hematopoietic system, although initially impaired by several limitations, has accomplished the marketing authorization of two hematopoietic stem cell (HSC) medicinal products, engineered by lentiviral vector gene addition and by CRISPR-Cas9 gene editing. Nonetheless, the success of these approaches stimulates a critical revision of our knowledge of HSC biology and bone marrow (BM) microenvironment in these diseases. Here, we review the clinical application of GT by gene addition and gene editing, and the novel findings about HSC and BM niche features and function in hemoglobinopathies. The identification of defective networks in HSC-niche is examined with the perspective of developing combined strategies to ameliorate the BM microenvironment to better support the genetically corrected cells.

INTRODUCTION

The most consolidated approach for clinical gene therapy (GT) of hemoglobinopathies derives from almost two decades of basic research and pivotal clinical trials using hematopoietic stem and progenitor cells (HSPCs) genetically modified with lentiviral vectors (LVs). The recent and novel strategies of gene editing were more rapidly built up on the acquired knowledge of how *ex vivo* engineered purified $CD34^+$ HSPC engraft and reconstitute the whole hematopoiesis, and on the optimization of clinical protocols for efficient HSPC mobilization and tailored conditioning regimen. Patients' hematopoietic stem cells (HSCs) are mobilized in the peripheral blood and harvested by aphaeretic collection following the administration of granulocyte- colony stimulating factor (G-CSF) and plerixafor, as mobilizer agents. One important difference in this procedure is the use of plerixafor alone for the treatment of patients affected by sickle cell disease (SCD), because of high risk of life-threatening complications related to G-CSF in this population.[1,2] As a result, the yield of $CD34^+$ cells collected from patients with SCD is generally lower compared to patients with beta-thalassemia (Bthal), thus limiting to achieve an optimal drug product target dose.[3] In addition, a key often neglected aspect of all strategies using *ex vivo* manufacturing of primitive HSC is the cellular composition and the molecular profiling of the target cells, which can be greatly influenced by the hosting diseased bone marrow (BM). Thus, the transcriptional response of HSC to *ex vivo* engineering and the maintenance of key stemness features are crucial for achieving a favorable and persistent clinical outcome. In addition, an intrinsic challenge of the manufacturing procedure is the impairment of the clonogenic activity and repopulating potential of HSPC following culture for LV transduction or electroporation for gene editing strategies. Several studies reported that *ex vivo* transduction protocols lead to a decrease in the frequency of clonogenic progenitors and primitive HSC compromising their long-term repopulating ability.[4–6] Optimization of culture conditions offers the opportunity to limit this detrimental effect and to preserve the stemness features of genetically modified HSC. On the same line, recent evidences suggest that protocols to deliver the gene editing molecular tools exploiting electroporation compromise the *in vivo* repopulating HSC potential.[7–12] Cell response to culture stress is even enhanced during genetic engineering because of the activation of DNA damage response.[8] Thus, in the design of clinical trials the optimal target dose of drug product should be adequately evaluated in consideration of the aforementioned issues.

In this review, we will focus first on gene addition and gene editing approaches that have been developed and pursued in clinical trials. In the second part, we will emphasize the recent findings related to the features of the hematopoietic compartment and the BM niche status in Bthal and SCD, which represent paradigmatic models of a chronic stress condition of BM microenvironment.

BETA-THALASSEMIA AND SICKLE CELL DISEASE

Bthal and SCD are caused by mutations in the β-globin chain gene of hemoglobin (Hb) and are the most common monogenic disorders in the world.[13,14] In SCD, an A>T mutation results in the substitution of valine for glutamic acid at position 6 of the β-globin chain, and the generated abnormal sickle Hb (HbS) undergoes deoxygenation-induced polymerization. This primary event drives red blood cell sickling, hemolysis, vaso-occlusive crises, inflammation, multi-organ damage, with a severely reduced life expectancy. In Bthal, more than 400 different mutations in the β-globin gene cause an imbalance between α-globin and β-globin chains with deficiency of adult hemoglobin (HbA) and a severity of clinical features depending on the level of α-globin chain excess and residual β-globin production. Patients with the most severe form are affected by profound anemia, requiring chronic red cell transfusions and iron chelation, ineffective erythropoiesis, and clinical morbidity including chronic haemolysis, hepato-splenomegaly, skeletal abnormalities for the expansion of erythroid BM, and complications of iron overload such as heart, liver, and endocrine dysfunctions. Allogeneic BM transplantation is the definitive cure for SCD and Bthal,[15–18] with best results in pediatric patients with an available HLA-matched donor. Despite the increased availability of HLA-matched donors and overall improvements in pre-transplant and transplant procedures, the outcomes of allogeneic BM transplantation are still hampered by significant morbidity and mortality, with reported incidences of 9%–39% for acute graft versus host disease (GVHD), 14%–46% for chronic GVHD, 7%–11% for mortality, and 10%–12% for graft failure.[15–18] Thus, these limitations favored the development of GT that is devoid of immunological complications and does not require immunosuppression therapy. Autologous transplantation with genetically corrected HSC has been pursued for decades, leading to the pioneer clinical trials using LV-mediated transfer of β-globin gene, and more recently with gene-edited cells. Thus, in hemoglobinopathies, the outcome of gene addition and pivotal gene editing clinical trials is available, with several years of follow-up in the first case while a limited follow-up time in the latter.

Indeed, a fundamental contribution to the development of GT strategies comes from our knowledge of the structure and regulation of the *HBB* cluster.[19,20] The *HBB* locus structure revealed essential regulatory sequences in the locus control region (LCR) that were included in the LVs to express the β-globin transgene efficiently and in an erythroid specific manner. In addition, rare genetic variants within or outside the *HBB* locus[21,22] are associated with the transcriptional reactivation in adulthood of the fetal γ-globin genes, leading to the condition of hereditary persistence of fetal hemoglobin (HPFH). The clinical history of hemoglobinopathies revealed that the severity of SCD and Bthal can be alleviated by HPFH. HPFH can result from mutations in the fetal γ-globin (HBG1/2) promoters, with some mutations generating activator (KLF1, TAL1, and GATA1) binding sites or disrupting repressor (BCL11A and LRF) binding sites. Outside the *HBB* locus, on chromosome 2, genome-wide association studies (GWAS) studies identified BCL11A as a key repressor of γ-globin genes, regulating the switching from fetal to adult Hb expression.[23–25] Indeed, genetic variation in BCL11A expression has been associated with beneficial persistent fetal Hb (HbF) production in Bthal,[26] and the imbalance between α-globin and β-globin chains can be diminished by the reactivated synthesis of γ-globin chains, which contributes with HbF to increase the total amount of Hb per cell. In SCD, HbF exerts a potent anti-sickling effect by associating with the β^S-chain and preventing polymerization of HbS. These molecular mechanisms are consistent with the evidence in newborns, protected from SCD and Bthal complications early in life, when HbF levels are still elevated, and in patients carrying HPFH-associated mutations and displaying milder symptoms. Thus, several gene-editing approaches have exploited the reactivation of fetal γ-globin expression as a potential therapy, using the non-homologous end joining (NHEJ) repair pathway to disrupt or create *cis*-regulatory sequences. Indeed, CRISPR-Cas9 inactivation of the erythroid-specific enhancer of BCL11A has been the first gene editing approach for SCD and Bthal moved toward clinical application[27,28] and achieving marketing authorization.

