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T-control: T-cell therapy in the context of allogeneic stem cell transplantation

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CHAPTER 1

GENERAL INTRODUCTION
AND AIM OF THE THESIS

GENERAL INTRODUCTION

HEMATOPOIETIC STEM CELL TRANSPLANTATION

The ideal therapy to treat patients with hematologic malignancies is to eliminate malignant cells in the body of the patient, while healthy cells remain unaffected. Unfortunately, strategies that only specifically and efficiently attack hematopoietic malignant cells are not available thus far. The best available treatment regimens consist of remission induction therapy followed by consolidation therapy using chemotherapy, immunotherapy and irradiation, with severe side-damage to healthy hematopoietic cells. In patients with high-risk malignancies, hematopoietic stem cell transplantation can be performed as part of the consolidation therapy to rebuild a healthy hematopoietic system. After transplantation, stem cells migrate to the bone marrow and have the ability to proliferate and differentiate into mature healthy blood cells. The infusion of stem cells harvested from the patient before the consolidation therapy is called autologous stem cell transplantation (autoSCT). As an alternative, stem cells from a healthy donor can be used for an allogeneic stem cell transplantation (alloSCT).¹⁻³ Hematopoietic stem cells can be harvested directly from the bone marrow (BM) but are nowadays usually acquired via leukapheresis from the peripheral blood (PB) after mobilization from the bone marrow by administration of granulocyte colony stimulating factor (G-CSF).^{4,5} In alloSCT, both stem cells from a related or an unrelated donor can be used.

Traditionally, myeloablative (MA) conditioning regimens have been used in SCT to maximally eradicate malignant cells and allow engraftment of the stem cells into the bone marrow of the patient using high doses of chemotherapy, immunotherapy, total body irradiation (TBI) and immune suppression. Due to considerable toxicity, feasibility of this therapy was limited to fit and young patients. However, the conditioning regimen alone has shown to be not sufficient to prevent relapse of the hematologic malignancy. This is illustrated by the high relapse rates after autoSCT or genetically identical (using an identical twin) stem cell transplantations in patients with high-risk acute leukemia, in contrast to alloSCT.⁶ The long-term curative effect of alloSCT is mediated by donor-derived T cells that are able to recognize and persistently eradicate residual malignant cells of the patient. This beneficial phenomenon is known as the graft-versus-leukemia (GVL) effect.⁷⁻⁹ Since this GVL effect has shown to be responsible for the curative potential of alloSCT, less toxic reduced intensity or non-myeloablative (NMA) conditioning regimens have been developed to broaden the curative potential of alloSCT to patients of higher age and with co-morbidities.^{10,11} These conditioning regimens aim to allow engraftment of donor hematopoietic stem cells without fully eliminating the hematopoietic system of the patient, but have the disadvantage of higher relapse rates compared to MA conditioning.

Although donor-derived T-cell responses are able to initiate GVL after alloSCT, donor-derived T-cell

responses can also be directed against healthy cells in the tissues and organs of the patient which can result in detrimental graft-versus-host disease (GVHD). The major challenge in the field of alloSCT is to find a balance between the prevention of GVHD while maintaining GVL and immunity for the protection against foreign pathogens like viruses. As these harmful and beneficial immune responses after alloSCT are mediated by T cells, knowledge about the biology of T cells is necessary to understand and further develop strategies to improve the curative effect of alloSCT.

BIOLOGY OF T CELLS

Within the hematopoietic system, two types of T cells can be distinguished: T-cell receptor (TCR) gamma-delta (γ/δ) T cells that are part of the innate immune system and TCR alpha/beta (α/β) T cells that play a major role in adaptive immune responses to control viral infections. In this thesis, we focus on α/β T-cell responses.

TCR-HLA interaction

To provoke an adaptive immune response, TCR α/β T cells need to be stimulated via their TCR that recognizes peptides (antigens) in the context of human leukocyte antigen (HLA) molecules, encoded by the major histocompatibility complex (MHC) on antigen-presenting cells. The TCR of an individual T cell is specific for a particular peptide-HLA combination. The strength by which a TCR interacts with a peptide-HLA complex is termed TCR-peptide-HLA affinity. This TCR-peptide-HLA affinity in combination with additional interactions between the T cell and antigen-presenting cells via adhesion and costimulatory molecules determines the strength by which a T cell binds to a target cell, called T-cell avidity. All nucleated cells express HLA class I molecules (HLA-A, -B, -C), which present peptides derived from intracellular proteins on their cell surface to CD8^{pos} T cells. HLA class II molecules (HLA-DP, -DQ, -DR) are under physiological conditions mainly present on cells of the hematopoietic system, which can process and present peptides derived from both intra- and extracellular proteins to CD4^{pos} T cells.^{12,13}

Thymic selection of T cells

During T-cell development, TCR are generated by a complex process including recombination of variable, joining and constant gene segments followed by random insertion and deletion of nucleotides, as well as the pairing of different α - and β -TCR chains.¹⁴ Therefore, the diversity of randomly generated TCR is enormous. Thymocytes (T-cell precursors) derived from the bone marrow are educated in the thymus to ensure a peripheral T-cell repertoire consisting of mature T cells containing TCR that are able recognize pathogens from outside the body, like viruses, but do not elicit harmful immune responses against cells of the own body.^{15,16} In the thymic cortex, thymocytes expressing an α/β TCR that are able to recognize peptides presented in the context of self-HLA with at least low affinity are positively selected, while thymocytes that do not interact with peptides in self-HLA at all are eliminated. After CD4^{pos} or CD8^{pos} lineage commitment, these

