Cytotherapy, 2018; ■■: ■■–■■

International Society for Cellular Therapy ISCT®

The simultaneous isolation of multiple high and low frequent T-cell populations from donor peripheral blood mononuclear cells using the major histocompatibility complex I-*Strep*tamer isolation technology

MARTHE C.J. ROEX[1], LOIS HAGEMAN[1], MATTHIAS T. HEEMSKERK[1], SABRINA A.J. VELD[1], ELLIS VAN LIEMPT[1], MICHEL G.D. KESTER[1], LOTHAR GERMEROTH[2], CHRISTIAN STEMBERGER[2], J.H. FREDERIK FALKENBURG[1] & INGE JEDEMA[1]

[1]*Department of Hematology, Leiden University Medical Center, Leiden, The Netherlands, and* [2]*June Therapeutics, Goettingen, Germany*

Abstract

Background. Adoptive transfer of donor-derived T cells can be applied to improve immune reconstitution in immune-compromised patients after allogeneic stem cell transplantation. The separation of beneficial T cells from potentially harmful T cells can be achieved by using the major histocompatibility complex (MHC) I-*Strep*tamer isolation technology, which has proven its feasibility for the fast and pure isolation of T-cell populations with a single specificity. We have analyzed the feasibility of the simultaneous isolation of multiple antigen-specific T-cell populations in one procedure by combining different MHC I-*Strep*tamers. *Methods.* First, the effect of combining different amounts of MHC I-*Strep*tamers used in the isolation procedure on the isolation efficacy of target antigen-specific T cells and on the number of off-target co-isolated contaminating cells was assessed. The feasibility of this approach was demonstrated in large-scale validation procedures targeting both high and low frequent T-cell populations using the Good Manufacturing Practice (GMP)-compliant CliniMACS Plus device. *Results.* T-cell products targeting up to 24 different T-cell populations could be isolated in one, simultaneous MHC I-*Strep*tamer procedure, by adjusting the amount of MHC I- *Strep*tamers per target antigen-specific T-cell population. Concurrently, the co-isolation of potentially harmful contaminating T cells remained below our safety limit. This technology allows the reproducible isolation of high and low frequent T-cell populations. However, the expected therapeutic relevance of direct clinical application without *in vitro* expansion of these low frequent T-cell populations is questionable. *Discussion.* This study provides a feasible, fast and safe method for the generation of highly personalized MHC I-*Strep*tamer isolated T-cell products for adoptive immunotherapy.

Key Words: *allogeneic stem cell transplantation, CD8+ T lymphocytes, cellular immunotherapy, good manufacturing practice, major histocompatibility complex I-*Strep*tamer technology, tumor-associated antigens, viral reactivations*

Introduction

Immune-compromised patients after allogeneic hematopoietic stem cell transplantation (alloSCT) are vulnerable to viral infections and disease relapses. Although donor T cells can mediate graft versus leukemia (GVL) responses and restore pathogen-specific immunity, the administration of T cells with the graft or unmodified donor lymphocyte infusions (DLIs) early after a T-cell–depleted graft is also associated with a significant risk of graft-versus-host disease (GVHD) [1–3]. Therefore, the adoptive transfer of selected T-cell populations with solely beneficial effects is highly desirable, especially in the period between T-cell–depleted alloSCT and DLI. This requires a widely applicable, fast and Good Manufacturing Practice (GMP)-compliant technique to isolate well-defined T-cell populations from donor peripheral blood mononuclear cells (PBMCs) and avoid the co-isolation of alloreactive T cells [4–7].

The major histocompatibility complex class I (MHC I-)*Strep*tamer technology is developed for the detection and isolation of human antigen-specific T cells from PBMCs. This technique is based on the direct labeling of CD8+ T cells with MHC I-*Strep*tamers, which are composed of peptide-loaded MHC I-*Strep*-tag fusion proteins (MHC I-*Strep* proteins) reversibly multimerized on magnetically labeled

Correspondence: **Marthe C.J. Roex**, MD, Department of Hematology, Leiden University Medical Center, C2-R, P.O. Box 9600, 2300 RC Leiden, The Netherlands. E-mail: M.C.J.Roex@lumc.nl

(Received 31 August 2017; accepted 16 January 2018)

ISSN 1465-3249
https://doi.org/10.1016/j.jcyt.2018.01.008

Strep-Tactin (*Strep*-Tactin nanobeads). After the magnetic separation, the MHC I-*Strep*tamers can be dissociated from the positively selected cells by the addition of D-Biotin, a high affinity competitor for the binding sites on *Strep*-Tactin. This isolation technique allows the purification of noncoated, unlabeled antigen-specific T cells under GMP conditions for clinical application in only 1 day [8–12].