The molecular strategies developed for the clinical translation of GT for hemoglobinopathies are the following (Figure 1): (1) LV-mediated gene transfer into patients' HSC, providing information for expression of a normal globin β-or β-like chain (β-globin or γ-globin); in addition, transfer of LV encoding siRNA to interfere with the expression of the γ-globin repressor BCL11A led to reactivation of HbF; (2) gene editing via homology-directed repair (HDR) to correct the mutated gene by providing the normal gene template; (3) gene editing via NHEJ to reactivate endogenous HbF expression by downregulation of BCL11A or by reproducing HPFH mutations; and (4) base editing of specific mutations in Bthal and of SCD point mutation, converting HbS to the benign variant Hb Makassar, or to reproduce HPFH associated variants.

HSC GENE THERAPY BY GENE ADDITION

The development of LVs has dramatically changed the course of GT for hemoglobinopathies since production of high-titer β-globin expressing vectors and regulated transgene expression was achieved uniquely in HIV-derived vectors. The pioneering trial using HSPC transduced with a globin LV expressing a mutated ß-globin^{T87Q} (HPV569), also endowed with anti-sickling activity, resulted in the first Bthal patient achieving transfusion independence (NCT02151526),[29] but subsequently requiring intermittent transfusions at longer follow-up after 12 years (NCT01745120).[30] In this patient, a myeloid-based dominant clone, harboring the vector integrated in the high-mobility group AT-hook 2 (HMGA2) gene, was generated by the activation of a cryptic splice site that deleted a let-7 miRNA target sequence, allowing overexpression from the vector transcriptional control sequences of a truncated HMGA2 mRNA. The limited number of engrafting HSC was potentially contributing to the cell expansion of a clone endowed with a selective advantage *in vivo*. Nonetheless, cell expansion was benign and the clone declined at later follow-up.[31] The experience from this pioneering trial guided the further optimization of LV-globin design as well as the CD34$^+$ cells transduction protocols. In the subsequent phase 1/2 clinical trials based on a refined version of the original

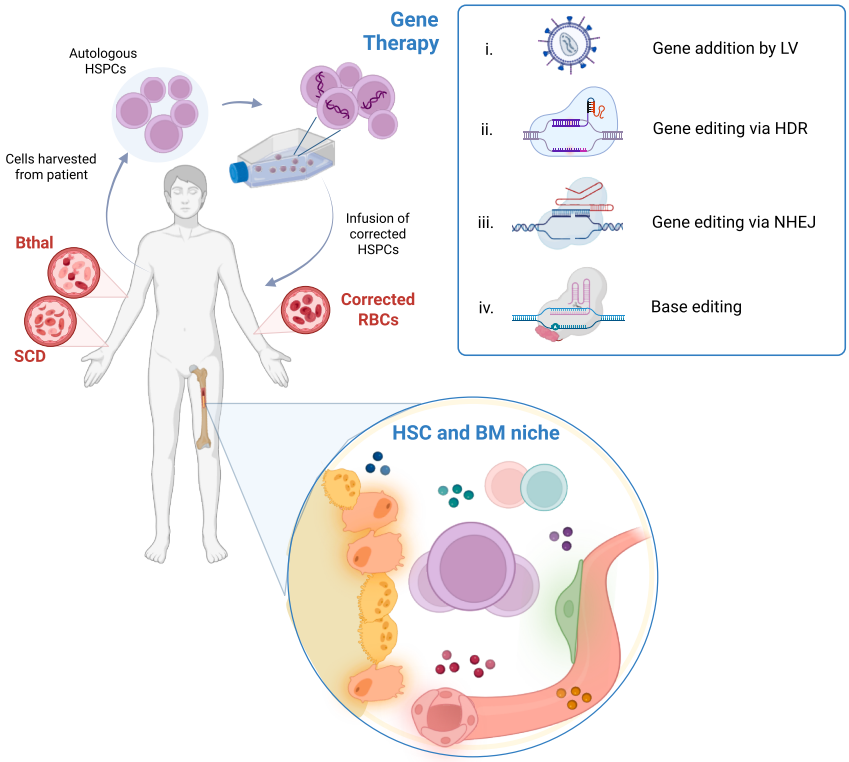

Figure 1. Gene therapy for hemoglobinopathies

Schematic representation of gene therapy procedure by gene addition and gene editing strategies to correct HSPCs from Bthal and SCD patients, with an eye to HSC and BM niche. Red blood cells, RBCs. Created in BioRender.

LV (BB305-LentiGlobin), 55% of patients (12/22) with less severe genotypes achieved transfusion independence, and 9 patients affected by severe genotypes reduced transfusions (NCT01745120 and NCT02151526).[31] Further optimization over the years of the drug product (beti-cel) manufacturing significantly improved the clinical outcome with 91% of patients (20/22) with less severe genotypes achieving transfusion independence in a phase 3 study (NCT02906202).[32] Recently, the results of a phase 3 study treating patients with severe genotypes confirmed the safety and the efficacy of the beti-cel drug product with 89% of patients (16 out of 18) achieving transfusion independence (NCT03207009).[33] As a relevant observation in these studies, as in other gene addition approaches, the efficiency of transduction and the vector copy number/cell in long-term engrafting cells is a main contributor to the clinical success, although influence of genotypes and patient to patient variability have additional roles. The complete correction of ineffective erythropoiesis and iron overload are still unmet endpoints for several patients, and longer follow-up will monitor these parameters. Beti-cel (as Zynteglo), developed by Bluebird Bio, was conditionally approved in 2019 by the European Medicines Agency (for non-β0/β0 patients), and it was later withdrawn since the company discontinued the operational activities in European Union (EU) unilaterally judging the reimbursement granted by the national health system/authorities insufficient. Conversely, beti-cel has been approved in 2022 in United States (US) by the Food and Drug Administration (FDA) for all transfusion-dependent patients.

A similar version of lentiglobin with the inclusion of a short insulator element from foamy virus in the long terminal repeat (LTR) was used to produce the drug product BD211 (NCT05015920), administered to two Bthal pediatric patients. The inclusion of an insulator sequence, flanking the proviral transgene cassette, was intended to prevent both potential transcriptional interference to neighboring genes and chromatin negative effect on transgene expression in the erythroid cells. At a follow-up of 2 years the patients were transfusion independent with transduced peripheral blood cells in the range of 33%–65% and 2–4 vector copy number (VCN)/cell. In this trial, correction of ineffective erythropoiesis was still modest.[34]

Lentiglobin LV and the refined manufacturing process of drug product were also applied in SCD GT (NCT02140554),[35] which moved from early proof-of-concept results in few patients (NCT02151526)[30,36] to a large, multicenter study of 35 treated patients, with stable marking of peripheral blood cells and resolutions of symptoms in most of them (NCT02140554).[37] Notably, two cases of acute myeloid leukemia (AML) were diagnosed in this study in patients with inefficient *in vivo* LV marking, but no causative effect by LV was demonstrated,[38,39] although blasts in peripheral blood resulted positive for insertion of lentiglobin in *VAMP4* gene, with no role in oncogenesis. The finding of other somatic mutations in patients' cells suggested a combinatorial risk effect, which in the transplant setting the stress of repopulating hematopoiesis in the presence of a likely limited number of engrafting cells might favor the clonal expansion of premalignant clones. In addition, the disease background of chronic erythropoietic stress and inflammation in the BM microenvironment might further contribute to the risk of proliferative stress.