T-cell precursors undergo negative selection in the thymic medulla. Hereby, thymocytes that recognize self-peptides in the context of HLA with high affinity are eliminated in order to prevent self-directed immune responses which may cause auto-immune diseases.¹⁷⁻¹⁹ Eventually, the T-cell repertoire in the peripheral blood of an individual is expected to consist of a huge variety of T cells that are capable of recognizing all kinds of peptide-HLA complexes different from the combination of self-peptides presented in self-HLA.^{20,21}

T-cell subsets

Several subtypes of mature CD4^{pos} and CD8^{pos} T cells can be distinguished based on their phenotype and their functional characteristics.²² T cells that have not yet encountered their particular antigen are called naïve T cells. To provoke an immune response, naïve T cells need stimulation via professional antigen-presenting cells, that besides high levels of peptide-HLA complexes also express co-stimulatory molecules and adhesion molecules. After this initial stimulation, naïve T cells rapidly expand and differentiate into effector cells that are able to migrate to infected tissue and carry out specialized T-cell functions like cytokine production and cytotoxic activity. After elimination of infected cells, most proliferated T cells die, while a minority of T cells differentiate into resting memory T cells. Whereas the naïve T-cell repertoire directed against a certain antigen contains a broad range of avidities, the memory T-cell repertoire specific for the same antigen primarily exists of high-avidity T cells. Upon a second encounter with the same antigen, these memory T cells can become easily activated, resulting in rapid and effective expansion, differentiation and elimination of infected cells.²³ Besides the mentioned T-cell subsets that stimulate immune responses, a special type of CD4^{pos} T cells suppresses and downregulates induction and proliferation of effector T cells. These regulatory T cells are thought to modulate immune responses, maintain tolerance to self-antigens and prevent auto-immunity.²⁴

ALLOREACTIVE T-CELL RESPONSES: GVL AND GVHD

Allogeneic stem cell grafts contain 1-5% donor-derived hematopoietic stem cells, implying that other immune cells like lymphocytes of donor-origin are part of the stem cell graft and are transferred into the patient. In general, PB-derived grafts contain 5-10 times more stem cells compared to BM-derived grafts, which favors stem cell engraftment in the patient but also results in higher T-cell counts in the graft.^{4,25} Because donor T cells in the graft are educated in the thymus of the donor, these T cells are only tolerant to self-antigens of donor origin. After alloSCT, alloreactive T-cell responses occur when donor-derived T cells that are educated in the donor, recognizing patient cells as foreign. Dependent on the tissue distribution of the recognized antigen, the immune response will result in GVL or GVHD. GVL is initiated if only hematopoietic cells (containing the malignant cells) of patient origin are recognized by donor-derived T cells, while GVHD is induced if healthy tissue cells of the patient are attacked by donor-derived T cells. The risk of GVHD is increased after HLA-mismatched transplantation, because donor-derived T

cells transferred with the graft might recognize allogeneic HLA molecules on healthy tissue cells of the patient as foreign. Therefore, HLA-matched alloSCT is preferred over HLA-mismatched alloSCT. To allow the separation of GVL from GVHD after HLA-matched alloSCT, knowledge about antigens that have the potential to induce beneficial alloreactive T-cell responses is essential.

Minor histocompatibility antigens

Also after HLA-matched alloSCT, strong alloreactive immune responses can occur. Genetic differences between patient and donor that give rise to polymorphic peptides presented in matched HLA-molecules on patient cells that are recognized by donor T cells are called minor histocompatibility antigens (MiHA).^{26,27} These polymorphisms occur at the level of single or multiple base pairs, due to single nucleotide polymorphisms, base pair insertions or deletions, or copy number variations. Generally, the immune system of an individual has not been exposed to MiHA of another individual. Therefore, MiHA-specific T-cell responses after alloSCT have exclusively been described in MiHA^{pos} patients receiving grafts from MiHA^{neg} donors, because high-avidity MiHA-specific T cells are supposed to pass thymic selection only in MiHA^{neg} donors.²⁸⁻³¹ As a consequence, MiHA-specific T cells in MiHA^{neg} donors are expected to be present in the naïve T-cell repertoire. High-avidity donor T cells that are capable of recognizing immunogenic peptides on patient cells may lead to destruction of the cells expressing this MiHA, without impairment of donor-derived cells. Both the tissue distribution of the gene encoding the MiHA as well as the expression of HLA molecules determines the clinical effect of the alloreactive T-cell response. Since HLA class I molecules are normally expressed by all nucleated cells, donor T cells that recognize HLA class I-restricted MiHA derived from proteins exclusively expressed by (malignant) hematopoietic cells of the patient are likely to cause GVL, while donor T cells that recognize HLA class I-restricted MiHA that are broadly expressed on both hematopoietic and non-hematopoietic tissues of the patient can mediate both GVL and GVHD at the same time.^{26,31-33} In contrast, HLA class II-restricted MiHA can be considered as relatively hematopoiesis-specific, because HLA class II expression is under non-inflammatory conditions limited to mainly hematopoietic cells. After the initial stimulation, differentiation and expansion of MiHA-specific T-cells of donor origin, the immune response will decline when MiHA^{pos} patient cells are eradicated and a memory T-cell response may develop. This memory response is relevant for sustained and prolonged suppression of MiHA^{pos} cells. Several clinical observational studies have shown a direct association between emergence of T cells specific for hematopoiesis-restricted MiHA (eg, HA-1 and HA-2), and elimination of malignant cells post-alloSCT in the absence of extended GVHD.²⁹ Therefore, many effort has been made to identify hematopoiesis-restricted MiHA via several strategies.^{26,34-37} However, the tissue distribution of MiHa is not the only determinant that separates GVL from GVHD. Inflammatory environmental circumstances can render non-hematopoietic cells susceptible to T-cell recognition of broadly expressed MiHa. Because the magnitude and diversity of alloreactive T-cells responses in patients with selective GVL reactivity have shown to be lower than in patients with GVL

