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# Gene-modified hematopoietic stem cells for cancer immunotherapy

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**The rapid expansion of available cancer immunotherapies has resulted in favorable early outcomes. Specifically the use of gene therapy to introduce chimeric antigen receptors (CARs) and T cell receptors (TCRs) in T cells creates new immunotherapy options for patients. While showing early success with these approaches, limitations remain that can be overcome by the use of modification of hematopoietic stem cells (HSCs) to express CARs and TCRs. With modern gene therapy technologies, increased safety and control of the modification of the HSCs can be achieved through the use of a suicide gene.**

**Keywords:** cancer immunotherapy, chimeric antigen receptor, HSC, T cell, gene therapy, suicide gene, HSV-TK

**Abbreviations:** CAR, chimeric antigen receptor; TCR, T cell receptor; HSC, hematopoietic stem cell; ADA-SCID, adenosine deaminase deficient severe combined immunodeficiency; HCT, hematopoietic stem cell transplantation; HLA, human leukocyte antigen; MHC, major histocompatibility complex; CD, cluster of differentiation; NSG, NOD scid gamma mouse strain; LTR, long terminal repeat; DLI, donor lymphocyte infusion; GVHD, graft-versus-host disease; GCV, ganciclovir; PET, positron emission tomography; HSVTK, herpes simplex virus thymidine kinase

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## Introduction

The first gene therapy trial in humans aimed to correct adenosine deaminase-deficient severe combined immunodeficiency (ADA-SCID).[1] Since that time this approach has been expanded and numerous clinical trials for varying diseases have been performed utilizing gene therapy. Although the initial development sought to correct gene deficiencies, the use of gene transfer for cancer immunotherapy has gained considerable excitement and attention given the recent success of TCRs and CARs in T cells for the treatment of malignancy.[2-4] The gene-modified T cell approach has produced clear successes; however, it continues to have limitations, primarily related to the length of persistence of effector cells, need for massive ex vivo expansion and non-sustained function of the modified T cells. Modification of HSCs can overcome these limitations by allowing a renewing source of immunotherapy that does not require ex vivo expansion or extensive manipulation.

Hematopoietic stem cell transplantation (HCT) is incorporated into therapy of both hematologic malignancies and solid tumors. **Table 1** outlines the diagnoses currently treated with HCT. The collection of HSCs for transplantation allows modification of these cells providing an ideal platform for the use of immunotherapy. The ability to transduce HSCs for gene therapy in the context of autologous stem cell transplant has clearly been demonstrated in the genetic diseases.[5-7] Successfully engrafted gene-modified HSCs will continuously generate new effector cells for cancer immunotherapy, presenting a solution for the limitations of effector cell persistence and functional exhaustion. While overcoming these limitations as compared with the T cell approach, the need for further control arises with transduction of HSCs due to the potential for insertional oncogenesis.[6-9] Here we will comment on the use of TCRs and CARs in HSCs, as well as the feasible control by a suicide gene.

## T Cell Receptors in HSCs

The clinical success of lymphocyte expressing exogenous TCR directed against NY-ESO[10] led to the evaluation of TCRs in HSCs. A series of publications have demonstrated the preclinical models of NY-ESO and MART-1,[11-13] and these

**Table 1.** Malignancies with indication for HCT, listed by type of transplant and clinical setting

| Disease | Type of transplant | Clinical setting |
|---|---|---|
| Acute leukemias | Allogeneic | High risk disease, R/R |
| Chronic leukemias | Allogeneic | R/R, TKI resistant |
| Myelodysplastic syndromes | Allogeneic | High risk disease, R/R |
| Non-Hodgkin lymphoma | Primarily autologous | R/R |
| Hodgkin lymphoma | Primarily autologous | R/R |
| Multiple myeloma | Autologous | Early vs. late debated |
| Germ cell tumors | Autologous | R/R |
| Central nervous system tumors | Autologous | R/R |
| Sarcoma | Autologous | Rarely used |

R/R, refractory or recurrent disease; TKI, tyrosine kinase inhibitor.

