PERSPECTIVES

CIRM CALIFORNIA'S STEM CELL AGENCY   STEM CELLS TRANSLATIONAL MEDICINE

Unleashing the cure: Overcoming persistent obstacles in the translation and expanded use of hematopoietic stem cell-based therapies

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Abstract

Hematopoietic stem cell transplantation (HSCT) is broadly used for treating and curing hematological cancers and various disorders of the blood and immune system. However, its true therapeutic potential remains vastly constrained by significant scientific and technical hurdles that preclude expansion to new indications and limit the number of patients who could benefit from, gain access to, or financially afford the procedure. To define and overcome these challenges, the California Institute for Regenerative Medicine (CIRM) held multiple workshops related to HSCT and has subsequently invested in a new generation of approaches to address the most compelling needs of the field, including new sources of healthy and immunologically compatible hematopoietic stem cells for transplant; safe and efficient genome modification technologies for correction of inherited genetic defects and other forms of gene therapy; safer and more tractable transplantation procedures such as nongenotoxic conditioning regimens, methods to accelerate immune reconstitution and recovery of immune function, and innovations to minimize the risk of immune rejection; and other life-threatening complications from transplant. This Perspective serves to highlight these needs through examples from the recent CIRM-funded and other notable investigations, presents rationale for comprehensive, systematic, and focused strategies to unleash the full potential of HSCT, thereby enabling cures for a greatly expanded number of disorders and making HSCT feasible, accessible, and affordable to all who could benefit.

KEYWORDS
bone marrow, gene therapy, hematopoietic stem cells, hematopoetic stem cell transplantation, immune reconstitution

1 | INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is the only broadly applied clinical stem cell therapy that is routinely used to treat, and even cure patients of disease, particularly in the areas of hematological cancer, certain leukodystrophies, and various diseases of the blood and immune system. The procedure can be divided into two categories: (a) allogeneic HSCT (allo-HSCT), in which hematopoietic stem and progenitor cells (HSPC) are procured from a healthy donor and used to reconstitute a patient's hematopoietic and immune systems; and (b) autologous HSCT (auto-HSCT), in which the patient's own HSPC are procured as the donor source for transplantation. Key to the long-term durability of this

treatment is the presence of definitive hematopoietic stem cells (HSCs) in the donor material, which engraft and establish a lifetime supply of blood and immune cells for the recipient. As of 2012, over 950 000 HSCTs had been performed worldwide of which 42% were allogeneic and 58% were autologous.[1] Notably, only 5%-6% of these procedures were performed for nonmalignant disorders, illustrating a large gap between the theoretical possibilities of HSCT to address such indications, and the realities of its current practice. The use of HSCT and its expansion into new areas of medicine has been significantly hampered by scientific and technical obstacles, primarily associated with the (a) limited availability of healthy, transplantable, immune-compatible cells; and (b) the harsh conditioning regimens necessary to facilitate effective engraftment. Consequently, HSCT carries significant risks of morbidity and mortality, precluding its use for treating non-life-threatening conditions that might otherwise benefit, and resulting in extremely high costs of implementation, currently ranging from $100 000 to $300 000 for a standard allogenic regimen,[2] to an estimated $660 000 to $1 800 000 for gene-modified autologous therapy.[3,4] In this Perspective, we describe a new generation of approaches to systematically and comprehensively tackle key limitations of HSCT, with a particular emphasis on recent investments from the California Institute for Regenerative Medicine (CIRM). We hope that these and other novel approaches will unleash the full potential of HSCT to transform the face of medicine, creating new treatments and cures that are accessible and affordable to all.

2 | LIMITED AVAILABILITY OF HSCS: IMPROVING DONOR CELL SOURCES

Numerous genetic diseases have the potential to be cured through allo-HSCT, including various primary immune deficiencies, disorders of the bone marrow, hemoglobinopathies such as sickle cell disease and thalassemia major, and a growing list of metabolic disorders including adrenoleukodystrophy and Hurler Syndrome.[5] Unfortunately, finding an immune-compatible donor is difficult, if not impossible for many patients, and transplantation with suboptimally matched donor cells carries unacceptably high risks of immune rejection and other life-threatening complications such as graft vs host disease (GVHD). Alternatively, transplantation of genetically corrected autologous HSC could avoid many of these immunologic problems, and improved methods to edit or correct disease-associated mutations in HSCs are an area of intense investigation. Even so, the ability to procure, expand, and preserve sufficient numbers of healthy HSCs for all patient's needing transplants is limited, necessitating the development of tools and technologies to create new sources or improve existing ones.