combined with GVHD, it is suggested that limited GVHD also benefits GVL.²⁸

Tumor-related antigens

Another group of antigens that have the potential to give rise to beneficial T-cell responses after alloSCT are tumor-related antigens. Tumor cells can express proteins that are essential or associated with their malignant phenotype. Within this group of tumor-related antigens, several categories can be distinguished, like tumor-associated virus antigens, tumor-specific antigens and tumor-associated antigens (TAA).³⁸⁻⁴² For example the Epstein-Barr virus (EBV) and human T-cell lymphotropic virus type 1 (HTLV-1) can be involved in the formation of hematologic malignancies, especially lymphoid neoplasms.⁴³ Since these antigens are non-self and are (over)expressed by tumor cells, donor-derived T cells can recognize these antigens as foreign. However, only a limited number of hematologic cancers is initiated by viruses and express these antigens. Tumor-specific antigens or neoantigens are antigens derived from mutated oncogenes or tumor suppressor genes, or chromosomal translocations. Since these mutations or translocations only occur in the tumor cells and not in healthy cells, these newly formed antigens are tumor-specific. Examples of mutations that give rise to mutant antigens in AML patients are internal tandem duplications of the FMS-like tyrosine kinase 3 gene (Flt3) and mutations in the nucleophosmin 1 (NPM1) gene.^{44,45} Nonetheless, the majority of tumor-related antigens belong to the group of TAA comprising non-mutated monomorphic self-antigens like differentiation antigens, aberrantly expressed antigens (eg, WT1, RHAMM, proteinase-3)⁴⁶⁻⁴⁹ and cancer-germline antigens (also known as cancer-testis antigens; eg, PRAME, NY-eso-1).⁵⁰⁻⁵² These antigens are expected to be overexpressed in malignant cells, while expression is absent or low in healthy cells. Several studies have suggested that T cells recognizing these self-antigens may contribute to antitumor reactivity after HLA-matched alloSCT.^{38,39,49,53-65} A relation has been proposed between expansion of TAA-specific T cells in the peripheral blood of patients and better relapse-free survival.⁶⁶ In addition, disease relapses have been observed in patients in the absence of TAA-specific T cells.⁶⁷⁻⁷² Furthermore, multiple phase I/II vaccination studies have targeted TAA in patients with hematologic malignancies.⁵⁶⁻⁶⁵ Although in a minority of patients clinical responses coincided with increased frequencies of TAA-specific T cells in peripheral blood, a causative relation between the induction of high-avidity TAA-specific T cells and clinical effect has not been proven so far.⁵⁶⁻⁶⁵ Due to these inconsistent results, the value of TAA as targets to boost GVL-responses needs to be exploited in more detail.

PREVENTION OF GVHD

Although donor-derived T-cells transferred with the graft can elicit alloreactive T-cell responses resulting in GVL, the administration of T cells together with stem cells of donor origin will simultaneously induce GVHD in the majority of patients. GVHD can present in an acute or chronic state, in several grades of severity, commonly affecting patient's skin, gut, liver and/or lungs.^{73-76,15,16} Since this dominant complication is associated with high morbidity and mortality,

several strategies have been studied to reduce the risk of GVHD.^{73,74,77,78}

HLA-matching

Since most tissue cells express HLA class I, mismatches for HLA class I-molecules between patient and donor often result in GVHD when donor T cells recognize healthy patient tissues, or graft rejection when residual patient T cells recognize donor stem cells. Therefore, it is preferred to match HLA-alleles between donor and patient. Only 25% of a patient's siblings are statistically HLA-identical, limiting the chance to find an HLA-matched related donor for a specific patient. Since the variation in HLA-polymorphisms is enormous, the chance of finding a matched unrelated donor in an international data bank is highly dependent on the patient's genetic background. For Caucasian patients, the chance of finding a 10/10 HLA-matched donor (only mismatched on HLA-DP allele(s)) is about 50-70%, but this chance will drop to 10-15% for patients with a non-Caucasian background.⁷⁹⁻⁸¹ Although the risk of (extensive) GVHD can be diminished by HLA-matching, HLA-matching alone is not sufficient to prevent GVHD.

