

## PRAME-Specific Allo-HLA-Restricted T Cells with Potent Antitumor Reactivity Useful for Therapeutic T-Cell Receptor Gene Transfer

Avital L. Amir<sup>1</sup>, Dirk M. van der Steen<sup>1</sup>, Marleen M. van Loenen<sup>1</sup>, Renate S. Hagedoorn<sup>1</sup>, Renate de Boer<sup>1</sup>, Michel D.G. Kester<sup>1</sup>, Arnoud H. de Ru<sup>2</sup>, Gert-Jan Lugthart<sup>1</sup>, Cees van Kooten<sup>3</sup>, Pieter S. Hiemstra<sup>4</sup>, Inge Jedema<sup>1</sup>, Marieke Griffioen<sup>1</sup>, Peter A. van Veelen<sup>2</sup>, J.H. Frederik Falkenburg<sup>1</sup>, and Mirjam H.M. Heemskerk<sup>1</sup>

### Abstract

**Purpose:** In human leukocyte antigen (HLA)-matched stem cell transplantation (SCT), it has been shown that beneficial immune response mediating graft-versus-tumor (GVT) responses can be separated from graft-versus-host disease (GVHD) immune responses. In this study, we investigated whether it would be possible to dissect the beneficial immune response of allo-HLA-reactive T cells with potent antitumor reactivity from GVHD-inducing T cells present in the detrimental immune response after HLA-mismatched SCT.

**Experimental Design:** The presence of specific tumor-reactive T cells in the allo-HLA repertoire was analyzed at the time of severe GVHD after HLA-mismatched SCT, using tetramers composed of different tumor-associated antigens (TAA).

**Results:** High-avidity allo-HLA-restricted T cells specific for the TAA preferentially expressed antigen on melanomas (PRAME) were identified that exerted highly single-peptide-specific reactivity. The T cells recognized multiple different tumor cell lines and leukemic cells, whereas no reactivity against a large panel of nonmalignant cells was observed. These T cells, however, also exerted low reactivity against mature dendritic cells (DC) and kidney epithelial cells, which was shown to be because of low PRAME expression.

**Conclusions:** On the basis of potential beneficial specificity and high reactivity, the T-cell receptors of these PRAME-specific T cells may be effective tools for adoptive T-cell therapy. Clinical studies have to determine the significance of the reactivity observed against mature DCs and kidney epithelial cells. *Clin Cancer Res*; 17(17); 5615–25. ©2011 AACR.

### Introduction

Alloreactive T cells can mediate detrimental graft-versus-host disease (GVHD) as well as beneficial graft-versus-tumor (GVT) responses after allogeneic stem cell transplantation (allo-SCT). The alloreactivity of T cells after human leukocyte antigen (HLA)-matched allo-SCT is mainly directed against peptides derived from polymorphic proteins that differ between patient and donor, and are processed and presented in the context of HLA class I and II molecules. Previously, we and others have shown that GVT reactivity can be separated from the GVHD reactivity within

the polyclonal alloreactive immune responses mediating GVHD (1). T cells that are reactive against polymorphic peptides derived from proteins expressed by multiple tissues induce GVHD, whereas T cells directed against polymorphic peptides derived from proteins exclusively expressed on hematopoietic cells steer the alloimmune response toward GVT (2). Analysis of the clinical responses of allo-SCT patients and the TCR repertoire of the alloreactive immune responses showed that beneficial GVT responses more frequently occur when the severity of the GVHD response increases due to the activation of polyclonal responses (3). This phenomenon is illustrated by the work of Goulmy and colleagues showing that a mismatch for the polymorphic peptide HA-1 was associated with a high risk of severe GVHD, indicating in first instance that HA-1-specific T cells may induce GVHD responses (4). However, in later studies it was shown that HA-1-specific immune responses also dominated in patients that experienced beneficial GVT responses (5), and that the expression profile of HA-1 was in accordance with a relative specific antileukemia reactivity of the T cells, because HA-1 is exclusively expressed by the hematopoietic lineage (6). These results imply that despite an overall detrimental clinical phenotype, beneficial GVT reactivities can appear