The first trial of GT in Bthal treating pediatric patients and adults mostly carrying severe genotypes, using a staggered enrollment strategy based on safe early hematological reconstitution, was based on the high-titer GLOBE LV (NCT02453477), expressing β-globin by minimal essential regulatory sequences. The non-clinical efficacy and safety studies, performed under the rigorous good laboratory practice setting, supported the regulatory dossier for the authorization to start the clinical trial.[40] In this clinical trial, the recovery of platelets and neutrophils following myeloablation was rapidly achieved thanks to the use of transduced G-CSF and plerixafor mobilized $CD34^+$ cells as drug product and the administration of an optimal cell dose in all patients. The supposed contribution of the intra-bone administration route was difficult to be proven since the lack of a control arm with intravenous administration. Clinical outcome showed

a reduction of transfusion requirements in adult patients up to more than 50%. Among the pediatric patients, 4 out of 6 discontinued transfusions shortly after GT and maintained transfusion-independence at later follow-up.[41,42] Among the 4 pediatric patients who achieved transfusion independence, two patients have restarted transfusion at 6 and 5 years of follow-up, respectively. A slow and progressive decrease in VCN and transduction efficiency (TE) has been observed both in peripheral blood and BM cells in one patient, while the other, carrying severe β0β0 mutations, showed a constant marking of transduced cells (around 50%) and restarted transfusion due to need of higher Hb during puberty. Consistently, integration site (IS) analysis demonstrated highly polyclonal engraftment but with a reduced number of insertions in patients showing a reduced engraftment of transduced cells with no clonal dominance.[43] This study set a threshold of 40% in transduction efficiency and 1 VCN in long-term engrafting HSC to obtain a favorable clinical outcome. These key points and the requirement of adequate conditioning to favor transduced cells engraftment were also evident from the unsatisfactory results obtained by another early study with one of the first developed β-globin LV.[44] The analysis of genotoxicity related to LV random integration in the human genome, has proved so far the safety of LV-based HSC gene therapy (HSC-GT) in Bthal with the absence of insertional mutagenesis and malignant clonal dominance in all treated patients.

In the framework of GLOBE-LV, other studies exploited the expression of distinctive engineered transgenes. In the DREPAGLOBE clinical trial (NCT03964792), three patients were treated with GT, with two showing some clinical benefit, using a GLOBE-derived LV (GLOBE-AS3) expressing an engineered β-globin protein with three beneficial anti-sickling amino acids that inhibit the contact required for HbS polymerization (T87Q and E22A) or increase the affinity for α-globin (G16D). Molecular analysis of gene marking during follow-up indicated that, despite similar VCN in the drug products, the efficacy was mostly associated to the level of *in vivo* engraftment and persistence of transduced HSC.[30]

In a distinct strategy, a pilot trial assessing the therapeutic potential of reactivating endogenous HbF in SCD was based on a GLOBE-derived LV expressing a short hairpin RNA targeting BCL11A specifically in erythroid cells (NCT03282656). Published results in 6 treated patients reported increased HbF and mitigation of symptoms in some subjects, although a general drop in VCN in peripheral blood in respect to the drug product.[45] The potential contribution of F-cells expanding following myeloablation can somehow limit the complete attribution of increased level of HbF to the activity of LV in transduced cells. In the US, this GT strategy has been extended to treat more patients in an ongoing phase 2 multi-center trial (NCT05353647).

The production of HbF with enhanced anti-sickling activity was obtained by the GbG^m LV in a clinical trial using a reduced-intensity conditioning in seven SCD patients (NCT02186418). The GbG^m LV was designed with a G16D amino acid substitution in a modified fetal γ-globin, binding to α-globin with high affinity and outcompeting the mutant sickle β-globin chain for production of Hb tetramer. In addition, the inclusion of the β-globin gene untranslated region (UTR), introns, and promoter is aimed to enhance transgene expression in adult cells.[46] The freshly released results showed that the transgenic HbF ranged from 9% to 30% of the total Hb, sustained by low VCN/cell. Amelioration of symptoms was observed in all patients, indicating that an LV expressing HbF with potentiated anti-sickling activity works at low VCN.[47]

The clinical trials of HSC LV GT by gene addition are listed in Table 1.

Overall, the published results from clinical trials utilizing HSC engineered by LV showed an optimal risk/benefit ratio, with no evidence of insertional mutagenesis and adverse events mostly related to myeloablative conditioning prior to autologous transplant or to the underlying pathology. In general, although lentiviral gene addition improves clinical outcomes in hemoglobinopathies, ineffective erythropoiesis persists across all GT trials for Bthal, evoking the opportunity for a combined therapy with erythropoietic enhancers, such as luspatercept.[48] The inclusion in globin LV of only selected and shortened sequences of the chromosomal LCR is limiting the transcription of physiological levels of transgene at low vector copy number per cell that may be clinically safer. In this respect, gene-editing strategies are endowed with an intrinsic advantage since in most cases transcription from the cellular promoters is enhanced by the whole regulatory sequences array.

HSC GENE THERAPY BY GENOME EDITING

The most advanced program of gene editing in hemoglobinopathies exploits CRISPR-Cas9 disruption of BCL11A erythroid enhancer to reactivate endogenous HbF expression and led to the first pioneering trial treating one patient affected by transfusion dependent thalassemia (TDT) and one by SCD with gene edited autologous $CD34^+$ cells (exa-cel).[49] The exa-cel drug product has been used in pivotal phase 3 clinical trials showing favorable clinical efficacy with transfusion independence in 32/35 patients affected by Bthal and with the resolution of the severe symptoms in most of 29/31 treated SCD patients. In both trials, the proportion of edited cells at early time points *in vivo* was >75%, and it was maintained at longer follow-up.[27,28] On the basis of these promising results, FDA approved exa-cel (Casgevy) for the treatment of patients 12-year and older with SCD in 2023 and with TDT in January 2024. Recently, Casgevy has been approved in the United Kingdom,EU, and Saudi Arabia for the same indications. Longer follow-up is needed to confirm therapeutic editing in long-term engrafting HSC and investigation on the cases of delayed neutrophil engraftment and thrombocytopenia merit a better understanding about the impact of the procedure on specific hematopoietic progenitors.

A similar gene editing strategy has been applied in a phase 1/2 trial for TDT (NCT04211480) sponsored by Bioray and conducted in China with 2 pediatric patients successfully achieving transfusion independence at a follow-up of more than 18 months.[50]

Disruption by CRISPR-Cas9 of previously unknown regulatory elements upstream of the HBG1/2 promoter region is the strategy adopted in the drug product developed by Novartis and administered to three adult SCD patients. Following transplantation, the frequency of edited cells decreased in the peripheral blood in respect to the infused product, and the amelioration

Table 1. Clinical trials of gene addition in Bthal and SCD

Disease	Clinical trial	Phase	Drug product	Target gene	Age	Sponsor	Location	Status	Market authorization
Bthal	NCT01639690	phase 1	TNS9.3.55	β-globin	18+ years	San Rocco Therapeutics	USA	active, not recruiting	–
	NCT02453477	phase 1, 2	GLOBE	β-globin	3-64 years	IRCCS San Raffaele	Italy	completed	–
	NCT03275051	interventional	GLOBE	β-globin	3+ years		Italy	long term-follow up active, not recruiting	–
	NCT01745120 HGB204	phase 1, 2	BB305	BB305 β-globin T87Q	12–35 years	bluebird bio	USA, Australia, Thailand	completed	–
	NCT02151526 HGB205	phase 1, 2	BB305	BB305 β-globin T87Q	5–35 years		France	completed	–
	NCT02906202 HGB-207	phase 3	Beti-cel	BB305 β-globin T87Q	0–50 years		USA, Thailand, France, Germany, Italy, United Kingdom	completed	FDA approved Zynteglo™ (betibeglogene autotemcel
	NCT03207009 HGB-212	phase 3	Beti-cel	BB305 β-globin T87Q	0–50 years		USA, France, Germany, Italy, Greece, United Kingdom	completed	FDA approved Zynteglo™ (betibeglogene autotemcel
	NCT02633943	observational	Beti-cel	BB305 β-globin T87Q	0–50 years	bluebird bio	USA, Australia, Thailand, France, Germany, Greece, Italy, United Kingdom	long term-follow up active, not recruiting	FDA approved Zynteglo™ (betibeglogene autotemcel
	NCT06271512	observational	Beti-cel	BB305 β-globin T87Q	0–50 years		USA	long term-follow up recruiting	FDA approved Zynteglo™ (betibeglogene autotemcel)
	NCT03351829	phase 1, 2	undisclosed	β-globin	4–70 years	Shenzhen Geno-Immune Medical Institute	China	not yet recruiting	–
	NCT03276455	phase 1, 2	undisclosed	β-globin	8+ years	Nanfang Hospital of Southern Medical University	China	not yet recruiting	–
	NCT06308159	phase 1, 2	Vebeglogene autotemcel	β-globin	0–35 years	Lantu Biopharma	China	recruiting	–
	NCT05745532	phase 1	HGI-001	β-globin T87Q	8–16 years	Shenzhen Hemogen	China	recruiting	–
	NCT06655662	phase 1	HGI-001	β-globin T87Q	6–35 years		China	recruiting	–
	NCT05015920	phase 1	BD211	β-globin T87Q	5–35 years	Shanghai BDgene Co., Ltd	China	completed	–
	NCT05773729	interventional	BD211	β-globin T87Q	3–18 years		China	recruiting	–

