Donor T-cell responses and interaction with graft versus host target tissue after allogeneic stem cell transplantation

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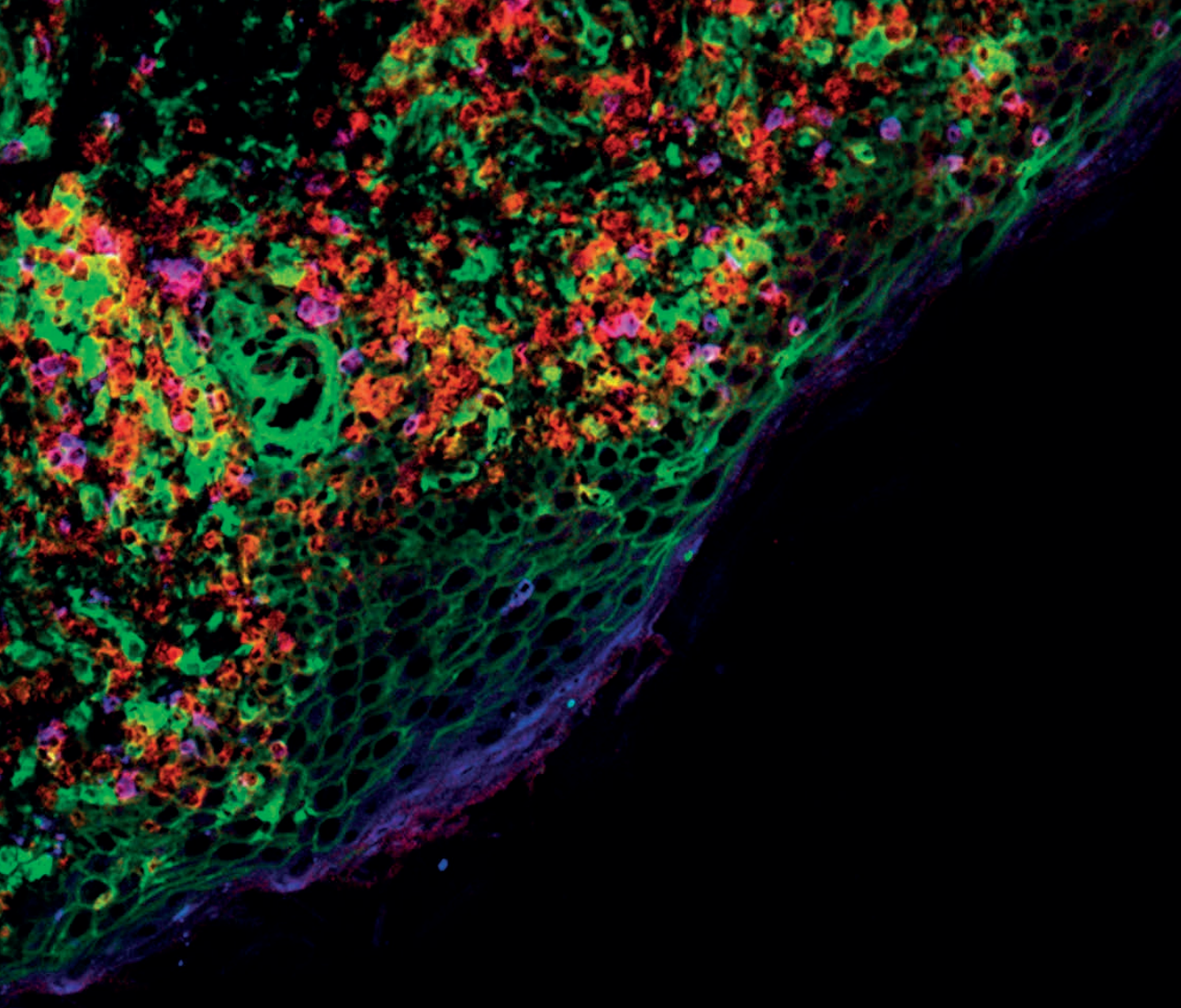