**Authors' Affiliations:** Departments of <sup>1</sup>Hematology, <sup>2</sup>Immunohematology and Blood Transfusion, <sup>3</sup>Nephrology, and <sup>4</sup>Pulmonology, Leiden University Medical Center, Leiden, The Netherlands

**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

**Corresponding Author:** Mirjam H.M. Heemskerk, Department of Hematology, Leiden University Medical Center, PO Box 9600, 2300RC Leiden, The Netherlands. Phone: 31-715262271; Fax: 31-715266755; E-mail: mhmheemskerk@lumc.nl

**doi:** 10.1158/1078-0432.CCR-11-1066

©2011 American Association for Cancer Research.

### Translational Relevance

Adoptive T-cell therapy with T cells expressing transgenic T-cell receptors (TCR) with antitumor reactivity is a promising therapy for cancer patients. However, identification of high-affinity TCRs specific for tumor-associated antigens (TAA) is a critical bottleneck in this strategy because high-avidity TAA-specific T cells are deleted by negative selection in the thymus. As allo-HLA reactivity of T cells is not subjected to negative selection, we investigated whether beneficial high-avidity TAA-specific T cells could be identified within an allo-HLA-directed immune response. Our search for TAA-specific T cells within a patient experiencing GVHD led to the discovery of 2 preferentially expressed antigen on melanomas (PRAME)-specific T-cell clones. On the basis of their single-peptide specificity and high antitumor reactivity, the TCRs of these PRAME-specific T cells may be effective tools for adoptive T-cell therapy.

in GVHD, and that these beneficial immune reactivities can be isolated from GVHD responses.

Treatment of patients with HLA-mismatched allo-SCT is associated with GVHD. Also, in this transplantation setting beneficial GVT alloreactivity is associated with GVHD, and depending on the HLA-mismatch, GVT is clinically observed (7). The alloimmune responses after HLA-mismatched allo-SCT will mainly be directed against peptides derived from monomorphic proteins presented in the context of allo-HLA molecules. T cells present in the polyclonal alloreactive immune response directed against allo-HLA-presenting peptides derived from proteins that are ubiquitously expressed may induce GVHD. In contrast, T cells directed against allo-HLA-presenting peptides derived from monomorphic proteins exclusively expressed on the tumor cells, and therefore capable of inducing selective GVT reactivity may also be part of this immune response. This may imply that despite occurrence of a clinical detrimental immunologic war after HLA-mismatched SCT, characterization of the fine specificity of alloreactive T cells may result in the isolation of allo-HLA-reactive T cells that mediate beneficial GVT responses.

As allo-HLA molecules are not expressed within the thymic environment during lymphopoiesis, T cells are able to recognize peptides with high affinity in the context of the allo-HLA, because allo-HLA reactivity of T cells is not subjected to negative selection. Therefore, in contrast to self-restricted T cells, allo-HLA-reactive T cells with antitumor reactivity may exert high avidity for the allo-HLA-presenting tumor-associated antigen (TAA) derived from overexpressed or tissue-specific self-proteins. Many research groups have tried to isolate or generate high-affinity TAA-specific TCRs. Stauss and colleagues, as well as other research groups, have confirmed that high-affinity T cells specific for TAA can be derived from the allo-HLA repertoire (8–10). However, these studies showed that stringent selections have to be done to identify high-affinity

single-peptide-specific T cells from these *in vitro* stimulations (8–10). In addition, selection from the murine repertoire can be alternative options to isolate high-affinity TCRs (11, 12). Examples of overexpressed tumor antigens that may be of potential interest in human leukemia are Wilms' tumor 1 (WT-1), and proteinase 3 (Pr3). In addition, the preferentially expressed antigen on melanomas (PRAME) is of potential interest because it is highly expressed in many different cancers, including acute and chronic myeloid and lymphoid leukemias (13), whereas normal tissues have low PRAME expression (14). High-affinity TCRs specific for TAA that are shared between various tumors would be attractive tools for TCR gene therapeutic strategies (15, 16).