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Table 1. *Continued*

Disease	Clinical trial	Phase	Drug product	Target gene	Age	Sponsor	Location	Status	Market authorization
	NCT05776173	interventional	BD211	β-globin T87Q	6–35 years		China	recruiting	–
	NCT06465550	phase 1	BD211	β-globin T87Q	3–35 years		China	recruiting	–
	NCT05762510	phase 1	LentiRed GMCN-508B	β-globin T87Q	5–35 years	Hospital of Guangxi Medical University	China	recruiting	–
	NCT05860595	N/A	KL003	β-globin T87Q	3–35 years	Institute of Hematology & Blood Disease Hospital	China	active, not recruiting	–
	NCT06219239	interventional	KL003	β-globin T87Q	3–35 years		China	recruiting	–
	NCT05991336	observational	KL003	β-globin T87Q	3–14 years		China	recruiting	–
	NCT06280378	phase 1, 2	KL003	β-globin T87Q	3–35 years	Kanglin Biotechnology, Hangzhou	China	recruiting	–
	NCT06364774	phase 1, 2	CHOP-ALS20	β-globin T87Q	18–35 years	Children's Hospital of Philadelphia	USA	recruiting	–
SCD	NCT02151526 HGB205	phase 1, 2	BB305	β-globin T87Q	5–35 years	bluebird bio	France	completed	–
	NCT02140554 HGB206	phase 1, 2	bb1111 BB305 Lovo-cel	β-globin T87Q	12–50 years		USA	completed	FDA approved LYFGENIA (lovotibeglogene autotemcel)
	NCT04293185 HGB-210	phase 3	BB305 Lovo-cel	β-globin T87Q	2–50 years		USA	active, not recruiting	FDA approved LYFGENIA (lovotibeglogene autotemcel)
	NCT04628585	observational	Beti-cel	β-globin T87Q	0–50 years		USA	long-term follow up enrolling by invitation	FDA approved LYFGENIA (lovotibeglogene autotemcel)
	NCT06399107	phase 1, 2	BAH243	β-globin T87Q	2–90 years	Essan Biotech	China	recruiting	–
	NCT02247843	phase 1, 2	Lenti/βAS3-FB	βAS3-globin	18+ years	UCLA	USA	active, not recruiting	–
	NCT03964792	phase 1, 2	GLOBE1 βAS3	βAS3-globin	12–20 years	Assistance Publique–Hôpitaux de Paris	France	active, not recruiting	–
	NCT04091737	phase 1	CSL-200	γ-globin G16D and shRNA734	18–45 years	CSL Behring	USA	terminated	–
	NCT02186418	phase 1, 2	ARU-1801	γ-globin G16D	18–45 years	Children's Hospital Medical Center, Cincinnati	USA	*terminated*	–

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Table 1. *Continued*

Disease	Clinical trial	Phase	Drug product	Target gene	Age	Sponsor	Location	Status	Market authorization
	NCT03282656	phase 1	LVV containing a short hairpin RNA	shBCL11A	3–40 years	Boston Children's Hospital	USA	active, not recruiting	–
	NCT05353647	phase 2	LVV containing a short hairpin RNA	shBCL11A	13–40 years	Boston Children's Hospital	USA	active, not recruiting	–

of clinical symptoms was modest, indicating an insufficient level of HbF produced *in vivo* in the progeny of the edited HSPC.[51]

Another strategy targeting the promoter region of HBG1/2 genes by disruption with CRISPR-Cas12a was tested in the RUBY and EdiThal clinical trials sponsored by EDITAS (NCT04853576 and NCT05444894), with 28 SCD and 7 Bthal patients treated and increase of HbF >40% associated to clinical benefit.[52,53] Despite these encouraging results, the sponsor discontinued the program in favor of an *in vivo* gene editing approach.

The exploitation of CRISPR-Cas9 and HDR to correct the SCD mutation by an AAV6 to deliver the donor template[54] was translated in the CEDAR clinical trial (NCT04819841). Indeed, the only SCD patient treated in the CEDAR trial using CRISPR-Cas9 gene editing via HDR to edit the mutated HBB gene to the corrected one experienced pancytopenia with prolonged low blood cell counts, leading to trial suspension in January 2023 and early termination, underlying the need of an improved drug product manufacturing. Indeed, the results of pre-clinical studies, including transplantation of the drug product in immunodeficient mice, anticipated low engraftment and chimerism of HDR-edited $CD34^+$ human cells in comparison to controls.[55] Recently updated results presented at international meetings revealed an unexpected reactivation of HbF.

As the most innovative approach, the advantage of base editing for GT for hemoglobinopathies is founded on the expectation of precise intervention at single base level with limited off-target activity and reduced cell response to DNA engineering. However, basic research studies are highlighting unpredicted findings, such as bystander editing or activation of interferon (IFN) pathway in human HSPC,[56] alerting for intensifying non-clinical studies before moving to clinical translation. Adenine base editor (ABE) can be used to convert the SCD mutation into the Makassar non-pathogenic variant[57] and to correct common mutations in Bthal, such as the IVS1-110 splicing mutation and the codon39 missense mutation[58] (Hardouin G. et al. 2025, in press). Furthermore, base editing strategies have been developed to reactivate HbF by generating HPFH mutations in the γ-globin genes promoter generating binding sites for transcriptional activators or disrupting DNA motifs recognized by transcriptional repressors.[59,60] Two clinical trials have been launched by Beam Therapeutics to treat adult SCD patients. The BEACON study (NCT05456880) is a phase 1/2 clinical trial of autologous transplantation of HSPC engineered to reactivate HbF expression by eliminating BCL11A binding sites in HBG1/2 promoters with base editors. Presented results showed rapid and robust HbF induction with initial clinical benefit in 7 SCD treated patients.[61] A second drug product, BEAM-102, was based on preclinical work that successfully converted the SCD point mutation in the HBB gene to the Makassar non-pathogenic variant, but the clinical trial was not activated. Despite the intrinsic limitation due to the base change constraint, base editing showed high efficiency in targeting human HSPC and promising initial results in a clinical context.

