Targeting HLA class II in allogeneic stem cell transplantation

Balen, P. van

CHAPTER 6

Summary and general discussion

SUMMARY

Allogeneic stem cell transplantation (alloSCT) is a curative treatment for a variety of hematologic diseases. Before alloSCT the patient is treated with chemotherapy or radiotherapy in order to reduce the tumor burden. The mechanism of curation by an alloSCT is the induction of an immune response of donor T cells attacking patients hematopoiesis, including the malignant hematopoietic cells. This is called the graft-versus-leukemia (GVL) reactivity. However, donor T cells can also be directed against healthy tissue cells of the recipient, causing graft-versus-host disease (GVHD). A major challenge in treatment with alloSCT is to induce GVL without or with very limited GVHD. The most efficient method to prevent GVHD is T-cell depletion (TCD) of the graft. AlloSCT regimens using infusion of positively selected CD34 cells, grafts depleted for alpha-beta T cells or CD3 T cells, or using the CD52 antibody Alemtuzumab for TCD have demonstrated efficient engraftment and reduced acute GVHD.[1-10] However, TCD substantially impairs post-transplant anti-viral and anti-tumor immunity.[4,11,12] Due to the reduced antitumor effect of TCD grafts, post-transplant donor lymphocyte infusion (DLI) may be needed for treatment of persistent disease or to prevent relapse of the disease. Indeed, DLI after alloSCT can mediate GVL reactivity, but frequently still at the cost of GVHD.[13-16]

In this thesis we focused on targeting HLA class II by donor CD4 T cells to induce GVL without GVHD, because under non-inflammatory conditions, HLA class II is mainly expressed on hematopoietic cells and not on other tissue cells.[17-20] Therefore, CD4 DLI after TCD alloSCT may be an attractive strategy to separate GVL from GVHD. However, HLA class II expression can be upregulated under inflammatory conditions and inflammatory conditions frequently occur after alloSCT. Also the conditioning regimens preceding alloSCT can cause tissue damage associated with inflammation and expression of HLA class II molecules. Therefore, we investigated in ***chapter 2*** the presence of tissue injury and inflammation, as well as the presence of HLA class II positive cells in skin during the period of hematologic recovery and immune reconstitution after alloSCT. We also investigated the interaction between detected T cells and HLA class II positive cells. Skin biopsies were immunohistochemically stained for HLA class II, CD1a, CD11c, CD40, CD54, CD68, CD86, CD206, CD3 and CD8. HLA class II expressing cells were characterized as activated T cells, antigen presenting cells (APC) or tissue repairing macrophages. In sex mismatched patient and donor couples, origin of cells was determined by multiplex analysis combining XY-FISH and fluorescent immunohistochemistry. No inflammatory environment due to pretransplant conditioning was detected at the time of alloSCT, irrespective of the conditioning regimen. A significant increase in HLA class II positive cells and CD3 T cells was observed 12-24 weeks after myeloablative alloSCT in patients without GVHD, but these HLA class II positive cells did not show signs of interaction with co-localized T cells. Immunohistochemical staining illustrated that these HLA class II positive cells were macrophages and not professional APC, which

are necessary for the initiation of an immune response from naïve donor T cells. In contrast, during GVHD, an increase in HLA class II expressing cells coinciding with T-cell interaction was observed, resulting in an overt inflammatory reaction with presence of activated APC, activated donor T cells and localized upregulation of HLA class II expression on epidermal cells. In the absence of GVHD, patient derived macrophages were gradually replaced by donor derived macrophages, although patient derived macrophages were detectable even 24 weeks after alloSCT. Despite conditioning regimens caused tissue damage in the skin, this did not result in a local increase of activated APC. In contrast to the inflamed situation in GVHD, when interaction took place between activated APC and donor T cells, the tissue damage caused by myeloablative alloSCT resulted in dermal recruitment of HLA class II positive tissue repairing macrophages co-existing with increased numbers of patient and donor derived T cells, but without signs of specific interaction and initiation of an immune response. Thus, the local skin damage caused by the conditioning regimen appears to be insufficient as single factor to provoke GVHD induction.