2.1 | Expanding donor HSCs

Efforts to address both immune compatibility and donor cell availability have led to the growing practice of banking umbilical cords (UC), which contain HSCs with an immature phenotype that requires less stringent

Significance statement

Hematopoietic stem cell transplantation (HSCT) is commonly used to treat leukemias and severe disorders of the blood and immune system, but it has not been possible to extend HSCT to many patients in need of transplant, or into various new areas of disease that might benefit. This vast, untapped potential results from inadequate sources of healthy, immune-compatible stem cells for transplant, technological barriers to efficient engraftment, and the significant health risks associated with the HSCT procedure itself. This Perspective elaborates on current limitations of HSCT and describes novel strategies to overcome them, including key innovations developed with support from the California Institute for Regenerative Medicine. Addressing these challenges could greatly expand the feasibility and accessibility of HSCT to all who might benefit, and enable HSCT to serve as a leading paradigm for developing new stem cell-based therapies in the future.

matching for transplant than those from adult donors. Although UC banks have increased the overall size and diversity of the donor pool, a single cord typically does not contain sufficient HSCs to treat an adult patient, and thus methods to culture and expand functional HSCs ex vivo have become key areas of research. In the past decade, multiple pharmacologic agents and other factors have been identified that promote ex vivo expansion, or modify properties of cord blood HSC or HSC-like cells to accelerate their growth in vivo. Some of these approaches have progressed into clinical studies including an engineered Notch ligand[6] and genetically modified umbilical vein epithelial cells,[7] both of which are being tested in CIRM-supported clinical trials, and StemRegenin 1 (SR1), an aryl hydrocarbon receptor anatagonist that was initially discovered as part of an early CIRM sponsored award[8] (Table 1). Other groups have identified expansion factors that exploit different mechanisms of action including the pyrimidoindole derivative UM171,[13] and dmPGE2, which augments the homing and engraftment potential of cord blood stem cells.[14] Several earlier stage CIRM awards have explored endogenous paracrine factors that might also be exploited to expand HSPCs in vivo or in vitro, such as pleiotrophin and dickkopf-1.[9,10] Most recently, a CIRM funded exploratory study identified novel culture conditions permitting 236-899-fold expansion of highly engraftable, functional murine HSCs in only 30 days,[11] and is currently attempting to replicate this phenomenon in a human HSC culture system (Table 1). Although these endeavors are encouraging, each approach affects one or more different subpopulations of blood progenitors and the long-term engraftability and clinical utility of expanded cells remains unknown. Investigators continue to pursue new leads that can more rapidly and efficiently increase the definitive HSC population from any source tissue, while maintaining the key properties that give rise to their long-term survival, safety, and functionality upon transplantation.

TABLE 1 CIRM-supported approaches to improve HSC source and availability

Approach	Investigator/company	Stage, trial number
HSC expansion		
Delta 1	Nohla Therapeutics[6]	Ph 2, NCT03301597
Engineered UVECs	Angiocrine Bioscience[7]	Phase 1b, NCT03483324
StemRegenin	Schultz, Boitano[8]	Discovery stage
Novel small molecules	Leavitt, Seigel	Discovery stage
Paracrine niche factors	Chute[9,10]	Discovery stage
Novel culture conditions	Nakauchi[11]	Discovery stage
Other approach		
hPSC differentiation	Multiple, see Reference 12	Discovery stage
Engineered HSCs	Multiple, see Table 2	All stages, see Table 2
Immune modulation	Multiple, see Section 2	All stages, see Section 2

Note: List of active and recent CIRM-funded projects supporting the research and development of novel approaches for expanding or deriving HSCs, or extending their usability.

Abbreviations: hPSC, human pluripotent stem cell; HSCs, hematopoietic stem cells; NCT, National Clinical Trial Indicator; Ph, phase; UVECS, umbilical vein endothelial cells.

2.2 | Differentiated HSCs

The ability to derive fully functional, definitive HSCs from human pluripotent stem cells (hPSCs) would have a transformative impact on the field of regenerative medicine, enabling unlimited quantities of allogeneic and autologous HSPCs to be prepared and made available for transplant. The scientific and technical challenges to achieving this goal were the subject of a previous CIRM Perspective that identified critical knowledge gaps in our understanding of the ontogeny of human HSCs, and of the intrinsic and extrinsic factors that govern HSC behavior and function.[12] Although there remains much to elucidate, continued CIRM investment has helped to support identification of defective medial HOXA gene activation as key impediment to deriving self-renewing HSCs from hPSCs, which can in part be overcome by manipulating retinoic acid signaling[15]; and the identification of distinct mesenchymal subpopulations that co-emerge during hPSC differentiation, a subset of which express genes associated with the HSPC niche and support maintenance of functional HSPCs ex vivo.[16] These efforts, combined with other new advances, provide new tools to model human hematopoietic development and to allow systematic testing of novel strategies to produce unlimited sources of functional, regenerative HSCs.[17]