In this study, we hypothesized that within the detrimental immunologic war that is induced after HLA-mismatched SCT by donor T cells reacting against patient allo-HLA donor T cells may also selectively be activated and expanded that mediate beneficial GVT responses. For this purpose, we analyzed in a patient experiencing severe GVHD after HLA-A2-mismatched SCT whether high-avidity T cells directed against allo-HLA-presenting TAAs could be observed by using HLA-A2 tetramers composed of different TAA peptides. We observed polyclonal allo-HLA-restricted T cells specific for PRAME. These T cells were shown to exert single-peptide specificity and recognition was strictly correlated with PRAME expression. The isolated allo-HLA-restricted T-cell clones were in contrast to self-restricted T-cell clones highly reactive against multiple different PRAME<sup>+</sup> tumor cell lines as well as freshly isolated metastatic melanoma and primary leukemic cells, whereas no reactivity against a large panel of nonmalignant cells was observed. However, the clones exerted limited on-target reactivity against mature dendritic cells (mDC) and kidney epithelial cells. On the basis of their potential beneficial specificity and high reactivity against numerous different tumors, the high-affinity TCRs from the allo-restricted PRAME-specific T cells may be effective tools for broad application of TCR gene therapy.

### Materials and Methods

#### Isolation and analysis of PRAME-specific T cells

All studies were conducted with approval of the institutional review board at Leiden University Medical Center. After informed consent, peripheral blood mononuclear cells (PBMC) were collected from a patient suffering from AML that experienced acute GVHD after single HLA-A2-mismatched SCT and subsequent donor lymphocyte infusion (DLI). On the basis of a cross-over, the patient was HLA-A\*0201<sup>+</sup> and the sibling donor was HLA-A\*0201<sup>-</sup>, whereas all other HLA class I and II molecules were completely matched. Patient PBMCs collected during GVHD were stained with anti-HLA-A2-FITC (Pharmingen), anti-HLA-DR-APC (Pharmingen), and anti-CD8-PE (BD Bioscience) for 30 minutes at 4°C, and activated (HLA-DRpos), donor-derived (HLA-A2neg) CD8<sup>+</sup> T cells were isolated by cell sorting (FACSaria). As PBMCs were limited, the sorted T cells were first expanded with anti-CD3/CD28

and irradiated autologous PBMCs ( $0.5 \times 10^6/\text{mL}$ ) in T-cell medium (see Supplementary Data). After 14 days of culture, T cells were labeled with anti-CD8-APC (BD Bioscience) and phycoerythrin (PE)-conjugated HLA-A2 tetramers specific for the different TAA peptides (17–20) were tested: VLDGLDVLL (VLD), SLYSFPEPEA (SLY), ALYVDSLFFL (ALY), and SLLQHLIGL (SLL), for PRAME; RMFPNAPYL, for WT-1; and VLQELNVTV, for Pr-1. For single-cell sorting, T cells were stained with APC-conjugated tetramers in combination with TCR- $\text{V}\beta$  repertoire kit staining (Beckman Coulter) for 1 hour at  $4^\circ\text{C}$ , and SLL tetramer $^+$   $\text{V}\beta 1^+$  and SLL tetramer $^+$   $\text{V}\beta 3^+$   $\text{CD}8^+$  T cells were sorted and stimulated nonspecifically (see Supplementary Data).