Chapter 1

General introduction

1. General introduction

Allogeneic stem cell transplantation (alloSCT) followed by cellular immunotherapy with donor-lymphocyte infusion (DLI) is an effective, potentially curative treatment for acute leukemia patients. The goal of this treatment is to replace the hematopoietic compartment of the patient by hematopoiesis of donor origin. This enables introduction of a donor-derived T-cell response aiming to eliminate patient hematopoiesis, including the malignant counterpart, and mediate surveillance against remaining and recurrent hematopoietic recipient cells, which prevents relapse of the disease. Prior to alloSCT, myeloablative (MA) conditioning treatment is given using high doses of chemo- and/or radiotherapy to eliminate the malignant population at the cost of damaging the patient hematopoiesis, creating space for the donor stem cells to engraft, and to reduce the risk of graft rejection. Alternatively, reduced-intensity conditioning (RIC), which uses lower doses chemotherapy, can be used for older patients or patients with co-morbidities mainly relying on the immune mediated therapeutic effect of alloSCT and DLI. The beneficial and necessary donor-derived immune response directed against the hematopoietic system of the recipient, including the leukemic cells, is called Graft versus leukemia (GvL) reactivity. However, this donor-derived immune response can also be directed against a broader range of patient organs, such as skin, liver, lungs and gastro-intestinal tract. This immune response is called Graft versus Host Disease (GvHD) and is the main cause of morbidity and mortality after alloSCT. To prevent GvHD the graft can be depleted of donor T cells or patients can be treated with systemic immune suppression (sIS). However, both interventions will also reduce the GvL effect and increase the risk of opportunistic infections.

Decades of research have been dedicated to optimize the transplantation strategy to skew the donor-derived immune response towards GvL with no or just limited GvHD. Factors that have been demonstrated to play an important role in this endeavor can be divided into 3 categories. First, the transplantation procedure, which includes the type of transplantation and the conditioning method in combination with GvHD prophylaxis. Secondly, the genetic disparity between donor and recipient, which is determined by the donor type (e.g. related or unrelated) and by the degree of HLA-matching. Finally, the type of post-transplant cellular intervention in combination with the in vivo inflammatory environment of the patient at that moment, including the composition and the timing of the DLI administration. Despite all the progress made during these decades, non-relapse mortality (NRM) of alloSCT can be up to 20% within the first year after alloSCT due to GvHD in combination with the occurrence of severe infections during sIS treatment for GvHD.

In this thesis we investigated donor-derived T-cell immune response characteristics and the interaction of these T cells with patient target tissues using clinical patient data, ex

vivo skin biopsies and in vitro models. Our goal was to get more insight in the factors that play a role in the safety of administration of DLI within the first 6 months after alloSCT to skew the donor-derived immune response toward GvL.

2. Acute leukemia

Acute leukemias are malignant clonal disorders involving cells of the hematopoietic system, and are characterized by the diffuse replacement of bone marrow with abnormal immature and undifferentiated hematopoietic cells, resulting in loss of erythrocytes, platelets and normal differentiated leukocytes in the peripheral blood (1). Globally, in 2020, leukemia was diagnosed in almost half a million cases and amounting to the tenth cause of death due to malignant disorders (2). Approximately 35% of these new leukemias are acute leukemias, which are classified based on the origin of the abnormal hematopoietic cells involved, such as lymphoid cells in acute lymphoblastic leukemia (ALL) and myeloid cells in acute myeloid leukemia (AML) (1, 3). The diagnosis of acute leukemia is made by the presence of >20% blasts in the bone marrow and the presence of particular genetic aberrations (4, 5).

AML and ALL are distinct disorders with subgroups based on differences in morphology and underlying genetic disorders as defined by the WHO classification (6), leading towards various treatment strategies. Moreover, the determination of genetic and molecular abnormalities in combination with specific disease characteristics in both disorders results in a risk stratification predicting treatment outcome.