In an ongoing clinical trial in the Leiden University Medical Center, patients are being treated three months after TCD alloSCT with purified CD4 DLI. In ***chapter 3***, we analyzed immune responses in four patients converting from mixed to full donor chimerism without developing GVHD upon purified CD4 DLI from their HLA-identical sibling donor after TCD alloSCT. In vivo activated T cells were clonally isolated after CD4 DLI. Of the alloreactive T-cell clones, 96% were CD4 positive, illustrating the dominant role of CD4 T cells in the immune responses. We identified 9 minor histocompatibility antigens (MiHA) as targets for alloreactivity, of which 8 were novel HLA class II restricted MiHA. In all patients, MiHA specific CD4 T cells were found that were capable to lyse hematopoietic cells and to recognize normal and malignant cells. No GVHD was induced in these patients. Skin fibroblasts forced to express HLA class II, were recognized by only two MiHA specific CD4 T cell clones. Of the 7 clones that failed to recognize fibroblasts, two targeted MiHA were encoded by genes not expressed in fibroblasts. Another clone recognized a MiHA whose presentation is dependent on HLA-DO, which is absent in fibroblasts. T cells recognizing the remaining 4 MiHA had an avidity that was apparently too low to recognize fibroblasts, despite clear recognition of hematopoietic cells. In conclusion, purified CD4 DLI from HLA-identical sibling donors can induce conversion from mixed to full donor chimerism with graft-versus-malignancy reactivity, but without GVHD, by targeting HLA class II restricted MiHA.

Since mismatches between donor and patient for HLA-A, B, C, DQB1 and DRB1 are associated with an increased risk of graft-versus-host disease (GVHD), unrelated donors for alloSCT are ideally matched with patients for these alleles. Other HLA class II alleles are usually not taken into account in donor selection. However, in patients receiving CD4 DLI 3 months after TCD alloSCT with such an HLA 10/10 matched unrelated donor (MUD), severe GVHD has been observed due to alloreactive donor CD4 T cells directed against

mismatched HLA-DP.[21] Also others described that mismatched HLA-DP can be the target in immune responses after alloSCT.[22-26] However, beside HLA-DP, other HLA class II alleles can be mismatched in case of 10/10 matching, being the alleles HLA-DRB3, 4 and 5. They are assumed to have limited relevance in alloSCT and mismatches in HLA-DRB3, 4 and 5 are not taken into account in donor selection. In ***chapter 4***, we described a patient in which conversion of chimerism in the presence of GVHD occurred after CD4 DLI from an HLA 10/10 matched donor, but with mismatches for HLA-DRB3 and HLA-DPB1 compared to the recipient. Alloreactive CD4 T cells were isolated from peripheral blood of the patient after CD4 DLI and recognition of donor derived target cells transduced with the mismatched patient variant HLA-DRB3 or HLA-DPB1 molecule was tested. A dominant polyclonal CD4 T cell response against patient's mismatched HLA-DRB3 molecule was found in addition to an immune response against patient's mismatched HLA-DPB1 molecule. CD4 T cells specific for these HLA class II molecules recognized both hematopoietic target cells as well as GVHD target cells. Thus, in contrast to the assumption that mismatches in HLA-DRB3, 4 and 5 are not of immunogenic significance after HLA 10/10 matched alloSCT, we showed that in this matched setting not only mismatches in HLA-DPB1, but also mismatches in HLA-DRB3 may induce a polyclonal allo-immune response associated with conversion of chimerism and severe GVHD.

Unrelated donors for alloSCT who are HLA 10/10 matched with the recipient are mismatched for HLA-DP in 71-88% of cases.[27-30] Although not taken into account in donor selection, immune responses by donor CD4 T cells against this mismatched HLA allele can result in both GVL and GVHD.[21,23,31] Not all mismatches in HLA-DP between donor and patient turned out to be equally immunogenic and therefore HLA-DP alleles had previously been classified into functional T-cell epitope (TCE) groups.[24,32-34] Mismatches between patient and donor within a TCE group had been regarded permissive, whereas mismatches between different TCE groups had been regarded non-permissive. TCE-1 and TCE-2 are clearly defined, but TCE-3 represents still an heterogeneous group and therefore we aimed to redefine these groups in ***chapter 5***. Since polymorphisms in HLA-DP influence the presented peptidome, we investigated whether the composition of peptides binding in HLA-DP may be used to refine the HLA-DP group classification. Therefore, peptidomes of HLA-DP typed B-cell lines were analysed with mass spectrometry after immunoaffinity chromatography and peptide elution. Gibbs clustering was performed to identify motifs of binding peptides. Hierarchical clustering was performed to investigate the similarities and differences in peptidomes of different HLA-DP molecules. HLA-DP peptide binding motifs showed a clear correlation with the HLA-DP allele specific sequences of the binding groove. Hierarchical clustering of HLA-DP immunopeptidomes resulted in the categorization of HLA-DP alleles into 3 DP peptidome clusters (DPC). The peptidomes of HLA-DPB1*09:01, 10:01 and 17:01 (TCE-1 alleles) and HLA-DPB1*04:01, 04:02 and 02:01 (TCE-3 alleles) were separated in two maximal distinct clusters, DPC-1 and DPC-3 respectively, reflecting their previous TCE classification. HLA-DP