Self-restricted PRAME-specific T-cell clones were isolated from an HLA-A\*0201 patient that was transplanted with a fully HLA-identical donor graft. PBMCs derived from the patient after SCT were labeled with PE-conjugated SLL tetramer for 1 hour at  $4^\circ\text{C}$ . Tetramer $^+$  T cells were isolated by magnetic-activated cell sorting by using anti-PE-coated magnetic beads (Miltenyi Biotec) and were expanded for 10 days with anti-CD3/CD28 beads as described earlier. For subsequent sorting, T cells were stained with PE-conjugated SLL tetramer and anti-CD8-APC for 1 hour at  $4^\circ\text{C}$ , and tetramer $^+$   $\text{CD}8^+$  T cells were sorted single cell per well and expanded. Three SLL tetramer $^+$  T-cell clones were selected, and used for further analysis.

#### Functional reactivity of the PRAME-specific T-cell clones

Stimulation assays were conducted with 5,000 T cells and 20,000 targets in 96-well plates in Iscove's Modified Dulbecco's Medium, supplemented with 10% human serum (HS) and 100 IU/mL interleukin 2 (IL-2). The different malignant and nonmalignant cells were collected and prepared as described in Supplementary Data. For peptide titrations, T2 cells were preincubated for 1 hour with different concentrations of peptide, and washed. After 18 hours of stimulation, supernatant was harvested and IFN- $\gamma$  production was measured by standard ELISA. In the cytotoxicity assays, T cells were tested at different effector/target (E/T) ratios against 1,000  $^{51}\text{Cr}$ -labeled targets in 96-well plates in a standard 4-hour  $^{51}\text{Cr}$ -release assay. In these experiments a control HLA-A2-restricted T-cell clone HSS12 specific for a peptide coded by the *USP11* gene was included.

#### Peptide elution, reverse phase high-performance liquid chromatography, and mass spectrometry

Peptide elution, reverse phase high-performance liquid chromatography (RP-HPLC), and mass spectrometry (MS) were carried out as previously described (21). Briefly,  $3 \times 10^{10}$  Epstein Barr Virus transformed B-cells (EBV-LCL) were lysed and the peptide-HLA-A2 complexes were purified by affinity chromatography, using HLA-A2-specific BB7.2 monoclonal antibody (mAb). Subsequently, peptides were eluted from HLA-A2 molecules, and separated from the HLA monomers and  $\beta 2$ -microglobulin by size filtration. After freeze drying, the peptide mixture was subjected to a first round of RP-C18-HPLC using a water/acetonitrile/

trifluoroacetic acid (TFA), and fractions were collected. A small sample of each fraction was loaded on T2 cells and tested for recognition by the T-cell clones. The recognized fraction was subjected to a second and a third round of RP-C18-HPLC fractionation. In the second fractionation, a water/isopropanol/TFA gradient was used, and in the third fractionation a water/methanol/formic acid gradient was used. After the third fractionation, the peptide masses present in the recognized fractions and in the adjacent nonrecognized fractions were determined by MS. Peptides of which the abundance correlated with the recognition pattern of the T-cell clone were selected for tandem MS and their sequences determined.

#### PRAME expression by quantitative real-time PCR, and inhibition of PRAME expression by silencing RNA

PRAME expression was quantified by real-time PCR (TaqMan); for details see Supplementary Data. Inhibition of PRAME was carried out by using retroviral vectors encoding for short hairpin RNA (shRNA) sequences specific for PRAME in combination with the puromycin resistance gene that were kindly provided by Dr. R. Bernards, NKI, Amsterdam, the Netherlands (22). Retrovirally transduced cells were cultured with different concentrations of puromycin for at least 1 week before testing. Proximal tubular epithelial cells (PTEC) were cultured at a concentration of  $3 \mu\text{g}/\text{mL}$ , renal cell carcinoma cell line RCC1257 at  $4 \mu\text{g}/\text{mL}$ , and  $\text{CD}34^+$ -derived dendritic cells ( $\text{CD}34\text{DCs}$ ) at  $0.4 \mu\text{g}/\text{mL}$ .  $\text{CD}34\text{DCs}$  were generated as described in Supplementary Data, and were transduced on day 1 of culture.