The most recent risk classification for AML by the European Leukemia Net (ELN) includes 3 categories based on cytogenetic and molecular abnormalities of the leukemic cells (see Table 1) (7). This risk profile has implications for the chance of therapy success due to therapy resistance or relapse of the disease. The backbone of therapy for AML is two or three cycles of multi-agent chemotherapy with cytarabine and daunorubicin, followed by consolidation treatment with AlloSCT for eligible candidates with an intermediate or unfavorable risk AML. In patients between 65-75 years, treatment choices become more challenging given the increased prevalence of unfavorable risk AML which is less sensitive to classical chemotherapy and an increased chance of NRM. In this patient population, hypomethylating agents (HMA), such as azacitidine or decitabine with or without the addition of Venetoclax, are options as induction therapy before alloSCT (8). The risk stratification for ALL is based on clinical factors such as age, white blood cell count and response to chemotherapy in combination with recurrent genetic alterations (9). The backbone of therapy remains multiple courses of multi-agent chemotherapy and alloSCT for eligible candidates without good-risk disease (5). The presence of the

cytogenetic aberration of the Philadelphia chromosome t(9;22) (Ph) has implications both in terms of prognosis and for treatment. Historically, Ph-positive ALL had a 1-year survival of around 10%. However, with the development of tyrosine kinase inhibitors (TKIs), survival has improved (5).

Table 1. 2022 ELN risk classification by genetics at initial diagnosis (7)

Risk category†	Genetic abnormality
Favorable	• t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1*†,‡ • inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/ *CBFB*::*MYH11*†,‡ • Mutated *NPM1*†,§ without *FLT3*-ITD • bZIP in-frame mutated *CEBPA*‖
Intermediate	• Mutated *NPM1*†,§ with *FLT3*-ITD • Wild-type *NPM1* with *FLT3*-ITD (without adverse-risk genetic lesions) • t(9;11)(p21.3;q23.3)/*MLLT3*::*KMT2A*†,¶ • Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Adverse	• t(6;9)(p23.3;q34.1)/*DEK*::*NUP214* • t(v;11q23.3)/*KMT2A*-rearranged# • t(9;22)(q34.1;q11.2)/*BCR*::*ABL1* • t(8;16)(p11.2;p13.3)/*KAT6A*::*CREBBP* • inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/ *GATA2, MECOM(EVI1)* • t(3q26.2;v)/*MECOM*(*EVI1*)-rearranged • −5 or del(5q); −7; −17/abn(17p) • Complex karyotype,** monosomal karyotype†† • Mutated *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, and/or ZRSR2*‡‡ • Mutated *TP53*[a]

Moreover, recently other (immune) targeted therapies have been introduced as standard care or are currently examined in major clinical trials for both AML and ALL. For example, monoclonal antibodies against CD33 with gentuzumab-ozogamicin and FLT3-, IDH1-, IDH2-inhibitors are added to the current standard treatment strategy for AML (8, 10). For ALL, monoclonal antibodies against CD20 and bispecific antibody constructs directed against CD3 and CD19 are added to the standard treatment schedules (11). Furthermore, T cells genetically engineered with a Chimeric Antigen Receptor (CAR) are part of an area of development in acute leukemia treatment (12). CAR T-cell therapy involves reprogramming a patient's own T cells to recognize and attack cancer cells. T cells are extracted from the patient's blood through leukapheresis and are genetically modified to express CARs on their surface, which are synthetic receptors that combine an antigen-binding domain with T-cell activation components. The antigen-binding domain is typically derived from an antibody fragment that specifically recognizes an antigen found on the surface of cancer cells. After expansion and preconditioning the patient with chemotherapy or lymphodepletion, the CAR T-cell product is infused in the patient (12). In B-ALL, CAR T-cell therapy has shown significant success, particularly

targeting CD19 (13). Several other trials started using different CAR constructs and other targets like CD20 and CD22. CAR T-cell therapy in AML faces challenges due to the lack of specific antigens that spare healthy hematopoietic cells (14). Other challenges and limitation of CAR T-cell therapy for AML and ALL, besides the identification of suitable target antigens, are the potential toxicity and side effects associated with CAR T-cell therapy and the development of resistance by cancer cells (12) (14).