2.3 | Genetically engineered HSCs for auto-HSCT

The development of feasible, effective, and safe methods for genetically modifying HSCs could provide a powerful alternative to allo-HSCT for the cure of genetic diseases. In some cases, such cells may even be more therapeutically potent than unmodified allogeneic HSC if they can be designed to overexpress a normal gene product. Two key methodologies for genetically modifying HSCs have been prioritized in recent years including lentiviral-mediated gene delivery and nuclease-based genome editing approaches, both of which have received considerable CIRM support (Table 2).

2.4 | Lentiviral-based modification

The demonstration that retroviral vectors could transduce HSPCs in the early 1980s heralded a combined cell and gene based therapy approach for curing monogenetic disease,[18,19] a concept that was subsequently tested in clinical trials for primary immunodeficiencies such as X-linked severe combined immunodeficiency (SCID-X1),[20] SCID caused by adenosine deaminase deficiency (ADA-SCID),[21] Wiskott-Aldrich syndrome (WAS),[22,23] chronic granulomatous disease (CGD),[24] as well as demyelinating leukodystrophies.[25,26] Unfortunately, despite some clear indications of clinical benefit, the first generation gamma-retroviral vectors were characterized by frequent insertional oncogenesis and leukoproliferative complications for some subjects, reviewed by Morgan et al.[27] However, improved "second generation" vectors were subsequently developed along with new assays to assess their oncogenic potential. The clinical results with these next generation vectors, although still early, are quite striking. No insertional oncogenesis has been observed, even in those trials where a small number of patients are >5 years post-treatment, well beyond the critical period in which patients developed leukemia in the earlier studies.[25] Moreover, trial subjects are showing clear signs of clinical benefit and even cure, including several children with ADA-SCID in a recent CIRM funded study.[28] Due in part to these successes, the FDA now provides written guidance on the set of preclinical safety studies that should be performed in order to submit a successful IND application for lentiviral-based gene and cell therapy clinical trials. Future improvements in vector manufacturing should greatly expand both the number of patients and number of diseases that could benefit from this HSCT approach.

2.5 | Genome editing

An alternative to lentiviral-mediated gene integration is genome editing, where an engineered nuclease is used to create a site-specific DNA double-strand break (DSB), subsequently activating the cell's endogenous repair machinery to create insertions, deletions, or when a homologous template is provided, to introduce precise changes to the targeted locus. There are now multiple nuclease platforms

TABLE 2 Active CIRM programs developing autologous, gene-modified HSCT for nononcological indications

Disease	Method, target	Institution or PI	Stage, trial number
Primary immune deficiencies			
ADA-SCID	LV, ADA	Orchard Therapeutics	Registration, NCT02999984
X-CGD	LV, CYBB	Kohn	Ph1/2, NCT02234934
X-SCID	LV, IL2RG	St. Jude's Hospital	Ph1/2, NCT01512888
Artemis SCID	LV, Artemis	Cowan	Ph1, NCT03538899
Leukocyte adhesion deficiency	LV, ITGB2	Rocket Pharmaceuticals	Ph1/2, NCT03812263
X-hyper IgM syndrome	CRISPR/Cas9, CD40L	Kuo	PreIND enabling
IPEX syndrome	CRISPR/Cas9, FOX3P	Bacchetta	Discovery research
Hemoglobinopathies			
Sickle cell disease	LV, modified HBB	Kohn	Ph1, NCT02247843
Sickle cell disease	CRISPR/Cas9, HBB	Porteus	IND enabling
Sickle cell disease	CRISPR/Cas9, HBB	Walters	IND enabling
Beta thalassemia	ZFN, BCL11A	Sangamo Biosciences	Ph1/2, NCT03432364
Inherited metabolic disorders			
Cystinosis	LV, CTNS	Cherqui	Ph1/2, NCT03897361
Danon disease	LV, LAMP2	Adler	Discovery research
Tay Sachs/Sandhoff disease	LV, HEXA/HEXB	Anderson	PreIND enabling
Acquired immune deficiencies			
HIV, lymphoma	LV, anti-HIV genes	Abedi	Ph1, NCT02797470
HIV	ZFN, CCR5	Zaia	Ph1, NCT02500849

Note: List of active CIRM-funded projects supporting the development of gene-modified, autologous hematopoietic stem cell based therapies, by disease target, modification approach, investigator or institution, and stage of research or development.