alleles categorized in DPC-2 shared certain similar peptide binding motifs with DPC-1 or DPC-3 alleles, but significant differences were observed for other positions. Within DPC-2, divergence between the alleles was observed based on the preference for different peptide residues at position 9. Immunopeptidome analysis revealed new functional hierarchies among HLA-DP alleles, providing new molecular insights into HLA-DP classification.

In summary, in this thesis we demonstrated that conditioning regimens cause tissue damage in the skin, but that this does not result in a local increase of activated APC. In contrast to the inflamed situation in GVHD, when interaction takes place between activated APC and donor T cells, the tissue damage caused by myeloablative alloSCT results in dermal recruitment of HLA class II positive tissue repairing macrophages co-existing with increased numbers of patient and donor derived T cells, but without signs of specific interaction and initiation of an immune response. Thus, the local skin damage caused by the conditioning regimen per se appears to be insufficient as single factor to provoke GVHD induction. We showed that purified CD4 DLI from HLA-identical sibling donors can induce conversion from mixed to full donor chimerism with graft-versus-malignancy reactivity, but without GVHD by targeting HLA class II restricted MiHA. In contrast to HLA-identical transplantations, transplantations with an HLA mismatch between donor and patient can result in immune responses by donor T cells directed against the mismatched HLA molecule of the patient. In contrast to the assumption that mismatches in HLA-DRB3, 4 and 5 are not of immunogenic significance after HLA 10/10 matched alloSCT, not only mismatches in HLA-DPB1, but also mismatches in HLA-DRB3 may induce a polyclonal allo-immune response associated with conversion of chimerism and severe GVHD. Finally, we analysed the immunopeptidome of different HLA-DP alleles. HLA-DP peptide binding motifs showed a clear association with the HLA-DP allele specific sequences of the binding groove. Hierarchical clustering of HLA-DP immunopeptidomes resulted in the categorization of HLA-DP alleles into 3 DP peptidome clusters (DPC). The peptidomes of HLA-DPB1*09:01, 10:01 and 17:01 (TCE-1 alleles) and HLA-DPB1*04:01, 04:02 and 02:01 (TCE-3 alleles) were separated in two maximal distinct clusters, DPC-1 and DPC-3 respectively, reflecting their previous TCE classification. HLA-DP alleles categorized in DPC-2 shared certain similar peptide binding motifs with DPC-1 or DPC-3 alleles, but significant differences were observed for other positions. Within DPC-2, divergence between the alleles was observed based on the preference for different peptide residues at position 9. Functional hierarchies among HLA-DP alleles were unravelled, providing new molecular insights into HLA-DP classification. Permissiveness is not a black and white phenomenon, but rather gradual based on similarities and differences in the peptidomes.