Immune suppressive medication

Many transplant centers do not manipulate the composition of the grafts before administration to the patient, which is called a T-cell replete alloSCT. Using this approach, long-term prophylactic immunosuppressive medication like cyclosporine A is indicated for several months to years to prevent GVHD. Since pre-clinical models have demonstrated that especially donor-derived TCR α/β T cells with a naïve phenotype are the major players in the development of GVHD, strategies have focused on either the reduction of potentially alloreactive T cells in the patient (*in vivo*) or in the graft before administration to the patient (*in vitro*).⁸²

In vivo T-cell depletion

In vivo T-cell depletion (TCD) using antibodies can be applied as part of the conditioning regimen before transplantation. A lot of experience has been obtained with alemtuzumab (ALT) and anti-thymocyte globulin (ATG). ALT is a humanized monoclonal antibody of the IgG1 type which targets the glycoposphatidylinositol (GPI)-anchored protein CD52.⁸³ This antigen is expressed on mature lymphocytes at different levels and not (or only marginally) on hematopoietic stem and progenitor cells.⁸⁴ ATG is a polyclonal antibody that targets several antigens that are mainly expressed on T cells in blood and peripheral lymphoid tissue.⁸⁵ When ALT and/or ATG are administered to the patient before infusion of the graft, the effect is both directed against residual patient-derived T cells that survived the chemotherapy and/or TBI included in the conditioning regimen, as well as against donor-derived T cells that are administered together with the stem cells during transplantation. Elimination of patient-derived T cells may favor the risk of graft rejection and may thereby support engraftment after transplantation.⁸⁶ Especially in HLA-mismatched transplantation, reducing the risk of graft rejection is of major importance. Elimination of donor-derived T cells is of importance

to reduce the risk of acute and chronic GVHD.

In vitro T-cell depletion

In vitro TCD is achieved by manipulation of the graft before infusion into the patient. One strategy is the elimination or selection of specific cell populations by using antibody-coated magnetic beads and magnetic separation.⁸⁷⁻⁹³ Selection of CD34^{pos} cells can be achieved by the physical positive isolation of CD34^{pos} cells to create a purified graft of stem and progenitor cells, while only a very limited number of T cells is preserved.^{88,89} Although clinical applications showed stable engraftment and low incidence of acute and chronic GVHD in the absence of immunosuppressive therapy, concerns regarding the risk for disease relapse and opportunistic viral infections remained. Therefore, the application of limited T-cell addback to CD34^{pos} selected T-cell grafts has been explored to support early T-cell reconstitution and preserve protective immunity after alloSCT.⁹² These observations suggest that a limited amount of T cells in the graft is necessary for early immune reconstitution and protection against viral reactivations. Another strategy is the *in vitro* selective depletion of TCR α/β T cells from stem cell grafts.^{90,91} This approach is thought to result in the efficient depletion of TCR α/β T cells while TCR γ/δ T cells are expected to be preserved in the grafts, leading to a significant reduction in GVHD incidence while a sustained reactivity against pathogens is maintained.

ALT can also be added directly to the stem cell graft before infusion, known as *in vitro* TCD or 'ALT to the bag'. Using this strategy, donor-derived CD52^{pos} cells can be depleted already before graft infusion into the patient. Although the exact effect of ALT addition to grafts on graft composition is not studied extensively, it has shown to be a very effective and fast method for prevention of GVHD as part of both MA and NMA conditioning.⁹⁴⁻⁹⁸

Although these mentioned approaches to prevent GVHD result in a significant decrease in incidence and severity of GVHD, beneficial T-cell responses are also impaired by the use of immunosuppressive medication and/or TCD. The GVL effect will be diminished, leading to a high risk of disease relapse. Furthermore, ALT- and/or ATG-based TCD may lead to an increased incidence of viral reactivations compared to non-TCD alloSCT protocols, resulting in substantial morbidity.^{99,100} However, the incidence of viral disease has shown to be comparable between these two protocols, suggesting that close monitoring and pre-emptive antiviral therapy might prevent the progression from viral reactivation to viral disease.^{96,101-103}

VIRAL COMPLICATIONS AFTER TCD alloSCT

The major viral pathogens causing serious morbidity and mortality after alloSCT are the cytomegalovirus (CMV), Epstein-Barr virus (EBV) and human adenovirus (AdV). In immune competent individuals, infections with these viruses normally occur during childhood or

adolescence and are accompanied by mild or even absent symptoms and have a self-limiting character. Professional antigen-presenting cells are required for the induction of a primary T-cell responses upon first infection, leading to a rapid increase of effector T cells and the formation of memory T cells. Because these viruses will not be entirely cleared from their host but remain latently present in immune and tissue cells, this immunological memory is very important to remain protected against viral reactivations. However, during a state of immunodeficiency as in the period after alloSCT, this protective immunity is destroyed by the conditioning regimen and reactivations are not any longer controlled by memory T cells. In the absence of protective immunity, viral infected cells are not eliminated and the virus can replicate uncontrolled.

Multiple factors influence the timing, rate and diversity of cellular immune reconstitution after alloSCT, like conditioning regimen (including TCD), patient age, stem cell source, occurrence of GVHD and the use of immunosuppressive therapy.¹⁰⁴⁻¹⁰⁷ T-cell reconstitution can be considerably delayed due to TCD of the graft, and is often incomplete. Sources for T-cell reconstitution can be both patient-derived T cells that survived the conditioning regimen and donor-derived T cells that are transferred with the transplant. These mature T cells from patient or donor origin may also comprise virus-specific T cells with a memory phenotype that can readily provide protection against viral reactivations. Besides that, *de novo* generated naïve T cells derived from the transplanted donor stem cells and educated in the patient thymus are another source of T-cell reconstitution. However, these latter T cells need time to develop, to receive adequate stimulation by professional antigen-presenting cells and to expand until appropriate cell numbers are reached to fight a primary viral infection or viral reactivation. Although these *de novo* developing T cells are of importance for rebuilding the immune system in the long term, these cells only have a limited role in the control of viral complications in the critical first months after TCD alloSCT.