Abbreviations: ADA, adenosine deaminase; CGD, chronic granulomatous disease; CRISPR/Cas9, clustered regularly interspaced short palindromic repeat/CRISPR-associated protein 9; IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked; HIV, human immunodeficiency virus; IND, Investigational New Drug; LV, lentiviral vector; NCT, National Clinical Trial Indicator; Ph, phase; SCID, severe combined immunodeficiency; ZFN, zinc finger nuclease.

demonstrated to edit HSPCs with high efficiency including zinc finger nucleases (ZFNs), TAL effector nucleases (TALENs), mega-TALs, and the CRISPR/Cas9 system (clustered regularly interspaced short palindromic repeat/CRISPR-associated protein), reviewed by Dever and Porteus.[29] The most clinically advanced are the ZFNs, which have been shown to be safe and effective in inactivating the *CCR5* locus in primary T-cells from patients with HIV infection.[30] Several new clinical trials using ZFNs to inactivate genetic elements in HSPCs are ongoing, including *CCR5* gene disruption as treatment for HIV infection, and disabling the erythroid specific enhancer in the *BCL11A* gene to derepress fetal globin expression as treatment for sickle cell disease and β-thalassemia. CIRM supports these and other IND enabling and earlier stage studies pursuing CRISPR/Cas9 and ZFN-based HSPC editing approaches for a variety of other diseases (Table 2).

Although methods to generate gene-modified HSCs for auto-HSCT are progressing at a rapid pace, the regulatory path for the use of genome editing is still in its infancy. Through support of these programs, CIRM is afforded the opportunity to work closely with the FDA to develop a standardized but evidence-based set of quantitative preclinical studies for these projects, thus minimizing the potential for serious adverse events while providing quantitative data for outcome assessment.

3 | MEDICAL AND PROCEDURAL RISKS OF HSCT

One major hurdle common to both allo- and auto-HSCT relates to the risk of the medical procedure itself, which necessitates clearing a patient's bone marrow niche to provide sufficient space for engraftment of the therapeutic cells. For allo-HSCT, a standard myeloablative regimen involves "conditioning" with high doses of chemotherapy and/or irradiation to eliminate the recipient's hematopoietic and immune systems, followed by infusion of donor HSCs, and prophylactic administration of immunosuppressive drugs to prevent the donor immune cells from attacking the host tissues (GVHD). In some cases, non-myeloablative regimens have been developed to reduce associated toxicities, although immunosuppressive drugs may still be required to prevent GVHD and/or the rejection of donor cells due to mixed chimerism. For auto-HSCT, where the HSC to be transplanted are self-matched, there is no need to eliminate the recipient's immune system to prevent rejection. However, there is still some form of conditioning required to eliminate sufficient numbers of endogenous, abnormal HSC from the bone marrow to allow engraftment. This is especially important in the context of gene-modified auto-HSCT, where the cells to be transplanted carry a corrected gene or therapeutic transgene and

depending on the condition to be treated, must meet a certain threshold of engraftment in order to confer efficacy over the diseased cell background. Moreover, although there is no risk of GVHD for auto-HSCT, prophylactic immunosuppression may still be necessary to address possible immune responses to the "normal" or modified transgene product, or to residual editing reagents, such as Cas9, especially when busulfan is used for conditioning, which is myeloablative but not immunosuppressive.[31] The potential immunogenicity of gene modified cells in the context of autologous transplant was recently investigated by Uchida et al in nonhuman primates[32] and will remain an important consideration in the development of such approaches.

3.1 | Developing nontoxic conditioning regimens

A critical need for the future expanded use of auto-HSCT is the development of conditioning methods that can open HSC niche space while minimizing toxicities. One of the most promising approaches toward this end involves the use of antibodies against the HSC receptor c-Kit (CD117), to disrupt binding to its ligand stem cell factor (SCF), resulting in HSC depletion in vivo.[33] Anti-human CD117 monoclonal antibody is currently being tested in the clinic with direct support from CIRM. In another approach, Patchaudhuri et al have developed an antibody-drug conjugate where a single dose of the immunotoxin, CD45-saporin, enables efficient engraftment of donor cells in a mouse model.[34] A second interesting strategy to open HSC niche, while much earlier in development, stems from observations that proliferation of both human and murine HSCs is dependent on the amino acid valine, and that mice fed a valine-deficient diet can receive HSCT in the absence of myeloablation.[35] These findings suggest it may one day be possible to design safer conditioning regimens through selective manipulation of HSC metabolism. Although there is much excitement around antibody-based and other nonmyeloablative conditioning regimens, there still remains a need for additional research to reduce the toxicities associated with HSCT.