3. Allogeneic stem cell transplantation

AlloSCT remains the most potent curative therapy for patients with acute leukemia. The pre-transplant conditioning regimen with chemotherapy with or without radiotherapy forms the first therapeutic effect. This conditioning regimen can be fully myeloablative (MA), causing irreversible cytopenia, but for the treatment of elderly patients and those with comorbidities, RIC resulting in less toxicity have been developed, in which the cytopenia can be reversible (15).

RIC has been developed since the most important therapeutic effect of alloSCT is the immune response of donor immune cells against the leukemic cells of the patient and not the preceding conditioning chemotherapy. Donor T cells, either transfused with the stem cells at time of the transplantation or later after transplantation as DLI, are responsible for the GvL immune response against patient hematopoietic cells, including the malignant leukemic cells (16). However, the immune response of donor T cells can also be directed against non-hematopoietic patient cells, resulting in the development of GvHD (17). GvHD can manifest with a maculopapular rash of the skin, hyperbilirubinemia with jaundice due to small bile duct damage leading to cholestasis, and damage to the gastro-intestinal tract and lungs (17). GvHD diagnosis depends on clinical, laboratory, and histological assessments and is graded clinically by the extent of involvement of the three main target organs, skin, liver and intestines (18). Overall grades are grade I (mild), II (moderate), III (severe), and IV (very severe). Prevention or treatment of GvHD after alloSCT can be done using sIS treatment. However, this can result in impaired GvL and higher susceptibility to severe infections. Both severe GvHD and the associated need for enduring sIS treatment, are undesirable side effects and are major causes of transplant-related morbidity and mortality (17). On the other hand, limited GvHD without need for long-term treatment with sIS could be a beneficial outcome after alloSCT because of the concomitant ongoing GvL. Therefore, undesirable GvHD may also be defined as the presence of GvHD requiring sIS treatment for a prolonged period.

T-cell depletion (TCD) of the graft is the most effective way to prevent GvHD, which can be achieved ex vivo by either infusion of positively selected CD34 cells, or by depleting

the graft of T cells using negative selection methods (19-21). Alternatively, in vivo depletion of donor T cells from the recipient can be performed by infusing monoclonal antibodies like alemtuzumab or anti-T cell globulin (ATG) prior to transplantation (19). Another method for in vivo depletion of rapidly activated alloreactive donor T cells is treatment of the patient shortly after transplantation with high-dose cyclophosphamide (22). It has been demonstrated that TCD-alloSCT can indeed reduce the development of acute GvHD, while still achieving efficient engraftment (19, 23, 24). However, profound TCD substantially impairs post-transplant anti-tumor and anti-viral immunity (25). To regain this T-cell-mediated anti-tumor and anti-viral immunity after the transplantation, postponed DLI can be administered. This infusion is indeed associated with GvL reactivity and with reduced incidence of GvHD compared to T-cell-containing alloSCT (26-28). GvHD and GvL often co-occur, which is a major obstacle to exploit the full immunotherapeutic potential of donor immune cells against patient leukemia and therefore it is the ultimate aim to skew the donor-derived immune response towards GvL with no or only limited GvHD. Therefore, to make further progress in this endeavor, it is important to increase our understanding of the cellular and molecular basis of GvL and GvHD.