Cytomegalovirus

CMV is responsible for the majority of viral reactivations following alloSCT. The risk of reactivation of CMV is dictated by the serostatus of both patient and donor.^{108,109} Between 45-60% of patients that receive an alloSCT, have encountered CMV before (seropositive patients) and are at risk for an endogenous CMV reactivation after transplantation.^{110,111} CMV infection of a CMV-seronegative patient via a stem cell graft from a CMV-seropositive donor can occur, but is less common. Ultimately, around 80% of CMV-seropositive patients will encounter a CMV reactivation after alloSCT.¹¹² The CMV reactivation may progress to CMV disease characterized by potentially fatal organ involvement, such as CMV pneumonia, colitis or encephalitis.¹¹³ The availability of antiviral agents like (val)ganciclovir and foscarnet have contributed to a significant reduction in CMV-related morbidity and mortality following TCD alloSCT. However, administration of these drugs is limited by adverse effects and possible development of resistance, and antiviral therapy has only a temporary effect.^{114,115} Subsequently, the incidence of CMV disease is still 10% in the first year

after alloSCT in CMV-seropositive patients.¹¹³

Epstein-Barr virus

Almost 90% of the population is EBV seropositive at adult age.¹¹⁶ After an active infection, the virus latently resides in B cells. In patients after alloSCT, both residual patient-derived B cells that survived the conditioning regimen as well as transferred donor-derived B cells with the graft, are a source for EBV reactivations. An impaired immune system may not be able to prevent the massive expansion of EBV-infected B cells leading to potentially fatal post-transplant lymphoproliferative disease (PTLD) in about 0.5-17% of patients.¹¹⁷ In case TCD strategies only focus on the elimination of T cells, donor-derived B cells in the graft remain an important risk factor. However, using *in vitro* ALT-based TCD strategies, the risk of EBV-PTLD is not significantly increased compared to T-cell replete alloSCT, because B cells also highly express CD52 and are eliminated by ALT.^{118,119} Both prevention and therapy of EBV-PTLD rely on rituximab targeting the CD20-antigen which is expressed by both EBV-infected B cells as well as healthy B cells.

Adenovirus

AdV reactivations after alloSCT show a high incidence particular in pediatric patients, while the incidence in adult patients is around 3-20%.^{100,120} AdV infections can progress to severe localized or disseminated disease, which is associated with high mortality rates. Reconstitution of AdV-specific T cells has been demonstrated to be essential to control AdV infections after alloSCT.^{121,122} The efficacy of antiviral treatment for AdV infections like cidofovir is still under investigation and is associated with severe toxic effects.¹²³

DONOR LYMPHOCYTE INFUSION

TCD can efficiently reduce the risk of acute and chronic GVHD. However, by reducing or eliminating T cells from the graft, the curative GVL effect and viral immunity are abrogated as well as. To (re)introduce GVL effect and viral protection after TCD alloSCT, the concept of donor lymphocyte infusions (DLI) has been developed.¹²⁴ In this approach, unselected lymphocytes from the stem cell donor are administered to patients after TCD alloSCT, with the aim to induce durable remission of persistent or relapsed disease. Timing, clinical setting and dosing of DLI determines whether GVL effect and viral immunity can be promoted while the risk and intensity of GVHD remains acceptable.¹²⁵ A longer time period between alloSCT and DLI is associated with lower intensity of GVHD and allows infusion of higher doses of DLI. By postponing the administration of donor lymphocytes, the inflammatory environment and the tissue damage caused by the conditioning regimen is gradually resolved. Moreover, the majority of patient antigen-presenting cells have been replaced by donor antigen-presenting cells reducing the presentation of patient-derived antigens and alloreactive T-cell activation directed against (healthy) patient tissue. Because immunosuppressive drugs are not indicated after TCD, transferred T cells of the DLI can proliferate

and function without exogenous inhibition, and the post-transplant lymphopenic condition of patients allows the homeostatic proliferation of T cells.¹²⁶ DLI can be applied in a prophylactic, pre-emptive or therapeutic settings. Prophylactic administration is usually administered at a pre-defined moment to prevent disease relapse, regularly 3-6 months post-alloSCT. Later application of prophylactic DLI lowers the risk of GVHD, but comes at the cost of increased risk of disease relapse in the meantime. Pre-emptive and therapeutic administration of DLI can be applied in case patients suffer from mixed-chimerism or early disease relapses.^{127,128} Besides the introduction of a GVL effect, the therapeutic administration of unmanipulated DLI from virus-seropositive donors has also been administered in the setting of refractory viral reactivations to boost the virus-specific immune system.¹²⁹⁻¹³¹ The optimal dosing of DLI depends on the conditioning regimen, donor source, time after transplantation and clinical setting. For example, DLI after NMA conditioning, obtained from an unrelated donor, infusion early after alloSCT or applied in a prophylactic setting only allows low doses of DLI to reduce the risk of GVHD.^{125,132,133}

ADOPTIVE T-CELL THERAPY

Although the two step approach of TCD alloSCT followed by unmanipulated DLI may be an efficient and relatively safe way to treat patients with hematologic malignancies, patients are vulnerable to disease relapses and viral reactivations in the period between TCD alloSCT and unmanipulated DLI.^{127,128} Therefore, the adoptive transfer of selected T-cell populations with exclusively beneficial effects is highly desirable early after TCD alloSCT. Prerequisites for the broad application of selective T-cell therapy is the knowledge of targetable antigens that are shared between patients and the feasibility of isolation methods for clinical application.