#### TCR gene transfer

The *TCRAV* and *TCRBV* gene usage of clone HSS1 was determined by reverse transcriptase (RT)-PCR and sequencing (23). HSS1 expressed *TCR-AV1S1* and *TCR-BV1S1*. A retroviral vector was constructed with a codon-optimized and cysteine-modified  $\text{TCR}\alpha$  and  $\text{TCR}\beta$  chain linked by the T2A sequence in combination with the truncated nerve growth factor receptor ( $\Delta\text{NGF-R}$ ; refs. 24, 25). Cytomegalovirus (CMV)-IE1-specific HLA-B8-restricted T cells were sorted by using CMV-IE1 tetramers, stimulated for 2 days with phytohemagglutinin and irradiated allogeneic PBMCs, and transduced with PRAME-TCR or mock. Transduced T cells were sorted on the basis of positivity for  $\Delta\text{NGF-R}$ , and tested for functional reactivity.

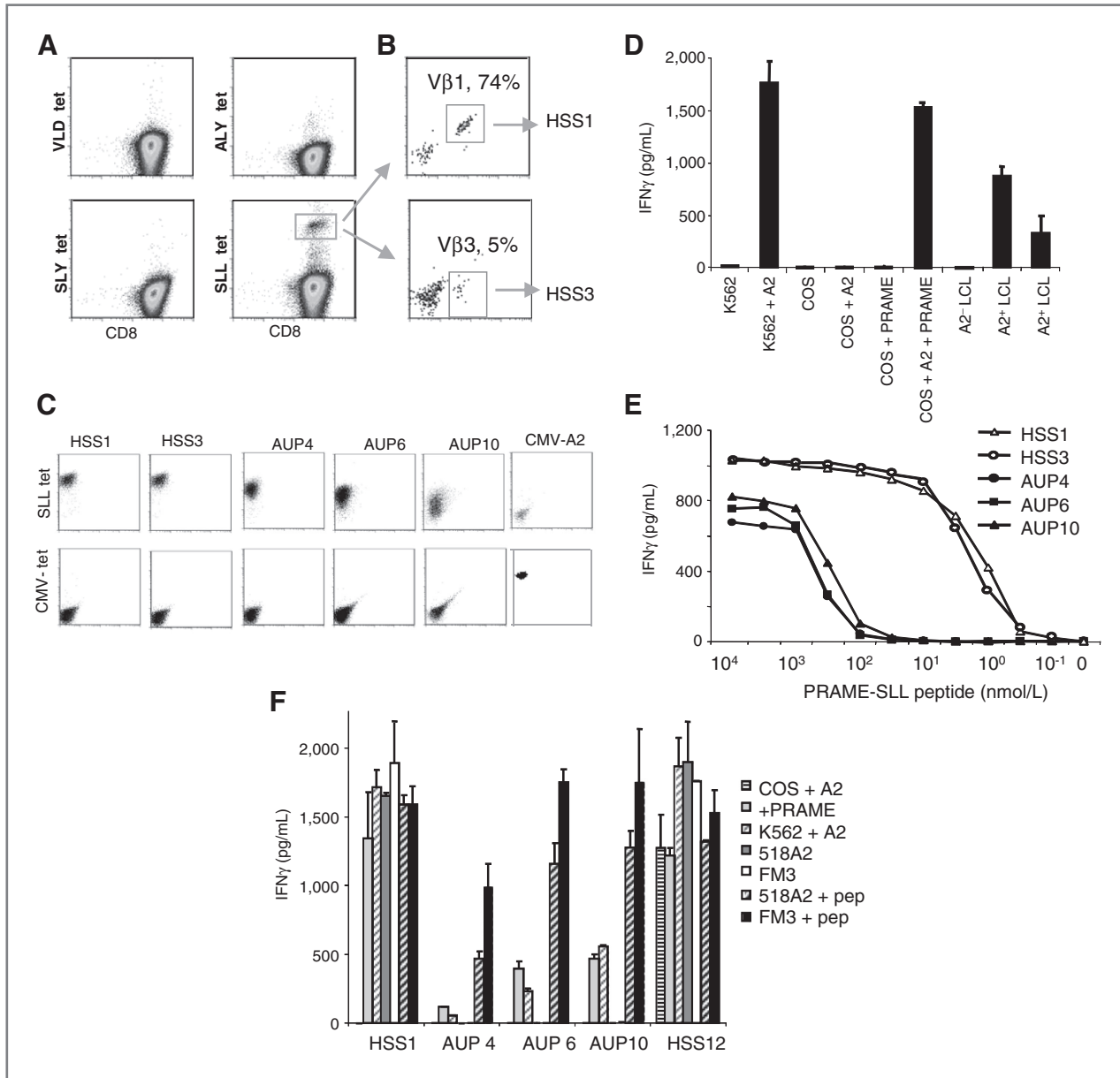
## Results

#### Isolation of high-avidity PRAME-specific allorestricted T-cell clones from a patient experiencing GVHD after HLA-mismatched SCT

The presence of allo-HLA-restricted TAA-specific T cells was analyzed in a patient transplanted with a single HLA-A2-mismatched SCT that experienced GVHD after treatment with DLI for relapsed acute myeloid leukemia (AML). The activated donor-derived  $\text{CD}8^+$  T cells at the time of GVHD were selected by fluorescence-activated cell sorting (FACS) and expanded by using  $\text{CD}3/\text{CD}28$  expansion

beads to be able to conduct multiple reactivity screenings. T cells were labeled with tetramers specific for the HLA-A2-restricted epitopes of different TAAs. The T cells did not stain with most TAA tetramers; however, 0.6% of CD8<sup>+</sup> T cells specifically stained with the PRAME-SLL tetramer

(Fig. 1A). TCR-V $\beta$  usage analyzed by TCR-V $\beta$ -specific mAbs illustrated that the SLL tetramer<sup>+</sup> T cells consisted of a minimum of 3 different clonal populations, one dominant TCR-V $\beta$ 1<sup>+</sup> population (74%), a TCR V $\beta$ 3<sup>+</sup> population (5%), and a T-cell population with unknown