4. Post-alloSCT monitoring and DLI

The goal of the alloSCT and posttransplant DLI strategy is to increase the probability of long-term treatment success, defined as that the patient is alive without relapsed disease or severe GvHD needing long term treatment with sIS. Treatment failure can be defined as relapsed disease, NRM or severe GvHD which needs long term use of sIS. An overt relapse of the acute leukemia after alloSCT, with high blast counts in peripheral blood or in bone marrow or with the development of extramedullary disease, is disastrous and will require combination treatment with chemotherapeutic agents followed by DLI from the same donor or even a second transplantation with a new donor (29). The long-term survival of patients with a full-blown relapse of acute leukemia after alloSCT is limited (30). To allow early therapeutic intervention before an overt hematological relapse occurs, early detection of disease activity following alloSCT is essential and several tools are available. Depending on the disease characteristics, minimal residual disease (MRD) can be measured using flow cytometry or molecular techniques. Early detection and treatment of MRD presence is associated with better outcomes (31). Another prognostic tool for disease relapse is measurement of chimerism, by analyzing the percentages of donor- and patient-derived bone marrow cells after alloSCT. The presence of cells of host origin following alloSCT may indicate persistence of host-derived malignant cells or the absence of a donor-derived alloimmune response towards patient hematopoiesis, which can be essential to prevent disease recurrence (32). Both MRD positivity and

presence of residual patient-derived hematopoiesis can be the trigger for interventions, such as decreasing immunosuppression, application of DLI or administration of anti-leukemic drugs, to avoid an overt relapse (33).

DLI that is administered to patients with mixed chimerism or MRD to prevent hematological relapse after alloSCT is called pre-emptive DLI, while therapeutic DLI is given in case of a relapsed leukemia. Prophylactic DLI is usually administered at a predefined time-period after alloSCT to prevent relapse in high-risk leukemia patients in the absence of active disease, independent of their chimerism status or MRD (34). Different prophylactic DLI strategies have been developed. For example, repeated low-dose prophylactic DLI has been administered to patients with high-risk leukemia at the same dose every two months for at least 36 months until relapse or development of GvHD (35). Another strategy is to administer DLI at increasing doses at fixed periods after alloSCT (19, 34).

5. T-cell-mediated immune responses

Under normal conditions, the major function of T cells is to detect and control viral infections. Intracellular non-self-proteins, like viral proteins or mutated proteins, can give rise to non-self-peptides presented on the cell membrane in the context of (self) human leukocyte antigen (HLA) molecules and can be recognized by the diverse repertoire of T cells. HLA class I and class II molecules, also called major histocompatibility complex (MHC) molecules, play a crucial role in T-cell activation, by presenting peptides on the cell surface for recognition by T cells.

HLA class I proteins, encoded by 3 major genes HLA-A, HLA-B and HLA-C, associated with β2-microglobulin (B2M) can fold together to create a binding groove, where the cytosolic peptide binds to the HLA molecule. Cytotoxic CD8+ T cells can recognize peptides presented in HLA class I and after binding of a CD8+ T cell to its target cell it can initiate its granzyme-B-mediated activation of apoptosis by releasing cytotoxic granules. HLA class I molecules are normally expressed on all nucleated human cells (36, 37).

The most relevant HLA class II proteins, encoded by 3 major genes HLA-DP, HLA-DQ and HLA-DR, consists of two domains that fold together to create a binding groove where the peptide binds to the HLA molecule. HLA class II proteins are normally expressed mainly on professional antigen-presenting cells (APCs), such as dendritic cells, mononuclear phagocytes, and B cells and the peptides presented by class II molecules are derived from endocytosed extracellular proteins (38). CD4+ T cells can bind to and be activated by these HLA class II-peptide complexes, resulting in a direct cytotoxic effect or

coordination and regulation of other effector cells like CD8+ T cells or macrophages and B cells (37, 39).