The rapid development of nuclease-based gene editing technology was based on significative advantages in genome engineering, such as precision, efficiency, and versatility. Nevertheless, the safety of the CRISPR-Cas9 system remains a major

concern. Some of the drawbacks related to off-target activity and on-target unintended editing have been improved, but not totally eliminated, by engineering Cas9 variants and refining guide RNA design. In addition, different studies documented that double-strand breaks (DSBs) can lead to large deletions, chromosomal rearrangements, genetic instability, and p53 response activation. Finally, the biological complexity of target cells and genetic variability among individuals can influence off-target profiles, with a difficult predictive assessment. Although no malignant transformation or clonal expansion of cells edited with CRISPR-Cas9 has been reported so far in non-clinical studies or in clinical trials, rigorous monitoring in longer follow-up is necessary.

Ideally, base editors can modify the genome without the induction of DSB, thus overcoming some potential safety issues for clinical translation. Thus, the base editing approach is endowed with precision, efficiency, and reduced genotoxicity. However, some intrinsic limitations, such as the restriction to specific type of mutations and edits, or the bystander edits or off-target modifications in regions with similar sequences, still impair the full exploitation of this technology for clinical translation. Several studies reporting pros and cons of gene and base editing technology platforms are summarized and discussed in a study by Aussel et al.[62]

The clinical trials of HSC GT by gene editing are listed in Table 2.

Finally, the technology overcoming the limitations of base editors allowing any base change is prime editing, which relies on Cas9 fused with reverse transcriptase. *Ex vivo* prime editing of SCD HSPC demonstrated that the edited cells engrafted *in vivo* in immunodeficient mice and generated >40% of human erythroblasts expressing normal HbA.[63]

Overall, the great potential of the different manifestations of gene editing to correct hemoglobinopathies will be not only further optimized for the *ex vivo* engineering of HSPC but also pursued for *in vivo* approaches, which represent the ultimate goal for a sustainable GT of genetic diseases. Advances in the fields of delivery, specific targeting of HSC, and chemotherapy-free biological conditioning will fuel the transition from the laboratory bench to the bedside.

HEMATOPOIETIC STEM CELL AND BONE MARROW NICHE BIOLOGY IN BTHAL AND SCD

In clinical trials for hemoglobinopathies, some patients experienced low levels of genetically modified HSCs without the clinical benefit of GT[31–33,41,44] and the root causes have not been clarified yet. The outcomes of GT-treated patients could depend on several factors, including the patient's clinical condition, the extent of genetic modification, the dose and quality of the engineered engrafting HSC, and the status of the recipient BM microenvironment.[64,65] In conditions where both the transplanted HSC and the recipient BM niche are defective, the additive effect of an impaired HSC function and a suboptimal supporting activity by the BM niche components can hamper the engraftment of genetically modified HSC. Furthermore, a recent halt in SCD GT trials, due to the development of myeloid neoplasms,[38] raises the need for a better characterization of the BM microenvironment in hemoglobinopathies toward the potential targeting of niche defects to ameliorate therapeutic outcomes.[66] Overall, a deeper knowledge of the features of the BM niche and HSC in hematological inherited disorders is fundamental to assess potential defects and to improve HSC GT.

To study HSC niche in Bthal and SCD, mouse strains recapitulating the main features of the human diseases were exploited, the *Hbb*$^{th3/+}$ (th3) mice and the Berkley or Townes mice, respectively.[67] Patient-derived samples were studied for the immunophenotypic characterization of HSC and BM microenvironment to validate findings obtained in mice and to model *in vitro* the discovered alterations.

Recently, it has been reported that HSC function and BM niche components are altered in Bthal[68–70] and SCD[71–73] leading to the hypothesis that specific HSC and BM niche features can contribute to the limited engraftment of genetically modified HSCs in these diseases. Many studies have investigated how stress signals affect HSC function and activity in these hematological inherited disorders.

HSC biology

Bthal

Recent studies have demonstrated that Bthal profoundly impacts HSC biology.[69,70] The disease's pathophysiology entails chronic anemia, iron overload, and ineffective erythropoiesis, all of which can alter the properties of HSCs and their supportive niche in the BM.

Using a mouse model, which mimics a severe Bthal intermediate phenotype, Aprile et al.[70] provided crucial insights into HSC functionality. Their research revealed that HSC in this model are quantitatively reduced and show signs of heightened cycling activity, indicating stress and exhaustion. Functionally, these HSC exhibit diminished capacity for engraftment when transplanted into recipient mice, pointing toward compromised self-renewal and long-term repopulating ability. Further molecular analysis through RNA sequencing (RNA-seq) revealed increased expression of stress response genes, DNA damage markers, and a reduction in stemness-related gene expression, suggesting that Bthal HSC endure substantial replication and oxidative stress. These findings are adequate to indicate that the function of HSC is compromised in the Bthal BM environment, which could impact the biological activity of HSC. Due to chronically active reactive oxygen species (ROS), patients with TDT require higher stimulation of the CD34^{+} response to stress and have higher circulating rates of primitive HSPC.[74]

Chronic oxidative stress is a hallmark of Bthal, driven by elevated ROS levels originating from disrupted iron homeostasis. A novel study revealed that iron overload leads to mitochondrial damage within HSC, impairing their metabolic function and promoting DNA damage.[75] This damage further impairs HSC quiescence and self-renewal, critical properties for effective hematopoiesis and engraftment post-GT. Notably, interventions that mitigate iron overload and ROS, such as iron chelation therapy or antioxidants, have shown promise in restoring mitochondrial function and HSC functionality, emphasizing the role of systemic and microenvironmental factors in maintaining healthy hematopoiesis.

Recently, a comparative study on long-term lineage commitment at the clonal level was carried out in patients treated with

Table 2. Clinical trials of gene editing in Bthal and SCD

Disease	Clinical trial	Phase	Drug product	Target gene	Age	Sponsor	Location	Status	Market authorization
Bthal	NCT04205435	phase 1, 2	CRISPR-Cas9	β-globin	5–15 years	Biorary Laboratories	China	terminated	–
	NCT04211480	interventional	CRISPR-Cas9	β-globin	5–15 years		China,	completed	–
	NCT03655678	phase 2/3	CRISPR/Cas9: CTX001	BCL11A erythroid enhancer	12–35 years	Vertex Pharmaceuticals	USA Canada Germany, Italy; UK	active, not recruiting	–
	NCT04208529	phase 3	CRISPR-Cas9: CTX001	BCL11A erythroid enhancer	–		USA, Belgium Canada Germany, Italy; UK	long-term follow up enrolling by invitation	–
	NCT05477563	phase 3	CRISPR-Cas9: CTX001 Exa-cell	BCL11A erythroid enhancer	12–35 years		USA, Germany, Italy, Saudi Arabia	recruiting	–
	NCT03432364	phase 1, 2	ZFN: ST-400	BCL11A erythroid enhancer	18–40 years	Sangamo Therapeutics	USA	completed	–
	NCT05145062	observational	ZFN: ST-400	BCL11A erythroid enhancer	18–45 years		USA	long-term follow up enrolling by invitation	–
	NCT06647979	phase 1	CRISPR-Cas9	BCL11A erythroid enhancer	13–40 years	Boston Children's Hospital	USA	not yet recruiting	–
SCD	NCT04774536	phase 1, 2	CRISPR-Cas9 CRISPR_SCD001	β-globin	12–35 years	University of California, San Francisco	USA	recruiting	–
	NCT04819841	phase 1, 2	CRISPR-Cas9: nula-cel	β-globin	12–40 years	Kamau Therapeutics	USA	recruiting	–
	NCT03653247	phase 1, 2	ZFN: BIVV003	BCL11A erythroid enhancer	18–40 years	Sangamo Therapeutics	USA	active, not recruiting	–
	NCT05145062	observational	ZFN: BIVV003	BCL11A erythroid enhancer	18–45 years		USA	long-term follow up enrolling by invitation<	–
	NCT03745287	phase 2, 3	CRISPR-Cas9: CTX001 Exa-cell	BCL11A erythroid enhancer	12–35 years	Vertex Pharmaceuticals	USA	active, not recruiting	FDA- EMA Approved Casgevy (Exagamglogene autotemcel)
	NCT04208529	phase 3	CRISPR-Cas9: CTX001 Exa-cell	BCL11A erythroid enhancer	2+ years		USA	long-term follow up enrolling by invitation	FDA- EMA Approved Casgevy (Exagamglogene autotemcel)