GENERAL DISCUSSION

In this thesis we demonstrated that HLA class II molecules of the patient can be targeted by donor CD4 T cells after alloSCT. Under non-inflammatory conditions HLA class II is mainly expressed on hematopoietic cells which makes HLA class II a potential good target for hematopoiesis restricted immune responses after alloSCT. However, HLA class II expression can be upregulated on GVHD target tissues in case of inflammation, making also these tissues vulnerable for immune responses by donor CD4 T cells. We demonstrated that the conditioning regimen before alloSCT resulted in dermal recruitment of HLA class II positive macrophages. Although HLA class II positive macrophages were recruited, there was no interaction between these macrophages and the surrounding T cells. Tissue damage as single factor was apparently not enough to provoke an immune response causing GVHD in the skin, which can be explained since macrophages are not professional APC. The induction of an immune response with antigen-specific clonal expansion of naïve T cells requires binding of the T-cell receptor to the peptide-HLA complex coincided with a second or co-stimulatory signal, which must be delivered by the same APC on which the T cell recognizes its specific antigen. The best characterized co-stimulatory molecules on APC are CD80 and CD86. The requirement for the simultaneous delivery of antigen-specific and co-stimulatory signals by one cell in the activation of naïve T cells means that only professional APC can initiate T-cell responses. Mature dendritic cells are highly efficient inducers of naïve T-cell activation. Macrophages do not deliver co-stimulatory signals to T cells. In the setting of alloSCT with HLA identical donors, alloreactive CD4 T cells recognize HLA class II molecules presenting MiHA which are polymorphic peptides derived from genes containing single nucleotide polymorphisms which differ between donor and recipient and are therefore foreign to donor T cells. MiHA recognizing donor CD4 T cells originate from the naïve T cell compartment of the donor and need to be activated by professional APC in the patient. Therefore, it is unlikely that HLA class II positive macrophages in GVHD target tissue, which are not professional APC, are able to induce an immune response after CD4 DLI from an HLA identical sibling donor. However, it is possible that patient derived HLA class II positive macrophages in GVHD target tissues amplificate an already initiated CD4 T-cell response, for instance if the immune response is initiated by APC elsewhere in the body or if the immune response is due to reactivation of donor memory T cells. In contrast to the HLA identical setting, after CD4 DLI from HLA 10/10 matched, but HLA-DP mismatched unrelated donors, observed immune responses turned out to be directed to the mismatched HLA class II alleles for the vast majority of alloreactive donor CD4 T cells. Since memory T cells recognizing mismatched HLA molecules can be present in the donor and activation of memory T cells is not dependent on the co-stimulatory signals by APC, immune responses after CD4 DLI from HLA class II mismatched donors, can be facilitated and amplificated by the presence of HLA class II positive macrophages without activation by professional APC.[35-41]

Immune responses by MiHA specific CD4 T cells are likely to target cells of hematopoietic origin preferentially. This is due to the relative hematopoiesis restricted expression of HLA class II alleles under non-inflammatory conditions. However, our results illustrate that also other factors play a role in the hematopoiesis specific immune response observed in patients receiving CD4 DLI from HLA identical sibling donors. The MiHA encoding gene can have an hematopoietic restricted expression profile, like the identified MiHA LB-LILRB1-1I and LB-LY75-2R. Those MiHA specific T cells will not cause GVHD, even under inflammatory circumstances in which expression of HLA class II molecules is upregulated on non-hematopoietic cells. However, also CD4 T cells targeting identified HLA class II restricted MiHA that were encoded by broadly expressed genes, preferentially targeted hematopoietic cells. Apparently, not only expression of the HLA restriction molecule and MiHA encoding gene in GVHD target tissue determine whether these non-hematopoietic cells are targeted by specific CD4 T cells. The lack of HLA-DO in non-hematopoietic cells is an explanation for this phenomenon for some MiHA specific CD4 T cells. HLA-DO is the natural inhibitor of HLA-DM and HLA-DM has a major role in the selection of dominant epitopes.[42-44] HLA-DM has been described as a chaperone that greatly accelerates peptide exchange in HLA class II molecules and various studies have shown that HLA-DM induces release of peptides binding to HLA class II with low affinity, resulting in a selection process that favors presentation of high-affinity peptides.[44-46] Crystal structure of the HLA-DO-DM complex demonstrated that HLA-DO and DM bind in a side-by-side arrangement similar to that proposed for HLA class II and DM. HLA-DO acts as a substrate mimic that tightly binds to HLA-DM, thereby preventing access of HLA-DM to HLA class II.[47] The effect of the presence of HLA-DO is that HLA-DO broadens the repertoire of presented peptides in HLA class II molecules.[48,49] Some peptides, and therefore also some MiHA, can only be presented in HLA class II in the presence of HLA-DO, which is expressed in hematopoietic cell types, but not in the majority of non-hematopoietic cells.[42] LB-LGALS8-1C is an example of an HLA-DO dependent MiHA, since fibroblasts were only recognized by specific CD4 T cells after enforced expression of HLA-DO. Even under inflammatory conditions GVL may be separated from GVHD due to the higher susceptibility of hematopoietic cells compared to non-hematopoietic cells from GVHD tissues to be targeted by MiHA specific CD4 T cells and due to the limited diversity and magnitude of the induced immune responses. After infusion of donor CD4 T cells after TCD alloSCT from an HLA identical sibling donor, we indeed observed hematopoiesis restricted immune responses consisting of mixed to full donor chimerism conversion without the occurrence of GVHD.