A properly functioning immune system requires recognition of a wide variety of foreign antigens, which is established by T-cell receptor (TCR) diversity. TCR diversity is generated by random and imprecise rearrangements of the V- and J-segments of the TCR alpha and V-, D-, and J-segments of the TCR beta genes in the thymus (40). The generated T-cell repertoire is then shaped by intrathymic positive and negative selection events to generate a peripheral T-cell pool of self-HLA-restricted, non-autoreactive T cells (40, 41). During positive selection, immature CD4+CD8+ double-positive T cells that express a TCR with low/intermediate affinity for self-peptide□HLA complexes interact with cortical thymic epithelial cells (TECs) and are induced to differentiate into naïve mature single-positive T cells that have high affinity for foreign peptides that are structurally related to the self-peptides involved in the selection (42, 43). During negative selection, T cells that express TCRs with high affinity for self-peptides interact with medullary APCs, such as specialized dendritic cells (DCs) and TEC, resulting in their elimination and thereby creating a largely self-tolerant peripheral T-cell repertoire (42, 43)

To provoke an immune-response by naïve T cells against (non-self) antigens, the T cells need to be activated, after which T-cell expansion, differentiation and memory formation can take place. The primary mediator of T-cell activation is the binding of the specific TCR with the HLA-peptide-complexes presented by APCs, which contain high levels of HLA molecules and co-stimulatory molecules on their cell surface. Subsequent to this recognition, APCs undergo actin-mediated membrane reorganization, facilitating the grouping of these TCRs and other relevant molecules, such as costimulatory and/or adhesion molecules to form immunological synapses (44). For appropriate T-cell activation, a second signal, the co-stimulatory signal, is required, and is induced by CD28-CD80/86 binding and/or other membrane-bound and/or membrane-soluble inflammatory signals, which are antigen-nonspecific and are provided by the interaction between co-stimulatory molecules expressed on the membrane of the APC and the T cell (45). After T-cell activation, naïve T cells undergo changes resulting in proliferation, acquisition of effector function and a differential homing program leading the effector T cells to migrate towards the side of infection. These effector T cells are short-lived. Another subset of T cells differentiates into memory cells, which can also migrate towards the side of infection (effector memory cells) or towards secondary lymphoid organs (central memory T cells). These memory cells are potentially long-lived and after second encounter of the same pathogen these T cells can rapidly expand, thereby mediating a very effective immune response (44, 46, 47).

6. T-cell-mediated immune responses after alloSCT

The aim of alloSCT is to induce an alloreactive immune response of donor-derived T cells against the patient-derived hematopoiesis, including the malignant cells. Alloreactivity refers to the ability of T cells to recognize polymorphic peptide-MHC-complexes as non-self, and is caused by genetic diversity between individuals. This reactivity can be provoked by polymorphic peptides resulting from single nucleotide polymorphisms (SNPs) or frame shift insertions and deletions in primary gene transcripts (48). These polymorphic peptides are called minor histocompatibility antigens (MiHAs). Alloreactivity can be initiated by non-self-peptides presented in self-HLA, but also by monomorphic or polymorphic peptides presented in non-self-HLA (48) (49).

In case HLA molecules are identical due to matching of the donor and patient, alloreactivity of donor T cells will be directed against non-self-peptides (MiHAs) presented in self-HLA. Since the immune system of the donor has not been exposed to these non-self MiHAs, MiHA-specific donor T cells will be present in the naïve T-cell repertoire and will need proper activation and differentiation before they can initiate an alloreactive immune response in patients (19). The tissue expression of the proteins leading to these MiHAs may dictate the specificity of the immune response. For example, MiHAs derived from proteins ubiquitously expressed by both hematopoietic and non-hematopoietic tissues can potentially lead to both GvHD and GvL responses, whereas MiHAs derived from proteins mainly expressed by hematopoietic cells can preferentially result in GvL. After sex-mismatched transplantation, MiHAs encoded by the male-specific Y-chromosome can be expressed, which are called H-Y antigens. These H-Y MiHAs can be targeted by T cells from female donors (48).

Additional alloreactivity can be expected when HLA-mismatched donors are used, since donor T cells can also react with the patient-specific HLA alleles by recognizing the non-self HLA molecules presenting any peptide. Since the entire T-cell repertoire may be at risk of being cross-reactive with allo-HLA molecules, including pathogen-specific T cells, these allo-HLA-reactive donor T cells can develop from the naïve T-cell compartment but may also be present in the memory compartment, resulting in a fast and efficient immune response (26).