The feasibility of the MHC I-*Strep*tamer approach for the isolation of cytomegalovirus (CMV) or Epstein-Barr virus (EBV) antigen-specific T cells from seropositive donors was demonstrated in various (pre-)clinical studies [10,13–16]. The achieved high purities of virus-specific T cells in the products and the acceptable numbers of contaminating cells resulted in T-cell products feasible for direct clinical application or further *in vitro* manipulation. The safety and efficacy of the clinical application of donor-derived CMV specific T-cell products in patients with persistent CMV viremia after alloSCT demonstrated no reports of initiation or aggravation of acute GVHD. Furthermore, in all patients donor-derived CMV epitope-specific T cells became detectable *in vivo* after infusion of the product, suggesting expansion of infused cells. Moreover, the majority of patients experienced a partial or complete response [14,16].

The MHC I-*Strep*tamer technology has so far been used mainly for the isolation of relatively frequent virus-specific T cells from the memory T-cell compartment of seropositive donors. However, T cells with more rare precursor frequencies in donor PBMCs, like virus-specific T cells from seronegative donors, minor histocompatibility antigen (MiHA)-specific T cells or tumor-associated antigen (TAA)-specific T cells, are also relevant candidates for adoptive T-cell therapy [17–21]. Previous attempts to enrich such low frequent T cells, like human adenovirus (HAdV) or TAA-specific T cells, with the MHC I-*Strep*tamer technique resulted in less pure T-cell products [10,15]. An approach to solve this problem is an *in vitro* target antigen-specific expansion induced by stimulation with peptide pools for 12–14 days prior to isolation [22]. Although this resulted in T-cell products with relatively high purities of HAdV or TAA-specific T cells, this strategy is time-consuming, might impair *in vivo* T-cell function and will abrogate the advantage of the MHC I-*Strep*tamer technology to create a selected lymphocyte product with regulatory advantages in contrast to an Advanced Therapy Medicinal Product (ATMP) [15,23].

The T-cell products generated with the MHC I-*Strep*tamer technology that were clinically applied until now contained only a limited number of specificities. Although T cells directed against a single antigen can control viral reactivations, the inclusion of T cells with different target antigen specificities in one product may be preferred for clinical application [24,25].

Freimuller *et al*. compared the simultaneous isolation of low frequent HAdV-specific T cells and high frequent EBV-specific T cells versus the isolation of HAdV-specific T cells alone. Although the frequencies of HAdV-specific T cells in the product were still low after the combined isolation, the addition of EBV-specific MHC I-*Strep*tamers resulted in increased purity of the final T-cell product to levels acceptable for clinical application [15].

In this study, we aimed to develop a robust and widely applicable GMP-compliant method for the simultaneous isolation of purified T-cell products containing multiple antigen-specific T-cell populations from donor PBMCs using the MHC I-*Strep*tamer technology. Therefore, we assessed how many T-cell populations can be targeted in one isolation procedure. Besides the isolation of high frequent viral T-cell populations, we also studied the isolation of low frequent viral T-cell populations and TAA-specific T cells. Our data show that the MHC I-*Strep*tamer technology allows the combined isolation of multiple T-cell populations with a wide range of precursor frequencies in donor PBMCs, resulting in a pure and safe T-cell product for direct clinical application. However, the expected therapeutic relevance of direct clinical application without *in vitro* expansion of very low frequent T-cell populations is questionable.

Materials and methods

Leukapheresis products from healthy donors

Peripheral blood or leukapheresis products were obtained from stem cell donors after approval by the Leiden University Medical Center (LUMC) Institutional Board and written informed consent according to the Declaration of Helsinki. PBMCs were collected by the use of Ficoll-Isopaque separation or red blood cell lysis using an NH_4Cl (8.4 g/L) and $KHCO_3$(1 g/L) buffer (pH = 7.4) (LUMC Pharmacy). PBMCs were used directly (donors M–P) or thawed after cryopreservation in the vapor phase of liquid nitrogen (donors A–L). Donor characteristics (HLA typing, CMV and EBV serostatus and amount of cells used for experiments) are provided in Supplementary Table S1.

Generation of MHC I-Streptamers

MHC I-*Strep*tamers were generated by the incubation of peptide-loaded MHC I-*Strep*-tag fusion proteins (MHC I-*Strep* proteins) with magnetically labeled *Strep*-Tactin (*Strep*-Tactin nanobeads) in phosphate-buffered saline (PBS; Sigma-Aldrich) supplemented with 0.4% human serum albumin (HSA; Sanquin Reagents)