CD4 DLI from HLA 10/10 matched, but HLA-DP mismatched donors did induce immune responses including GVL and, in contrast to CD4 DLI from HLA identical sibling donors, also GVHD. In the HLA 10/10 MUD setting, 80% of patients and donors are mismatched for HLA-DPB1.[27-30] It was already known that the mismatched HLA-DPB1 allele of the patient can be the target for these immune responses.[21-23] Regarding the immunogenetic impact of

HLA-DPB1 mismatches, HLA-DPB1 alleles were previously categorized into T-cell epitope (TCE) groups. This TCE categorization is based on in vitro experiments using recognition patterns of anti-HLA-DPB1*09:01 directed T cells and amino acid sequences of the binding groove defining functional distance among the different HLA-DPB1 alleles from HLA-DPB1*09:01.[25,34,50] Based on this classification, HLA-DP mismatches have been classified as permissive (mismatch within the same TCE group) or non-permissive (mismatch across different TCE groups) with predictive value for the outcome of transplantation.[24,32-34,51] The functional groups of TCE-1 alleles (HLA-DPB1*09:01, 10:01 and 17:01) and TCE-2 alleles (HLA-DPB1*03:01, 14:01 and 45:01) were clearly defined, but TCE-3 included any HLA-DPB1 allele not belonging to either group 1 or 2, and represents a relatively heterogeneous group and therefore the TCE classification was aimed to be refined by analyzing the peptidome of HLA-DP molecules.

It has been shown that polymorphisms within the peptide binding groove of HLA-DP molecules are more important for HLA-DP restricted alloreactivity than polymorphisms outside the peptide binding groove.[50,52] In addition, it has previously been demonstrated that biologically relevant allo-HLA class I and II reactivity selected during a GVHD response were peptide specific.[53,54] Therefore, we hypothesized that differences in composition of peptides bound to the various HLA-DP molecules determine the potency to induce immune responses between mismatched HLA-DP molecules and that analysis of the peptidome could result in refining the TCE categorization. Based on the results of hierarchical clustering of similarities and differences in the peptidomes of different HLA-DP molecules, a new categorization in 3 different DPC groups could be made. We were able to partly confirm, but also to refine the existing TCE group categorization. The categorization in TCE groups is based on experiments analyzing recognition of HLA-DP directed T cells and the amino acid sequence of the binding groups, whereas the categorization in DPC groups is based on similarities and differences in the peptides presented in HLA-DP molecules. However, instead of strict categorization into closed groups, our data suggest a more gradual categorization. The peptidomes of DPB1*09:01, 10:01 and 17:01, previously classified as TCE-1 alleles, were most similar and were grouped together in DPC-1. These peptidomes were most different from the peptidomes of DPB1*04:01, 04:02 and 02:01, previously assigned to TCE-3, which were clustered in DPC-3. All other HLA-DP peptidomes, including some originally assigned to TCE-3, were categorized in DPC-2, among which further stratification can be made, mainly based on similarities or differences at amino acid position 9 of the bound peptides.

Based on the new categorization proposed from our results, it is expected that a mismatch between patient and donor is non-permissive if the mismatch is between DPC-1 and DPC-3 alleles. Whether other mismatches are permissive or non-permissive depends on the differences in the peptidome and whether these alleles cluster close to each other or not.

We hypothesize that permissiveness is not a black and white phenomenon, but rather gradual based on similarities and differences in the peptidomes. Permissiveness between HLA-DP mismatches can also potentially be influenced by the location of binding of the T-cell receptor (TCR) to the HLA-peptide complex. If a TCR binds strongly to certain amino acids on a specific position in the binding groove or bound peptide, differences at these positions between HLA-DP alleles will determine permissiveness more than amino acid differences where the TCR will not bind. Further studies investigating the relation of HLA-DP mismatched with clinical outcome parameters after alloSCT are warranted to test the hypothesis that permissiveness is based on similarities and differences in the peptidomes.