Patient-derived T cells can recognize and attack the donor hematopoiesis resulting in graft rejection. This is a rare immunologic complication of alloSCT since it is mostly prevented by depletion of the host immunity by high intensity conditioning therapy in combination with administration of T-cell depleting antibodies (50).

7. The balance between GvL and GvHD

Conditioning treatment prior to the alloSCT will induce tissue damage and potential upregulation of pro-inflammatory cytokines, resulting in increased HLA class II expression and upregulating of costimulatory and adhesion molecules on both APCs and non-hematopoietic tissues. Target tissues of GvHD may contain large numbers of activated recipient APCs, which play a key role in the induction of the alloreactive immune response by presenting a large variety of non-self-peptide/MHC complexes in these tissues (18, 51). The conditioning regimen can create lymphopenia resulting in homeostatic proliferation of donor T cells further promoting the immune response (49). Therefore, DLI which is given with or very early after alloSCT in this pro-inflammatory environment, is associated with high incidence and severity of GvHD. With the passing of time after alloSCT, tissue damage will gradually be repaired and patient hematopoiesis, including the APCs, will be replaced by donor hematopoiesis and will be less activated and the recurrence of lymphocytes will result in lower homeostatic proliferation, resulting in a less pro-inflammatory environment (26). Thus, delaying the administration of donor T cells after alloSCT will lead to a mitigated magnitude of the immune response, since there may be less tissue damage, patient APCs are gradually replaced by donor APCs and lymphocyte counts are normalized. Moreover, under these more steady state circumstances patient-derived hematopoietic cells, including the malignant cells, may still display high expression of HLA class I and HLA class II in comparison with non-hematopoietic cells and thereby could be preferentially recognized, resulting in GvL with less chance of severe GvHD (26). Indeed, it has been demonstrated that TCD-alloSCT followed by prophylactic DLI at 6 months can achieve an enduring GvL response without induction of severe GvHD (23, 24, 27, 28). In high-risk leukemia patients after TCD-alloSCT, early relapses within 6 months after alloSCT are seen more often and therefore require earlier intervention (10, 52). Prophylactic DLI earlier after TCD-alloSCT could be administered in these patients using limiting amounts of effector cells to control the disease without introduction of severe GvHD (18, 53).

Since the donor immune system has not been exposed to the MiHAs of recipient origin, the donor T cells that recognize a specific MiHA will be usually present within the naïve donor T-cell repertoire (26). Therefore, an immune response needs to be initiated by professional patient-derived APCs presenting patient-derived MiHAs. Since professional APCs are hematopoietic cells, these immune responses are likely to be partly directed against MiHAs that are expressed in hematopoietic cells of the recipient origin (26). A specific anti-hematopoietic immune response targeting the leukemic cells, may occur when MiHAs are derived from genes selectively expressed on hematopoietic cells (26). Indeed, several hematopoiesis-restricted MiHAs are discovered and potential immune responses against these antigens were investigated (54). Although specific GvL reactivity may occur due to immune responses selectively targeting MiHAs expressed only on hematopoietic

cells, under most circumstances these T-cell responses will be accompanied by immune responses targeting broadly expressed MiHAs resulting in coinciding GvHD (26).

Other factors in addition to gene expression are also relevant for skewing the immune response towards GvL reactivity. For efficient effector function, T cells need robust attachment to the target cells, which is mediated by adhesion molecules (26). Most non-hematopoietic tissues do not highly express these antigens under steady state conditions and therefore do not interact with these T cells. However, danger signals will result in activation of toll-like receptors and the increase in local pro-inflammatory cytokines leading to upregulation of adhesion molecules on these tissues, facilitating alloreactive donor T cells to target these tissues (26). Due to a higher expression of costimulatory and adhesion molecules on hematopoietic cells they are more accessible to T-cell interaction than many other tissues, which may explain why GvL reactivity without significant GvHD can be induced in patients after unmodified DLI with a restricted, but not hematopoiesis-specific, repertoire of T cells (26, 55). Besides restricted MiHA expression on hematopoietic cells, selective GvL reactivity targeting MiHAs is also influenced by many environmental circumstances, including the presence of a pro-inflammatory environment, which can be induced after conditioning, by infections, by inflammation or due to ongoing alloimmune responses (26). Skewing the T-cell response specific for MiHAs in the direction of GvL may be performed by selecting DLI containing donor T cells that target MiHAs that are specifically expressed on hematopoietic cells, or by timing the unmodified DLI and thereby trying to control the amplitude of the immune response and the inflammatory circumstances in the patient. Postponing DLI administration and controlling infections first may also contribute to selective GvL reactivity (24, 26, 28).