**Table 2.** Theoretical benefits of CAR-modification of HSCs as compared with T cells

| |
|---|
| Persistent production of CAR-enabled immune cells |
| Multilineage immune cells armed with CAR |
| In vivo proliferation of tumor-specific T-cells leading to establishment of immunological memory |
| Intervention using a standard medical procedure |
| Decreased side-effects |
| Harnessing the immune system to fight cancer |
| Enables the use of different targets for same disease |
| Enables targeting of any type of cancer |

targets are both being evaluated in ongoing clinical trials. In the setting of modification of lymphocytes to express exogenous TCRs there is the concern of mispairing of the patient's endogenous TCR with an engineered TCR, creating targeting of antigens other than the one for which the TCR is designed and potentially autoimmunity.[14] This potential toxicity is eliminated by the use of TCRs expressed through modification of HSCs, as allelic exclusion favoring the exogenous TCR has been demonstrated.[15] Furthermore, in vivo T cell proliferation and expansion may lead to establishment of immunological memory.

The use of engineered TCRs expands the potential antigens of interest as it does not have to be expressed on the cell surface. However, TCRs are HLA-dependent and well-described mechanisms of tumor immune system evasion include decreased antigen processing, downregulation of MHC and immunomodulatory pathways resulting in decreased effector T-cell function.

## Chimeric Antigen Receptors into HSCs

Compared with TCRs, modification of HSCs with CAR brings the added advantage of a multi-lineage immunotherapy that is not HLA restricted. The vast majority of experience lies in the expression of CAR-modified T cells, but the efficacy of CAR expression on NK and myeloid cells has been demonstrated.[16-19] Following HCT, the first cells to recover consist of myeloid cells followed by NK cells, which would result in an earlier anti-cancer therapy until de novo thymopoiesis. The theoretical benefits of modification of HSCs rather than T cells with CAR are described in **Table 2**.

Early studies with first-generation CARs showed minimal success. The necessity of co-stimulation for effective T cell response is well described and likely explains the limited efficacy of the early attempts at CAR therapy using first-generation constructs containing the CD3ζ signaling domain alone.[20,21] The constructs currently in use are mostly second-generation constructs that incorporate the CD28 or 4-1BB co-stimulatory domains, with each bringing a potential advantage. Third-generation CARs containing two of the domains (CD28, 4-1BB, OX-40) have also been developed, but there is concern that the increased number of co-stimulatory domains may escalate toxicity. The most studied CD28 and 4-1BB domains have yielded successful early results in clinical trials, with persistence of gene-modified cells being a relevant issue.[4,22,23]

We have demonstrated that first and second-generation anti-CD19 CARs can effectively transduce HSCs and lead to CAR expression on myeloid, T cells, and NK cells. In our work, the transduction efficiency of human umbilical cord blood CD34+ cells is consistently between 40–50%.[24,25] The gene-modified HSCs were then transplanted into irradiated NSG mice, which were subsequently harvested to evaluate for the presence and function of CAR. The CAR expressing cells were detected in the bone marrow, spleen, and peripheral blood, and the presence of CAR did not affect the ability of the cells to differentiate or to maintain effective cytotoxicity. Further, the presence of the CAR induced B cell aplasia serving as a surrogate of CAR activity. Engrafted mice presented significant protection against CD19-positive tumors, with inhibition or elimination of tumor development and consequent survival advantage.[24]

**Table 3.** Examples of suicide gene approaches listed by clinical experience, strengths, and limitations

| | Prodrug | Clinical experience | Strength | Limitation |
|---|---|---|---|---|
| **HSV-TK** | Ganciclovir | Extensive | Experience, PET imaging | Potential immunogenicity, prodrug, time to activity |
| **iCaspase 9** | AP1903 | Phase I, DLI | Rapid onset, no immunogenicity | Lack of experience |
| **CD20** | Rituximab | Limited | Rapid onset | Monoclonal antibody biodistribution, prodrug infusion reaction |
| **EGFRt** | Cetuximab | Limited | In vivo tracking Rapid onset | Monoclonal antibody biodistribution, prodrug infusion reaction |