3.2 | Immune reconstitution

Another limitation of HSCT is increased risks of bacterial, viral, and fungal infections due to delayed recovery of the adaptive immune system, particularly T, B, and NK lymphocytes. A variety of methods to augment post-HSCT immune recovery are under active investigation, including cytokine administration, "add backs" of various lymphocyte populations, and co-transplantation with ex vivo generated T-lymphoid progenitors.[36-38] Other strategies seek to accelerate the pace of hematologic recovery after transplant by altering the proportion of HSCs or other cell types in a graft,[37,39] or by administering factors that promote HSC self-renewal and/or prevent apoptosis of HSCs after chemotherapy or radiation.[40,41] A recent CIRM-funded study exemplifies this latter approach, describing a class of tyrosine phosphatase-sigma (PTPσ) that when administered systemically, accelerates hematological recovery and improves survival in irradiated or 5-fluorouracil-treated mice.[41]

3.3 | Tissue mismatch

A central reality of allo-HSCT is that the more disparate the recipient and donor are for both major (HLA) and minor histocompatibility antigens, the greater the likelihood of developing GVHD and other complications. Fewer than 20% of patients have a histocompatible family donor, and although some can rely on cells from matched, unrelated donors, there are many more for whom this is not feasible. One strategy to address this has been to develop approaches for enabling transplants with partial mismatch, such as haplo-identical HSCT (eg, parent to child)[42]; creation of homozygous HLA donor cells banks[43]; "graft engineering," which involves ex vivo removal of specific immune cell subsets that contribute to GVHD,[39] and/or the use of post-transplant cyclophosphamide to eliminate T lymphocytes that proliferate in response to recipient histocompatibility antigens.[44] Other approaches are under development to induce full operational tolerance to grafts, which if successfully achieved, could enable fully mismatched transplants as well as partially matched ones. Recent clinical experiments using allo-HSCT in conjunction with renal transplantation have demonstrated feasibility in some patients to achieve transplantation tolerance between donors and recipients with the removal of all post-HSCT immunosuppressive drugs.[45] Other strategies to induce tolerance were the subject of a CIRM Workshop and Perspective in 2015, and are discussed further there.[46] A third general approach to address complications of tissue mismatch involves development of targeted therapies for the treatment of chronic GVHD including the in vivo expansion of endogenous regulatory T lymphocytes (Treg) with low-dose interleukin-2 or ex vivo expansion of Treg subsets followed by their in vivo administration.[47]

3.4 | Immune evasion

Other major strategies to circumvent the adaptive immune system are in much earlier stages of development but could represent game changers for the field. One approach includes the use of genetic engineering to create "universal donor" cells, which would, in theory, be tolerated by the recipient immune system, or alternatively to make transplanted cells "invisible" to the host. Such strategies include ablating or engineering HLA genes and/or expressing immunosuppressive genes.[48] Another novel strategy, developed with CIRM support, represents an evolution of the antibody-based approaches to clearing niche space that were described above. In this case, a pretreatment comprising six monoclonal antibodies that target and suppress HSCs, CD47, T cells, and NK cells was administered prior to allogenic HSCT in mice.[49] Remarkably, this procedure enabled murine recipients to engraft donor cells with mismatches at half (haploid identical) or all major histocompatibility complex (MHC) genes without conditioning or radiation. Moreover, the transplanted hosts were subsequently able to accept an organ transplant form that same mismatched donor without rejection. If these findings can be replicated with human cells, it is possible that the donor eligibility for allo-HSCT can be greatly expanded, as well as the potential use of allo-HSCT to induce tolerance for other types of organ replacement.

4 | SUMMARY

The utility and power of HSCT, both allogeneic and autologous, is well established but there remains substantial room for improvement. The challenges include: (a) developing methods to efficiently and safely derive and/or expand HSCs from immune-compatible donor sources; (b) developing safe, effective, and scalable methods of genetically modifying the stem cells to treat both genetic and nongenetic diseases; (c) developing nontoxic methods of facilitating engraftment of HSCs without reliance on genotoxic conditioning agents; and (d) developing methods to rapidly reconstitute the recipient immune system while minimizing or ideally abrogating the risk of developing GVHD. In all of these areas, sustained support with a long-term commitment to finding solutions from a variety of funding sources, including government agencies, private foundations, and philanthropy, in combination with productive partnerships with small biotechnology and large pharmaceutical companies, will be essential. In this way, HSCT can continue to be a leading paradigm for stem cell based therapies in the future.

CONFLICT OF INTEREST
The authors indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS
S.T., K.A.S.: conception and design, manuscript writing, and final approval of manuscript.

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