(*Continued on next page*)

Table 2. *Continued*

Disease	Clinical trial	Phase	Drug product	Target gene	Age	Sponsor	Location	Status	Market authorization
	NCT05329649	phase 3	CRISPR-Cas9: CTX001 Exa-cell	BCL11A erythroid enhancer	2–11 years		USA, Germany, Italy, UK	recruiting	FDA- EMA Approved Casgevy (Exagamglogene autotemcel)
	NCT05477563	phase 3	CRISPR-Cas9: CTX001 Exa-cell	BCL11A erythroid enhancer	12–35 years		USA, Germany, Italy, Saudi Arabia	recruiting	FDA- EMA Approved Casgevy (Exagamglogene autotemcel)
	NCT05951205	phase 3	CRISPR-Cas9: CTX001 Exa-cell	BCL11A erythroid enhancer	12–35 years		NA	not yet recruiting	FDA- EMA Approved Casgevy (Exagamglogene autotemcel)
	NCT06287086	interventional	CRISPR-Cas9: BRL101	BCL11A erythroid enhancer	3–35 years	Bioray Laboratories	NA	not yet recruiting	–
	NCT06287099	interventional	CRISPR-Cas9: BRL101	BCL11A erythroid enhancer	3–35 years		NA	not yet recruiting	–
	NCT06300723	interventional	CRISPR-Cas9: BRL101	BCL11A erythroid enhancer	3–35 years		China	enrolling by invitation	–
	NCT06647979	phase 1	CRISPR-Cas9:	BCL11A erythroid enhancer	13–40 years	Boston Children's Hospital	USA	not yet recruiting	–
	NCT04443907	phase 1, 2	CRISPR-Cas9: OTQ923	y-globin promoter	2–40 years	Novartis Pharmaceuticals	USA	terminated	–
	NCT06155500	observational	CRISPR-Cas9: OTQ923	y-globin promoter	18+ years			long-term follow up recruiting	–
	NCT04853576	phase 1, 2	CRISPR-Cas12 EDIT-301	y-globin promoter	12–50 years	Editas Medicine	USA, Canada	active, not recruiting	–
	NCT06363760	phase 1, 2	CRISPR-Cas12 EDIT-301	y-globin promoter	12–50 years		USA	long-term follow up enrolling by invitation	–
	NCT06506461	phase 1	CRISPR-Cas9: HBG-115	y-globin promoter	18–24 years	St. Jude Children's Research Hospital	USA	recruiting	–
	NCT05456880	phase 1, 2	Adenine base editing BEAM-101	y-globin promoter	18–35 years	Beam Therapeutics	USA	recruiting	–

lentiviral vector-based HSC-GT for Bthal, metachromatic leukodystrophy (MLD, a neurodegenerative lysosomal storage disorder) and *Wiskott-Aldrich* syndrome (WAS, X-linked primary immunodeficiency).[34] In the phase 1/2 GT trial for Bthal[41] (TIGET-BTHAL, NCT02453477), patients showed a hematopoietic process with a preferential output and lineage commitment toward erythroid lineage, with respect to other diseases, even years after treatment. The fraction of active engrafted transduced HSC contributing to hematopoiesis at steady state was comparable to GT trials for other hematopoietic diseases, thus suggesting that this specific feature is independent of infused cell dose.[34]

Other factors may contribute, such as environmental signals together with intrinsic or epigenetic factors. Interestingly, in Bthal patients, a notable shift was observed in their peripheral blood HSC and multipotent progenitors (MPPs), where there was an increase in myeloid lineage production accompanied by a corresponding decrease in erythroid lineage differentiation.[76] A more recent study, performing transcriptome analysis at a single cell level on human cells from patients affected by TDT highlighted that BM HSC/MPP are more active than healthy donor ones and are characterized by a reduced stemness with an enhanced commitment toward erythroid differentiation trajectory, favoring the disease's main clinical feature of ineffective erythropoiesis. Furthermore, recent findings point to altered transforming growth factor beta 1 (TGF-β1) signaling within the BM niche, which affects autophagy mechanisms, reducing the quiescent, dormant state of HSC, and consequently compromising long-term self-renewal capacity.[68]

Notably, no dangerous somatic mutations were identified in GT Bthal patients enrolled in TIGET-BTHAL.[34] The average number of mutations remained stable over time. Somatic mutations have a variant allele frequency (VAF) less than 2%. Four mutations detected at several time points showed no increase in abundance, thus indicating no selective advantage. The analysis of ISs showed that most active clones identified early and contributing to hematopoiesis, remained stable without an increase in frequency of LV integration in hotspots. No persisting dominant clone was identified in the GT-treated patients.[34] High $CD34^+$ cell doses infused in the Bthal patients may protect HSC against replicative stress, clonal hematopoiesis (CH), and malignancy.

Few trials studied the effect of GT on patient's HSC biology. In the phase 1/2 trial (NCT04211480) of gene editing in children with TDT by disrupting the GATA1-binding site at the +58 *BCL11A* erythroid enhancer to induce γ-globin expression,[50] the authors performed scRNA-seq of peripheral blood mononuclear cells (PBMCs) to evaluate changes in transcriptomes of the blood lineages reconstituted from unedited or edited HSPCs. Firstly, the study showed that edited HSPCs successfully engrafted and differentiated into all blood lineages, and transcriptome analysis revealed that BCL11A editing did not impact the transcriptome in all blood cell lineages, especially in B and plasmacytoid dendritic cells (pDCs), in which BCL11A plays an important role.

In the pilot trial (NCT05015920) aimed to evaluate the safety and efficacy of a β-globin expression-optimized and insulator-engineered lentivirus-modified cell product (BD211) in TDT, the authors performed scRNA-seq on PBMCs. Differential expression analysis of blood cancer-related genes in treated patients compared with healthy donor samples revealed no upregulation of cancer-related genes, thus suggesting absence of oncogenesis related to the drug product.[77]

In a study assessing the specificity of CRISPR-Cas9 gene editing of BCL11A erythroid enhancer in the engineering of Exa-cel (CLIMB THAL-111, NCT03655678), the authors, using a hybrid-capture sequencing in three treated TDT patients, demonstrated that none of the 36 candidate off-target sites occurred in the encoding region of myeloid cancer-associated genes.[78]

SCD

In SCD, the BM niche is similarly compromised by chronic inflammation and hematologic stress.[73] Patients and murine models show HSC dysfunction, characterized by increased proliferation, DNA damage, and oxidative stress and iron overload.[71,75] The aged or damaged HSC tend to circulate more in peripheral blood,[79,80] reflecting impaired BM retention and a shift toward emergency hematopoiesis.

Stress erythropoiesis and chronic inflammation in subjects with SCD affect the immunophenotypes of $CD34^+$, their yield, and mobilization.[81,82] More than 50% of $CD34^+$ HSPCs from SCD BM resulted in $CD34^{dim}$ due to higher erythroid glycophorin A marker expression on erythroid committed progenitors.