## Suicide Gene Therapy

Addition of a suicide gene, a gene activated upon administration of a prodrug, to the modification of HSCs for gene therapy allows increased safety as concern for insertional oncogenesis exists. In the trials using LTR regulated gamma retrovirus to correct X-linked SCID, four patients developed acute leukemia.[6] Further, patients treated with CAR T cells have shown increased inflammatory responses that resemble sepsis, which has been termed cytokine release syndrome.[2-4,22,23] The nonrandom vector integration patterns and immune stimulation potential highlight the need for control of the gene-modified cells, which can be achieved through the addition of a suicide gene to the CAR vector. The currently used suicide genes include HSV-TK, icaspase 9, CD20, and a truncated epidermal growth factor receptor (EGFRt).[26-29] **Table 3** provides an overview of some of the currently available suicide genes.

The most extensive experience exists with the HSV-TK gene in order to eliminate graft-vs.-host disease (GVHD) in the setting of donor lymphocyte infusion (DLI). In the 1990s Bonini et al.[26] treated 8 patients with relapsed disease or Epstein Barr Virus post-transplant lymphoproliferative disorder with DLI using cells modified to express HSV-TK. In the three patients that developed GVHD the prodrug ganciclovir (GCV) was administered and the transduced cells were no longer detected. Since then, over 100 patients have been treated with cells modified with the HSV-TK.[30,31]

Despite the extensive experience with HSV-TK, the efficacy in HSC was unclear until recently due to the quiescent nature of the cells. In a rhesus macaque autologous stem cell transplant model Barese et al.[32] demonstrated the ability of the hyperactive sr39tk HSV-TK mutant to effectively remove HSCs transduced with a gamma retrovirus vector and transplanted into rhesus macaques. Following treatment with GCV, no transduced cells were detected up to 18 months later.[32] This provided the proof of principle for HSV-TK as an effective suicide gene in HSCs, which was previously lacking.

The inducible caspase 9 (icasp9) suicide gene is gaining interest due to the rapid onset of action within one hour, and inert, non-toxic, chemical inducer of dimerization (CID) prodrug AP1903. This gene is created by fusion of the human caspase 9 gene to a modified human FK binding protein. Dimerization leads to initiation of the apoptosis pathway. Di Stasi et al.[33] demonstrated the efficacy of this suicide gene in the setting of DLI for patients receiving a haploidentical HCT. The patients received T cells transduced with the icasp9. In the four patients that developed GVHD, administration of the AP1903 resulted in rapid eradication of the T cells and in resolution of the skin and liver GVHD symptoms within 24–48 h.[33]

Other possible suicide system approaches are cell surface inert molecular markers encoded by genes delivered by the same gene therapy vector, for targeting with monoclonal antibodies, such as the expression of a truncated CD20 molecule and targeting with rituximab,[28] or truncated epidermal growth factor receptor and targeting with cetuximab.[29]

## Conclusions and Perspectives

The incorporation of gene transfer into cancer immunotherapy is creating promising new therapies, primarily driven by the results seen with TCRs and CARs modifying mature T cells. Modification of HSCs with these immunotherapies provides a sustained anti-cancer therapy that can be controlled with the use of a suicide gene. The TCR-based therapies provide a wide range of potential targets, but are limited by the HLA restriction. CARs in particular have gained recent attention to the excellent success of the anti-CD19 CAR in the early phase I trials. The optimal design of the CAR including the co-stimulatory domain and the epitope recognition is not yet know and is part of ongoing trials. Modification of HSCs with either CAR or TCR could provide the solution to the limitations of T cell modification approaches, including persistence, sustained anti-cancer effector function and generation of immunological memory.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.


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