**Figure 1.** Isolation of allorestricted and self-restricted PRAME-specific T-cell clones. A, activated (HLA-DRpos), donor-derived (HLA-A2neg) CD8<sup>+</sup> T cells were isolated from a patient experiencing GVHD after HLA-A2-mismatched SCT and subsequent DLI. The isolated T cells were expanded by using CD3/CD28 beads and labeled with anti-CD8 and TAA tetramers (tet); only the PRAME tetramers (VLD, SLY, ALY, and SLL) are shown. B, to determine the TCR-V $\beta$  usage, the T cells were labeled with anti-CD8, SLL tetramer, and the V $\beta$  repertoire kit. The V $\beta$ 1<sup>+</sup> (74% of tet<sup>+</sup>) and V $\beta$ 3<sup>+</sup> (5% of tet<sup>+</sup>) T cells were single-cell sorted. C, allorestricted PRAME-SLL clones HSS1 and HSS3, CMV-A2 control clone, and the self-restricted PRAME-SLL clones AUP4, AUP6, and AUP10 were stained with SLL- and pp65-tetramer. D, HSS1 was tested against K562, K562 transduced with HLA-A2 (K562 + A2), untransduced COS cells (COS), COS transduced with HLA-A2 (COS + A2), COS + A2 transfected with PRAME (COS + PRAME), COS transduced with HLA-A2 and transfected with PRAME (COS + A2 + PRAME), HLA-A2<sup>-</sup> EBV-LCL (A2<sup>-</sup> LCL), and 5 HLA-A2<sup>+</sup> EBV-LCLs of which 2 are shown (A2<sup>+</sup> LCL). E, HSS1, HSS3, AUP4, AUP6, and AUP10 were stimulated with T2 cells loaded with titrated concentrations of the PRAME-SLL peptide. F, HSS1, AUP4, AUP6, and AUP10, and the USP11-specific T-cell clone HSS12 used as a control, were tested for IFN- $\gamma$  production against COS + A2, COS + A2 transfected with PRAME (+PRAME), K562 + A2, 2 HLA-A2<sup>+</sup> melanomas (518A2 and FM3) unloaded, or loaded with PRAME peptide (+ pep).



