The Application of ‘Drug-Reversible’ CAR-T Cells Directed Against Recipient Hematopoietic Cells as a Selective Conditioning Strategy for Stem Cell Transplantation

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ABSTRACT

The transplant of autologous or allogeneic hematopoietic stem cells (HSCs) has the proven ability to treat a wide array of malignant and non-malignant hematological diseases. The preparative regimen, however, routinely entails aggressive and genotoxic treatment with total body irradiation and/or chemotherapy, which brings severe and even life-threatening complications that limit its clinical application. Previous studies have established that depletion of recipient HSCs is an essential requirement of these conditioning regimens in allowing successful engraftment of donor hematopoietic cells. However, these regimens, especially when performed in immunocompetent patients, have also indicated that allogeneic anti-HSC donor T cells additionally facilitate stem cell engraftment, but this is often accompanied by the risks of graft versus host disease. This has prompted the consideration of alternative conditioning methods for the depletion of HSCs with less toxic side-effects, such as anti-c-kit and anti-CD133 antibody-directed treatments. In this way, more precise HSC targeting may also be achieved by the application of short-lived, genetically engineered chimeric antigen receptor (CAR) T cells for stem cell transplantation conditioning.

We developed a novel and controllable CAR-T approach for recipient HSC targeting via genetic modification using the non-viral piggyBac™ (PB) transposon system. As opposed to viral vector delivery systems, the relatively large carrying capacity of PB allows the stable introduction of at least three separate genes encoded within the same tri-cistronic transgene cassette. This includes a second-generation CAR that targets either human c-kit (CD117) or prominin-1 (CD133), markers known to be antigenically expressed on the surface of HSCs. In addition, a drug resistance element serves as a selection gene that, in combination with a non-genotoxic drug, provides an effective method of CAR-T cell purification during manufacture. Importantly, a small molecule drug-inducible safety switch gene is also included to facilitate rapid in vivo clearance of the CAR-T cells after depletion of recipient HSCs and prior to donor HSC transplant. Lastly, as a result of the manufacturing process, the majority of the CAR-T cells express chemokine receptors such as CXCR4 that can make all selective trafficking to the bone marrow (BM) for radicication of residual HSCs.

To select a lead candidate from a panel of anti-HSC CAR constructs, CD3/CD19-stimulated T cells from human peripheral blood were first electroporated with mRNA encoding each of the CAR candidates directed against either c-kit or CD133. CAR surface expression was confirmed in transfected T cells by flow cytometry. In vitro functional assays were performed by co-culturing mRNA-transfected CAR-T cells with mouse or human cell lines (EL4-C1, TF-1 and K562), expressing either c-kit or CD133, as well as mouse and human primary BM cells. Lead CAR candidates were identified from their specific activation of the CAR-T cells through degranulation, measured by CD107α expression and secretion of IFNγ. Furthermore, these CAR candidates were also capable of selectively depleting c-kit or CD133 positive cells. Importantly, mRNA-transfected CAR-T cells retained effector activity against target c-kit or TF-1 cells even in the presence of their soluble ligand, stem cell factor. These lead CAR candidates were then tested in an in vivo setting with the selection and drug inducible safety switch genes in the same tri-cistronic transgene and were then compared to T cells using PB. The manufacturing and genetic stability of CAR-T cells, that were mainly of the T memory stem cell (Tscm) phenotype, as determined by positive expression of CXCR4 and CCR7, was examined. These CAR-T cells demonstrated the stem cell memory phenotype of PB CAR-T cells; (B) PB CAR-T cells express CXCR4, a marker commonly associated with bone marrow homing.

Future studies will evaluate PB-produced lead anti-HSC CAR-T cells in immune-deficient NSG mice with pre-established human hematopoietic chimerism, engrafted with standard myeloablative busulphan or radiation conditioning controls. This approach constitutes a novel targeted biological therapy that is envisioned to lead the way towards minimally toxic, transplant expanding, and selective depletion of endogenous HSCs in the BM and to procure their replacement with genetically engineered or gene-corrected stem cells.

INTRODUCTION

Need for Alternative Conditioning Therapies prior to HSC Transplants

• More than 5,000 patients per year in the U.S. are treated with myeloablative conditioning regimens prior to HSC transplants. Most of these conditioning regimens consist of high doses of genotoxic radiation or busulphan. The use of stem cell transplants is limited by the major life-threatening complications associated with these regimens.

• Antibodies directed against antigens expressed on HSCs, such as c-kit and CD45, have been considered as alternatives.

• CAR-T cells may provide more effective, selective, and safer depletion of HSCs residing in the bone marrow.

• PiggyBac™ (PB) is a non-viral delivery system with a large cargo capacity that allows introduction of multiple genes, including a selection marker and a safety switch that can clear CAR-T cells prior to donor HSC transplant.

• PB-produced CAR-T cells exhibit a stem cell memory (SCM) phenotype for enhanced in vivo homing that may better home to bone marrow than other HSC-directed pre-conditioning alternatives.

METHODS & RESULTS

PiggyBac anti-c-kit & CD133 CAR Vector Design and CAR-T Production

Phenotype of PB CAR-T Cells

% Survival CD34+CD38-CD133+ cells

Depletion of Hematopoietic Progenitors from Human Mobilized Peripheral Blood CD34+ Cells

Depletion of More Primitive Hematopoietic Cells in Long-Term Stromal Cultures by Both anti-c-kit and CD133 CAR-Ts

Depletion of Hematopoietic Progenitors from Human and Non-Human Primate Bone Marrow

Migration of PB CAR-T Cells to the Bone Marrow of NSG Mice

CONCLUSIONS

• PB CAR-T cells targeted against c-kit or CD133 deplete hematopoietic progenitor cells from human and non-human primate bone marrow, and primitive CAR-T cells from human CD34+ cells.

• PB CAR-T cells exhibit a stem cell memory phenotype and naturally express CXCR4, although expression can be increased by 24h culture with added factors.

• PB CAR-T cells successfully home to bone marrow within 16 hours after injection.

• These data support the use of PB CAR-T cells to target endogenous HSCs in the bone marrow as a minimal, non-genotoxic HSC transplant regimen.