8. Aims of this study

To optimize the treatment success of the TCD-alloSCT procedure followed by prophylactic DLI, it is essential to establish a potent donor-derived immune response resulting in sufficient GvL to induce longtime remission of the acute leukemia, while the accompanying GvHD is acceptable. Since the two generally overlap to some extent, it is essential to examine which factors steer the balance into the right direction. Therefore, the aim of the thesis is to investigate in detail which immunological factors are important in this balance, and to explore whether procedures can be influenced to optimize treatment success.

In **Chapter 2** we want to investigate whether early reduced-dose prophylactic DLI in high-risk acute leukemia patients after TCD-alloSCT may or may not lead to induction of undesirable GvHD, and thereby determine the treatment success. This includes the

examination of additional direct toxicity, but also long-term presence of undesirable GvHD needing sIS treatment. To investigate this, we will use a cohort of patients who underwent TCD-alloSCT for acute leukemia. Furthermore, long term efficacy of the overall transplantation strategy will be examined by comparing the probabilities of general outcomes like overall survival, relapse free survival, non-relapse-mortality and treatment success at 1, 3 and 5 years after transplantation and compare our data to comparable data already published.

In **Chapter 3** we aim to analyze the local environmental conditions in GvHD target tissues using skin biopsies of patients after the conditioning regimen and at specific periods (3, 6, 9 and 12 months) after TCD-alloSCT in presence or absence of GvHD. We aim to get more insight into the dynamics of expression of HLA and adhesion molecules on both hematopoietic and non-hematopoietic cells and the presence of T cells after TCD-alloSCT in both circumstances. Moreover, to get more insight in the kinetics of APC-replacement from patient to donor, we will investigate whether the HLA class II positive cells and T cells are of patient or donor origin, using XY-FISH combined with staining of HLA class II in these skin biopsies from patients after TCD-alloSCT with a sex-mismatched donor.
Selective GvL reactivity targeting MiHAs is in part dependent on the restricted expression on hematopoietic cells but is also influenced by many environmental circumstances including an inflammatory environment, which can be induced after conditioning, during infections, and due to an ongoing alloimmune response. In **Chapter 4** we want to investigate the question whether the susceptibility of non-hematopoietic tissues to the cytotoxic effects of the MiHA-specific T cells needs the establishment of high avidity interactions between T cells and target cells due to upregulation of adhesion molecules by pro-inflammatory cytokines. To examine this, we will develop an in vitro model in which we will analyze reactivity of alloreactive human T cells specific for ubiquitously expressed MiHAs against skin-derived primary human fibroblasts under different conditions.

Once an overt GvL response is established, this often coincides with GvHD reactivity. In **Chapter 5**, using our in vitro model, we aim to analyze whether activated HLA-class II-specific T cells, while attacking their hematopoietic targets, can also cause direct damage to surrounding non-hematopoietic cells due to misdirection of their cytotoxic granules. We want to examine whether close proximity of these cells, due to upregulation of adhesion molecules on non-hematopoietic tissues, which may be induced by the released pro-inflammatory cytokines of the activated T cells, is necessary for this potential collateral damage.

In **Chapter 6** the most important results of the performed analyses are summarized and in **Chapter 7** the relevance of the results in the light of the current knowledge are discussed.

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