Importantly, recent epigenetic studies reveal accelerated biological aging in SCD peripheral blood samples,[83] likely increasing the risk for myeloid malignancies. An altered fitness and function of HSPCs in SCD individuals and mice has been reported.[71] Young SCD mice show a modest increase of HSC numbers that decrease over time with age. Proliferation kinetics, DNA damage, and high oxidative stress characterize SCD HSC. Interestingly, the BM of young SCD mice cannot support the long-term repopulating activity of HSC.[71]

The GT approach aims to reduce or correct SCD pathophysiology, but the altered HSC might persist during GT manufacturing or hematopoietic reconstitution after BM transplantation, thus making them vulnerable to leukemic transformation due to regenerative stress.[84–88] In allogeneic transplants, some patients developed leukemia in the context of graft failure. Cases of myelodysplastic syndrome/AML in SCD GT trials[38,89] raise the demand to assess the impact of BM microenvironment on HSC biology in hemoglobinopathies to target niche defects to avoid genetic insults. Prolonged exposure to hydroxyurea (HU) might contribute to a hypothetical increased risk of neoplastic malignancy, although recent studies revealed that the rate of clonal hematopoiesis was unaltered in SCD patients treated with HU compared to untreated.[88] A recent publication reported the absence of increased clonal expansion in genetically modified cells versus the unmodified ones. However, potential driver mutations associated with CH or myeloid neoplasms (i.e., DNMT3A- and EZH2-mutated clones) were identified in all transplanted cells, irrespective of the genetic modification, thus suggesting a positive selection during GT.[90]

Recently, genomic analysis in SCD patients treated with GT using the DREPAGLOBE lentiviral vector (NCT03964792) expressing an anti-sickling β-globin showed no somatic mutations with a VAF greater than 1%, thus excluding any evidence of CH.[91] Transcriptome profile revealed enrichment in inflammatory signature (via interleukin [IL]-1 or tumor necrosis factor alpha [TNF-α] and IFN signaling pathways) in the most immature

component of the mobilized HSPC source along with a lineage bias in the HSC in patients with poor engraftment. The inflammatory activation in the stem cell source may explain the lower engraftment of GT HSC. Considering no data on defect of lineage commitment was reported in this trial, the authors do not exclude that other factors might have influenced the outcome of the trial, including the low number of infused GT HSC in patients with graft failure.[91]

The risk assessment of potential malignant transformation was also tested in three patients with SCD treated with Exa-cel (CLIMB SCD-121, NCT03745287). None of the off-target sites was found in cancer genes.[78]

The occurrence of myeloid leukemia and myelodysplastic syndrome has prompted further refinement in the manufacturing process, highlighting the relevance of safety in advancing genetic therapies for SCD. A recent study modified the manufacturing process to reduce stress, thus improving stem cell fitness.[92] The authors demonstrate that SCD $CD34^+$ HSPCs transduced in the presence of UM171 showed reduced DNA damage and ROS, decreased apoptosis, and improved engraftment of long-term HSC.

BM niche biology

HSC reside in a specialized microenvironment within the BM, termed niche, which tightly regulates their fate through complex interactions with stromal and hematopoietic cells, soluble factors, and physical cues.[93,94] Over the past two decades, the regulation of the BM niche in native conditions has been extensively studied thanks to technical advances in mouse genetics and imaging technologies,[95,96] revealing that the niche composition is highly dynamic and responsive to hematopoietic stress or perturbations.[97]

While numerous studies have explored BM microenvironmental changes in response to stress and malignancy,[98] the regulation of HSC-niche interactions in inherited hematological disorders remains poorly understood. However, growing evidence indicates that in Bthal and SCD, despite differences in disease etiology, chronic stress alters BM erythropoiesis and disrupts the niche, contributing to impaired hematopoiesis. Allogeneic HSC transplantation from HLA-matched donors offers the definitive cure for Bthal and SCD with very good outcomes in terms of overall survival and disease-free survival.[16,18] However, higher mortality was associated with older patient age and worse clinical status.[99] Although few studies reported long-term follow-up after transplantation, cases of late graft failure have been described.[100–102] The causes underlying late graft loss have not been clarified. In this view, recent evidence of alterations in the BM microenvironment in murine models and in patient-derived cells suggests a potential contribution of the recipient HSC niche. Organ damage cannot be excluded as other potential reason for poor outcomes, especially in SCD patients.

Bthal

In murine models of Bthal and SCD, HSC exhibit decreased frequency, increased cycling activity, elevated DNA damage, and accumulation of ROS, associated with impaired hematopoietic support by osteolineage cells (OLCs) and mesenchymal stromal cells (MSCs).[70,73] It was demonstrated that the impaired self-renewal of Bthal HSC is driven by their persistence into an altered BM microenvironment, characterized by reduced bone density and deposition, along with decreased expression of key niche molecules, such as osteopontin and JAGGED1 (JAG1) by OLCs, and diminished circulating levels of parathyroid hormone (PTH).[70] PTH is a key regulator of calcium and phosphate homeostasis and bone remodeling influencing HSC maintenance through its specific receptor on BM stromal cells.[103] In Bthal, the downregulation of PTH-JAG1-Notch1 axis impairs the crosstalk between OLCs and HSC and also MSCs exhibit reduced frequency and lower *Jag1* expression, as compared to wild-type controls. Importantly, *in vivo* reactivation of PTH signaling restores HSC-stromal niche interactions and rescues Bthal HSC function.[70]

Additionally, it was reported that iron overload adversely affects BM MSCs derived from TDT patients.[74] Bthal MSCs accumulate iron and display elevated ROS levels and marked depletion of the primitive $CD146^+$ $CD271^+$ MSC subset. Bthal MSCs also showed reduced clonogenic capacity, proliferation, early cell-cycle arrest, and impaired adipogenic and osteogenic differentiation.[74] Furthermore, they express lower levels of key hematopoietic-supportive factors, including *SCF*, *CXCL12*, *CDH2*, *VCAM*, *ANGPT1*, *VEGFA*, *IL-6*, and *FGF2*, resulting in defective maintenance of HSC, as highlighted by migration assays, co-culture systems, and xenotransplantation models.

Further analysis of the BM niche revealed high systemic and BM local levels of fibroblast growth factor 23 (FGF23) in Bthal mice and patients.[69] FGF23 is a negative regulator of bone metabolism and PTH secretion[104,105] and is upregulated in response to the anemia-related factor erythropoietin (EPO).[106] The enhanced FGF23 production by bone and erythroid cells under high EPO stimulation, characteristic of the disease, underlies the observed bone pathology and suppressed PTH levels in Bthal mice, ultimately impairing HSC-niche crosstalk.[69] Pharmacological inhibition of FGF23 signaling by c-terminal FGF23 peptide restored bone density and deposition, and the hematopoietic support capacity of OLCs, offering a promising niche-targeting strategy for improving HSC function in Bthal.[69]

SCD

Recent work identified significant alterations in the BM microenvironment of SCD mice, including reduced frequency of MSCs, accumulation of ROS, and impaired adipogenic and osteogenic differentiation, indicating compromised MSC functions.[73] Gene expression profiling revealed downregulation of critical niche-supportive factors, such as *Opn*, *Vcam1*, *Angpt1*, *Scf*, and *Cxcl12*, associated with a diminished capacity to support HSC maintenance both *in vitro* and *in vivo*. Although human MSCs derived from the BM of SCD patients exhibited altered expression of SCF and CXCL12, others reported they retain normal functionality *in vitro*.[107] Furthermore, BM MSCs from SCD mice assumed with aging an increased expression of pro-inflammatory genes associated to reduced HSC maintenance.[72]

Alterations in the BM vasculature also appear to play a critical role in SCD hematopoiesis. SCD mice exhibit a profoundly disrupted vascular network, characterized by highly tortuous arterioles and fragmented sinusoidal vessels, accompanied by elevated hypoxia-inducible factor 1-alpha (HIF-1α) levels and