TCR-V $\beta$  usage (20%; Fig. 1B). To analyze the specificity and avidity of the PRAME-specific T cells, the SLL tetramer<sup>+</sup> V $\beta$ 1<sup>+</sup> and V $\beta$ 3<sup>+</sup> CD8<sup>+</sup> T cells were single-cell sorted and expanded. Of both cell subsets, one T-cell clone was selected for further analysis. Both T-cell clones HSS1, expressing TCR-V $\beta$ 1, and HSS3, expressing TCR-V $\beta$ 3, efficiently stained with the SLL tetramer, but not with control CMV-pp65 tetramer (Fig. 1C, left). FACS analysis with V $\beta$ -specific mAbs confirmed their difference in clonal origin (data not shown).

To determine whether the PRAME-specific T-cell clones effectively recognized endogenously processed and presented PRAME, clone HSS1 was tested against K562, previously shown to highly express PRAME (14), and K562 transduced with HLA-A2. To show that recognition was actually directed against PRAME-derived peptide presented in HLA-A2, clone HSS1 was tested against COS cells, either only expressing HLA-A2 or PRAME, or expressing both HLA-A2 and PRAME. In addition, clone HSS1 was tested against different HLA-A2<sup>+</sup> and HLA-A2<sup>-</sup> EBV-LCLs. As shown in Fig. 1D, HSS1 efficiently recognized K562 + A2 and COS cells expressing both PRAME and HLA-A2, whereas untreated K562 and COS cells as well as COS cells expressing only HLA-A2 or PRAME were not recognized. These results indicate that HSS1 recognized endogenously processed and presented PRAME peptide in the context of HLA-A2. Interestingly, HSS1 also recognized all 5 different HLA-A2<sup>+</sup> EBV-LCLs, although with variable strength, whereas HLA-A2<sup>-</sup> EBV-LCLs were not recognized (reactivity against 2 representative HLA-A2<sup>+</sup> and 1 HLA-A2<sup>-</sup> EBV-LCL are shown in Fig. 1D).

As it has not yet been shown that EBV-LCLs express PRAME, and since these T-cell clones were allo-HLA reactive, we investigated whether clone HSS1 and HSS3 in addition to PRAME also recognized other peptides presented in HLA-A2. For this purpose, the HLA-A2<sup>+</sup> EBV-LCLs recognized most prominently by the allorestricted clones were expanded to  $3 \times 10^{10}$  cells, HLA-A2 was isolated by affinity chromatography, and peptides eluted from HLA-A2 were fractionated by multidimensional RP-HPLC, and loaded onto T2 cells (for detailed information see Supplementary Data). As shown in Supplementary Fig. S1A, HSS1 was reactive against T2 cells loaded with only one RP-HPLC fraction. This recognized fraction was subfractionated by using a different HPLC gradient composition, and loaded on T2 cells. Again, one fraction of the second RP-HPLC separation was efficiently recognized by HSS1, and this fraction was again further subfractionated. The peptide masses present in the recognized third dimension RP-HPLC fractions and in the adjacent not recognized fractions were determined by MS. Comparing the presence and abundance of these peptide masses to the recognition pattern of the T-cell clone resulted in one unique peptide candidate, which was analyzed by tandem MS to be the SLLQHLLGL peptide of PRAME. The synthetic PRAME peptide showed a fragmentation pattern identical to the fragmentation pattern of the natural eluted peptide. Similar results were obtained with clone HSS3 (data not shown).