Virus specific T-cell therapy

Much experience has been gained with the generation of virus-specific T-cell products to prevent or treat viral reactivations or viral disease.^{134,135} Initially, virus-specific T-cell lines were created by *in vitro* repetitive antigenic stimulation of T cells with (pools of) overlapping peptides followed by long-lasting expansion in the presence of interleukin-2 to generate virus-specific T-cell products.¹³⁶⁻¹³⁹ However, the *in vivo* efficacy and long-term survival of virus-specific T-cell lines after administration was disappointing, attributed to the abrupt withdrawal from IL-2 in combination with functional and phenotypical changes of T cells initiated during the culture period.^{140,141} Furthermore, long-term culture periods make this strategy not applicable for the treatment of patients with rapid progressive viral disease. Further efforts have been made to develop methods to directly isolate CD4^{pos} and/or CD8^{pos} virus-specific T cells from the blood of seropositive donors followed by short time culturing or direct infusion without *in vitro* expansion. These T cells are supposed to proliferate more efficiently under physiological conditions *in vivo* compared to extensively *in vitro* cultured T cells. In this respect, peripheral blood of virus seropositive donors were stimulated with viral peptides, after which activated CD4^{pos} and CD8^{pos} T cells were isolated

based on an activation-induced effect, like the secretion of cytokines (eg, interferon gamma) or the expression of activation markers (eg, CD137) at the cell surface.¹⁴²⁻¹⁵⁰ These studies show that the generation of CMV, EBV and AdV-specific T-cell lines is feasible, administration is safe and enduring efficacy could be demonstrated by simultaneous appearance of virus-specific T cells in the peripheral blood in concordance with viral control. The isolation of CD8^{pos} peptide-specific T cells can also be achieved using MHC I-multimers (tetramers) or MHC I-*Streptamers* (Figure 1). Both are techniques to isolate T cells based on the specificity of their T-cell receptor and are independent of T-cell kinetics of cytokine production or activation marker expression. However, these approaches require knowledge of defined viral peptides restricted to prevalent MHC I molecules and are not available for the isolation of CD4^{pos} T cells due to the lack of functional MHC class II-multimers or *Streptamers*. In contrast to MHC I-tetramers, the MHC I-*Streptamer* technology is designed for clinical grade isolation of T-cell populations, since MHC I-*Streptamer*-complexes can be dissociated from the T cells resulting in noncoated, unlabelled antigen-specific T-cell products with a preserved T-cell function, suitable for direct clinical administration.^{151,152} The feasibility of this Good Manufacturing Practice (GMP) compliant technology was demonstrated in various (pre-)clinical studies by the isolation of very pure CD8^{pos} T-cell products containing acceptable numbers of contaminating T cells.¹⁵³⁻¹⁵⁷ Although CD4^{pos} helper T cells are thought to contribute to *in vivo* survival, persistence and function of CD8^{pos} T cells, the infusion of CD8^{pos} CMV- or EBV-specific T-cell populations reported promising results regarding T-cell expansion and clinical outcomes as applied in the therapeutic setting. Furthermore, previous observations suggested that the adoptive transfer of a minimum of 250–5,000 virus-specific T cells/kg body weight of the patient is sufficient for virus control in the therapeutic setting, encouraging the infusion of direct *ex vivo* isolated T cells without *in vitro* expansion.^{146,156,158,159} Recently, the generation of T-cell products specific for multiple antigens derived from different viruses has been proposed to provide viral disease prophylaxis or treatment after (TCD) alloSCT.^{156,160,161} However, the optimal strategy for fast generation, safe administration and clinical efficacy of multi-virus specific T-cell products needs improvement to incorporate their application in standard clinical practice.

MiHA-specific T-cell therapy

MiHA can be therapeutically relevant for treatment strategies aimed to promote GVL without initiating GVHD. Theoretically, the adoptive transfer of high-avidity T cells directed against hematopoiesis-restricted MiHA isolated from MiHA^{neg} donors seems a suitable strategy. However, T-cells directed against MiHA are expected to derive from the donors naïve T-cell repertoire, implying a very low frequency in peripheral blood. This makes the enrichment of MiHA-specific T cells even more complex than the obtainment of virus-specific T-cells from seropositive healthy donors, and theoretically similar to the isolation of virus-specific T cells from seronegative donors. This was illustrated before by the generation of leukemia-specific cytotoxic T-cell lines by the stimulation of T cells of HLA-matched donors with leukemic cells of the patients, followed by long-term *in vitro*