A difference was observed in tissue restriction of immune responses after CD4 DLI from HLA 10/10 MUD compared to HLA identical sibling donors. The immune response consisting of GVL without GVHD after CD4 DLI from HLA identical sibling donors was due to MiHA specific CD4 T cells, whereas in the HLA 10/10 MUD setting the immune response was more fulminant and due to CD4 T cells directed against mismatched HLA class II alleles with occurrence of both GVL and GVHD. Of course, also in case of a partially mismatched donor, an immune response to MiHA presented in shared HLA can occur. However, it is likely that the immune response is overwhelmed by CD4 T cells directed against the mismatched HLA alleles. The precursor frequencies of MiHA specific T cells are very low compared to the precursor frequencies of allo-HLA directed T cells. The T-cell repertoire of an healthy individual contains T cells interacting with peptides in the context of non-self-HLA molecules. Mixed lymphocyte reactions showed that roughly 1-10% of all T cells in an individual may respond to stimulation by cells from allogeneic HLA mismatched individuals.[35-38] An other explanation for the stronger immune response in the HLA mismatched setting is that allo-HLA directed T cells can originated from both naïve as well as memory T cells, resulting in a stronger and also quicker immune response after infusion, whereas MiHA specific CD4 T cells originate from the naïve T-cell compartment of the donor and the immune response will only be initiated after activation of the T cells by professional APCs. In the setting of an HLA identical sibling donor with an immune response from MiHA specific CD4 T cells, both the magnitude as well as the diversity of the immune response will be lower than in case of allo-HLA directed CD4 T cells, resulting in a lower risk of development of GVHD.[55]

We described in this thesis in detail the immune response occurring in a selection of patients that received CD4 DLI. Several questions still remain that need to be further investigated. Obviously, the overall effect of CD4 DLI needs to be analyzed when the ongoing clinical trial is completed in which we treat patients three months after TCD alloSCT with purified donor CD4 cells. Effects of CD4 DLI on infection rate, disease status, conversion of chimerism and occurrence of GVHD will be evaluated by comparing the intervention group with the placebo group. Furthermore, it needs to be investigated whether alloreactive CD4 T cells directed against mismatched HLA-DP alleles can be used to induce GVL without GVHD.

In the setting of CD4 DLI after HLA 10/10 matched but HLA-DPB1 mismatched alloSCT, occurrence of both GVL and GVHD was observed due to CD4 T cells directed against the mismatched HLA class II molecule, usually HLA-DPB1. However, it has been described that GVL without GVHD due to allo-HLA-DP directed CD4 T cells from HLA-DP mismatched donors may also occur.[23] Among allo-HLA-DP directed T cells, we previously found T cells with tissue restricted recognition, including hematopoiesis restriction.[56] Since T cells directed against HLA molecules are peptide specific, it would be interesting to investigate whether donor CD4 T cells that recognize hematopoietic specific peptides in the context of the mismatched HLA-DP can be purified and infused into patients.[54] These T cells are expected to result in GVL without GVHD, the ultimate goal in alloSCT. However, it may not be feasible to obtain a cellular product containing enough purified CD4 T cells specific for an hematopoiesis restricted peptide in mismatched HLA-DP by isolation or enrichment from donor mononuclear cells. The precursor frequencies of these CD4 T cells will be as low as the precursor frequencies of MiHA specific CD4 T cells. However, in vitro generation of T-cell clones against HLA-DP-peptide complexes of interest will make it possible to identify the T-cell receptor (TCR) of these T cells and use this TCR for TCR gene transfer. HLA-DP mismatched donor T cells lacking the HLA-DP-peptide complex of interest can then be transduced with the specific TCR to obtain reactivity against patient hematopoietic cells expressing the HLA-DP-peptide complex. For this strategy first hematopoiesis specific peptides will be identified as potential suitable targets. The results obtained from peptide elution from hematopoietic cells like EBV-LCL, K562 cells and primary AML samples followed by mass spectrometry will be helpful for the identification of frequently presented peptides in HLA-DP. Once identified, it should be possible to generate T-cell clones directed against the peptide-HLA complex and to use the TCR from these T cells for TCR gene transfer. The ultimate aim of this strategy will be to infuse TCR gene modified T cells after TCD alloSCT from 10/10 matched, but HLA-DP mismatched donors to target monomorphic hematopoiesis specific peptides in patient specific HLA-DP resulting in an hematopoiesis restricted immune response with GVL but without GVHD.

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