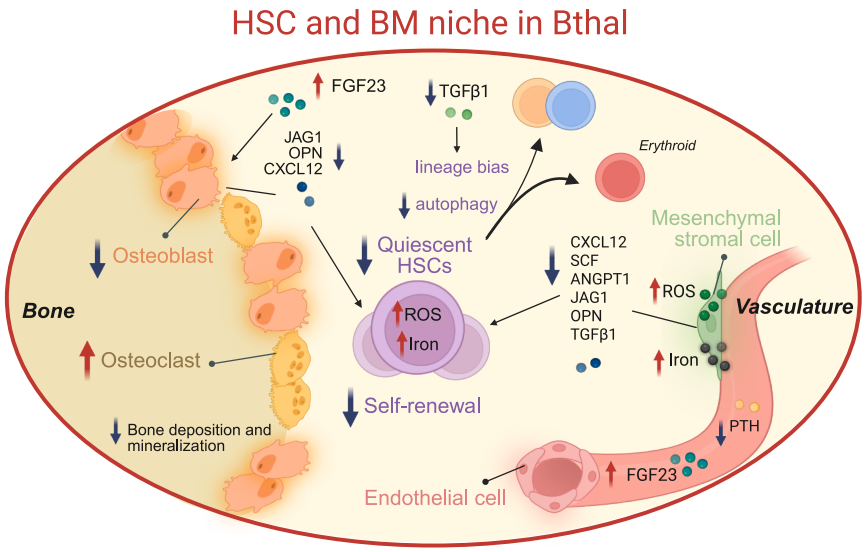

Figure 2. HSC and BM niche in Bthal
Overview of the altered BM niche components affecting HSC function in Bthal. Created in BioRender.

enhanced neovascularization.[108] Due to aberrant interactions between SS-RBCs and endothelial cells, SCD patients display chronically elevated levels of inflammatory cytokines, particularly IL-6, IL-8, and TNF-α. While inflammation under physiological conditions contributes to bone remodeling, persistent inflammation within the bone microcirculation in SCD may promote osteoclast activity through upregulation of RANKL.[109] Consistent with this, micro-CT imaging in transgenic SCD mice has revealed altered bone microarchitecture, with a reduced number of trabeculae and compromised structural integrity, suggestive of progressive damage of bone tissue.[110] Another work reported that recurrent hypoxia/reperfusion episodes mimicking vaso-occlusive crises, stimulate osteoclastogenesis and bone turnover in SCD mice, marked by upregulation of the pro-resorptive cytokine IL-6 and suppression of osteogenic lineage markers, such as Runx2 and Sparc.[111] Despite these findings, the direct contribution of these BM microenvironmental alterations to the regulation of the HSC niche in SCD remains largely unexplored.

Chronic inflammation is a hallmark of SCD pathophysiology, and exposure to inflammatory signals and infections has been shown to impact HSC biology by promoting cycling, inducing DNA damage, and impairing long-term self-renewal capacity.[112,113] Additionally, inflammatory stimuli can directly damage BM niche components.[114] However, the specific effects of chronic inflammation on the SCD HSC niche remain to be fully elucidated and represent an important area for future investigation.

IMPLICATIONS FOR GENE THERAPY AND FUTURE DIRECTIONS

Recent findings challenged the paradigm of Bthal and SCD, as disorders confined to erythropoiesis and highlighted that HSC and BM niche components are altered (Figures 2 and 3). The efficacy of GT in Bthal and SCD hinges on understanding and overcoming the intrinsic HSC and niche defects. In Bthal, ineffective erythropoiesis and iron overload affect HSC self-renewal and engraftment, but with refined protocols and careful safety assessments, GT has shown favorable results, with no apparent increase in oncogenic risk. In SCD, targeting the oxidative damage and inflammatory signatures in HSC sources can improve engraftment and reduce the risk of CH or malignancies.

The reduced number of HSPCs available for collection and the impaired engraftment potential in patients treated by HSC GT indeed pose potential limitations for full clinical benefit, resulting in variability in terms of GT efficiency and *in vivo* reconstitution. Moreover, in SCD patients treated with hydroxyurea, which is a gold-standard treatment to reduce vaso-occlusive crisis by increasing HbF levels, $CD34^+$ cell counts resulted lower in both the peripheral blood and BM, due to the suppressive action of HU on BM.[115] It is reasonable to expect that the status of the BM microenvironment influences the quality of HSPCs harvested for genetic engineering, as well as their engraftment and reconstitution capacity once transplanted. Thus, the impact of *ex vivo* genetic manipulation on HSPC already hampered by the disease needs to be further explored. The development of optimized protocols for increasing the efficacy and safety of *ex vivo* genetic manipulation in combination with supportive therapies, which aim to ameliorate the BM niche and preserve long-term HSC function has the potential to improve the clinical outcomes of HSC GT. Treatment pre- and post-transplantation can restore a functional HSC niche before HSPC harvest and promote early engraftment after GT, respectively.

Furthermore, since myeloablative conditioning is required to obtain high levels of gene-modified autologous grafts upon HSC GT, potential damages to hematopoietic and stromal BM components might have further impact on the supportive capacity of the BM niche. In this view, recent advances in biological conditioning strategies by antibody-drug conjugates showed better preservation of the BM environment.[116] Moreover, chemotherapy-free conditioning based on mobilization efficiently deplete the BM niche while maintaining its integrity,[117] and might provide a potential alternative for combination with niche targeting approaches.

In conclusion, we underline the importance of improving our comprehension of HSC and BM niche biology in hemoglobinopathies toward the novel concept of combined therapies. Future therapies must integrate strategies to modulate the BM niche, reduce oxidative and inflammatory damage, and optimize HSC fitness to achieve durable and safe cures for patients suffering from these devastating disorders.

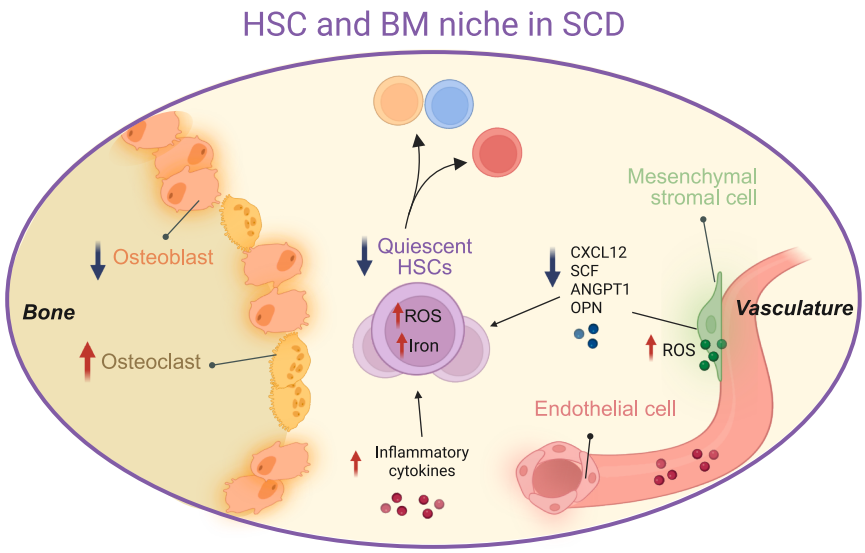

Figure 3. HSC and BM niche in SCD
Overview of the altered BM niche components affecting HSC function in SCD. Created in BioRender.

AUTHOR CONTRIBUTIONS

A.A. and M.R.L. equally contributed to write the original draft. G.F. wrote and reviewed the original draft and supervised the general structure and the work progress.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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