culturing under stringent GMP conditions before infusion into the patient.^{162,163} Although some patients did benefit from this therapy, the strategy was logistically complex, time consuming and only successful in a limited number of cases. Afterwards, studies focussed on the application of T-cell lines after alloSCT directed against specific hematopoiesis-restricted MiHA with a balanced allele frequency in the population, like HA-1H. However, the isolation and adoptive transfer of HA-1H-specific T-cell lines has so far not resulted in durable *in vivo* persistence of HA-1H-specific T cells.^{164,165} As already mentioned for virus-specific T cells, long-term *in vitro* culture may limit the *in vivo* expansion capacity of T cells. Therefore, other strategies for adoptive transfer of hematopoiesis-restricted MiHA-specific T cells need to be developed. Besides the isolation and administration of unmanipulated T-cells, T cells can be genetically engineered by TCR gene transfer for hematopoiesis-restricted MiHA to obtain high numbers of MiHA-specific T cells.^{153,166,167} Furthermore, vaccination strategies with donor and patient antigen-presenting cells loaded with MiHA peptides have been explored to boost donor-derived MiHA-specific T-cell responses after alloSCT.^{168,169}

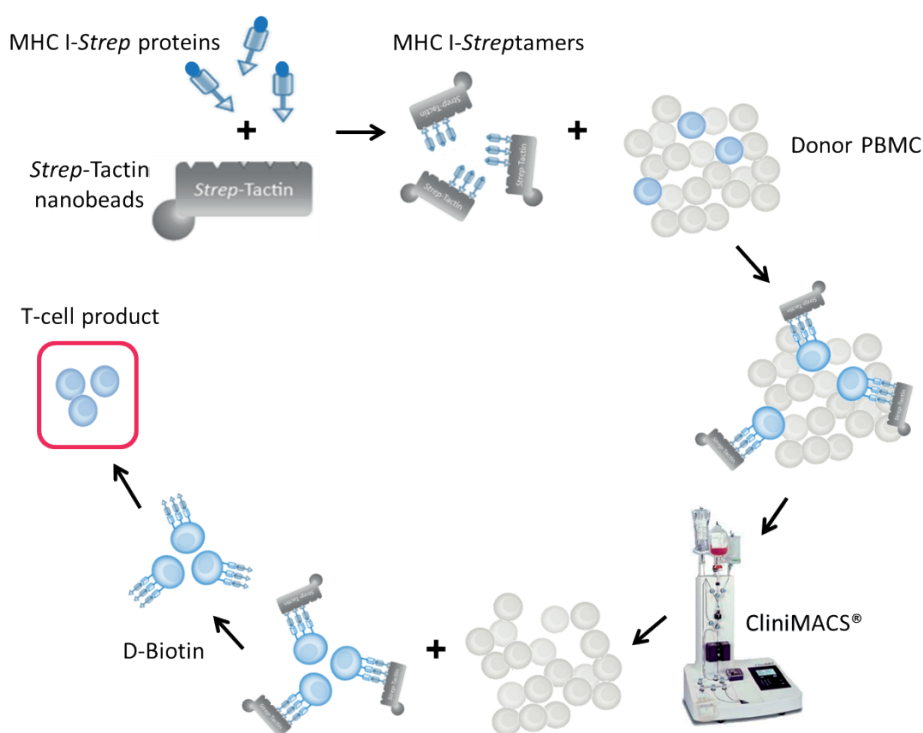


Figure 1. MHC I-Streptamer technology for isolation of antigen-specific T cells from peripheral blood mononuclear cells (PBMC). MHC I-Streptamers are generated by the incubation of peptide-loaded MHC I-Strep-proteins with magnetically labelled Strep-Tactin nanobeads, and are incubated with PBMC to allow binding of MHC I-Streptamers to antigen-specific T cells. Isolation of MHC I-Streptamer bound cells is performed on a CliniMACS or MidiMACS separator device. After dissociation of MHC I-Streptamers from antigen-specific T cells using D-Biotin, the result is a T-cell product containing unmanipulated antigen-specific T cells.

Tumor-related T-cell therapy

Tumor-specific and tumor-associated antigens are both explored as targets for adoptive T-cell therapy in the setting of hematologic malignancies.¹⁷⁰ As WT1 is classified as the 'priority antigen' by the American National Cancer Institute, many clinical trials have focussed on WT1 as tumor-target.¹⁷¹ Besides many active immunotherapy approaches like vaccination studies, only a limited number of clinical trials made use of passive immunotherapeutic approaches like the adoptive transfer of genetically unmanipulated donor-derived TAA-specific T cells with the aim to induce antitumor responses. In two study, T-cell lines directed against BCR-ABL, WT1 and proteinase-3 versus only WT1 were generated by stimulation of donor-derived T cells with donor-derived mature dendritic cells loaded with peptides of the mentioned antigens.^{170,172} After several rounds of stimulation, T-cell products were prophylactically or therapeutically administered to leukemia patients after alloSCT. Although generation and administration of T-cell products was feasible and safe, it is hard to prove efficacy. Again, using this approach, several *in vitro* stimulation and expansion rounds are needed, making it a time-consuming and labour-intensive approach. Wang et al demonstrated the direct *ex vivo* isolation of WT1-specific T cells from alloSCT donor-derived leukapheresis products using MHC I-*Streptamers*.¹⁷³ Although WT1-specific T cells could be enriched from donor peripheral blood mononuclear cells (PBMC), T-cell products contained only a few million cells due to the very low precursor frequency of WT1-specific T cells in the peripheral blood of healthy individuals. Therefore, the direct isolation of TAA-specific T cells for adoptive T-cell therapy approaches is as complex as for MiHA-specific T cells and needs further investigation. Currently, several trials are enrolling patients to test genetically unmanipulated T-cell immunotherapeutic approaches targeting TAA.^{50,174} However, at the same time, it is still under debate whether TAA are effective targets for passive or active immunotherapy strategies. The most important question that needs to be addressed is whether TAA-specific T cells that are able to recognize TAA-expressing malignant cells are actually present in the T-cell repertoire of healthy individuals, as negative thymic selection is supposed to delete high-avidity TAA-specific T cells to prevent auto-immunity. An argument supporting the value of TAA in immunotherapy is the dysregulated overexpression of TAA in malignant cells which may allow the immune system to discriminate TAA-expressing malignant cells from their healthy counterparts.^{48,51,175,176}

AIM OF THE THESIS

Donor-derived T cells play a key role in alloSCT. In the period around the transplantation, donor-derived T cells are depleted or suppressed to reduce the risk of harmful GVHD. However, in the complete absence of donor-derived T cells, the curative GVL effect of alloSCT and the virus-specific immunity is abrogated. Therefore, the major challenge in the field of alloSCT is to find a balance between the GVL effect and viral protection versus GVHD. The research described in this thesis focusses on the manipulation of donor-derived T cells during the process of alloSCT.

To reduce the risk of GVHD after alloSCT, several strategies for TCD have been proposed. In **chapter 2** we study the effect of *in vitro* ALT addition on the composition of allogeneic stem cell grafts before infusion into the patients. The depletion efficiencies of different lymphocytes and T-cell subsets are investigated by comparing the composition of grafts before and after the incubation with ALT. Subsequently, we analyze whether the composition of the grafts at the moment of infusion into the patients are predictive for T-cell reconstitution and the development of GVHD early after ALT-based TCD alloSCT. These observations result in better understanding of the effect of ALT on different lymphocyte subsets and suggestions for the *in vitro* application of ALT for the depletion of T cells in allogeneic stem cell grafts.

As long-term immunosuppression is not indicated after TCD alloSCT, this transplantation strategy is an ideal platform for the application of adoptive T-cell therapy to reduce complications early after TCD alloSCT. In **chapter 3** the development of a widely applicable method for the simultaneous isolation of multiple antigen-specific T-cells populations from donor PBMC is studied. The MHC I-*Streptamer* technology was previously developed for the detection and isolation of antigen-specific T-cell populations under GMP conditions. So far, MHC I-*Streptamers* were used for the isolation of virus-specific T-cell populations from virus-seropositive donors with a relatively high precursor frequency in the donor's peripheral blood. Furthermore, the clinically applied T-cell products isolated with this approach targeted a limited number of different virus-specific T-cell antigens. 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 viral control. Therefore, we assess how many T-cell populations can be simultaneously targeted in one isolation procedure while the purity of the product is maintained and the isolation of potentially harmful alloreactive T cells remains limited. In addition, we investigate whether T-cell populations with high frequencies in the peripheral blood can be isolated in the same procedure as T-cell populations with very low frequencies in the peripheral blood of healthy individuals, like virus-specific T cells from seronegative individuals, MiHA- and TAA-specific T cells. Based on our findings, we define optimal technical conditions to isolate multi-antigen specific T-cell products from donor PBMC using the MHC I-*Streptamer* technology for direct clinical application.

In **chapter 4** we investigate the clinical application of MHC I-*Streptamer* isolated multi-antigen specific T-cell products for the prevention of viral reactivations and disease relapses early after TCD alloSCT in a phase I/II trial. The feasibility of patient/donor inclusion and donor-derived T-cell products generation is assessed. We aim to isolate personalized T-cell products targeting CMV-, EBV-, AdV-, TAA- and MIHA-specific antigens based on the technical knowledge obtained in **chapter 3**, to boost both virus-specific and tumor-specific T-cell immunity. Furthermore, the safety of prophylactic infusion early after TCD alloSCT is analyzed with respect to infusion-related complications and the initiation of GVHD. Patient follow-up provides information on the relation

between T-cell product infusions, the occurrence of clinical events like viral reactivations and disease relapses, and the expansion of target-antigen-specific T cells in the peripheral blood of the patients. The results of this study give insight in the safety and feasibility of personalized adoptive T-cell therapy using MHC I-*Streptamers* and might hint for patient groups that benefit of prophylactic interventions to prevent complications early after TCD alloSCT.

In literature, TAA are proposed as powerful target antigens for immunotherapeutic approaches to stimulate antitumor reactivity. Since the isolation and administration of TAA-specific T cells is also aimed in the clinical study of **chapter 4**, we investigate in **chapter 5** whether TAA-specific T cells in the repertoire of healthy individuals actually have the potential to recognize endogenously processed and presented TAA. TAA-specific T cells are isolated using MHC I-*Streptamers* from healthy donors and are functionally analyzed to demonstrate whether clinically relevant antitumor responses directed against these self-antigens in the context of self-HLA are expected to develop from the autologous or HLA-matched repertoire after immunotherapeutic interventions like TAA-specific vaccination, stem cell transplantation or adoptive immunotherapy.

In **chapter 6** the results of this thesis are summarized and discussed and conclusions based on the results of this thesis and recent literature are drawn.

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