These results show that reactivity of the alloreactive PRAME-specific T-cell clones against EBV-LCLs was mediated by the recognition of PRAME presented in HLA-A2, and in addition show that the alloreactive T-cell clones derived from GVHD exerted single-peptide-specific recognition.

#### Comparing the functional avidity of allo-HLA and self-HLA-restricted PRAME-specific T-cell clones

Because we speculated that the avidity of allorestricted PRAME-specific T cells would be significantly higher than self-restricted PRAME-specific T cells, we isolated by flow cytometry using the SLL tetramer 3 PRAME-SLL-specific self-restricted T-cell clones (AUP4, AUP6, and AUP10) from an HLA-A2<sup>+</sup> patient after HLA-matched SCT. The self-restricted PRAME-specific T-cell clones showed specific staining with the SLL tetramer, although with lower intensity than the allorestricted T-cell clones (Fig. 1C, right).

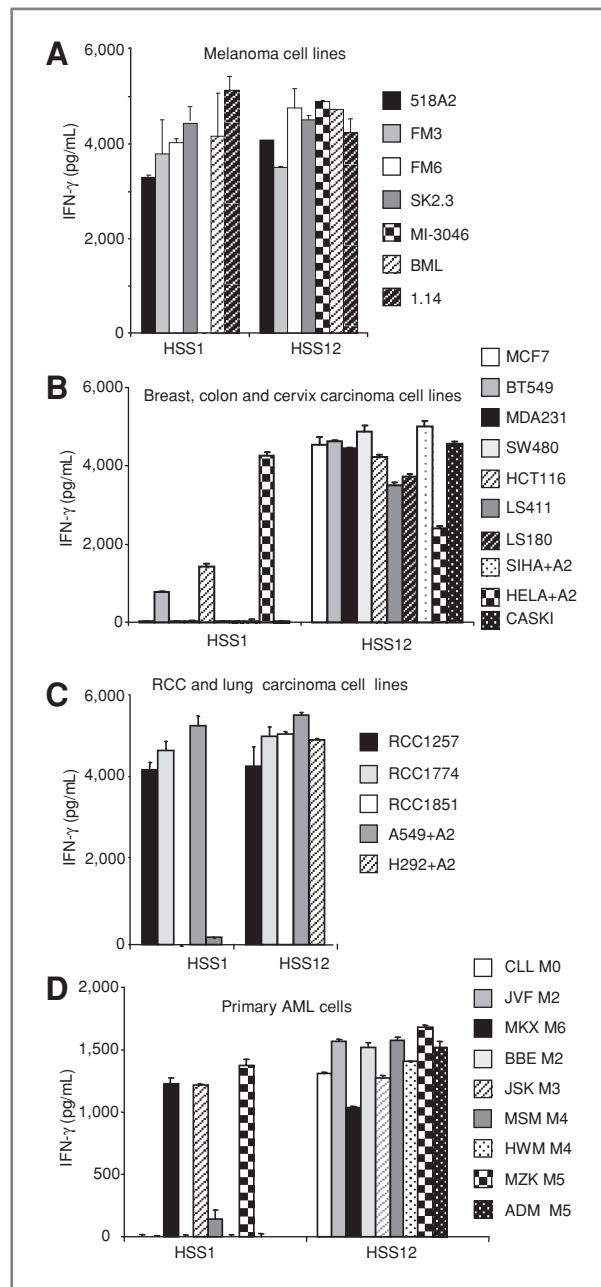
To compare the functional avidity of the self- and allorestricted PRAME-specific T-cell clones, AUP4, AUP6, AUP10, HSS1, and HSS3 were tested against T2 cells loaded with different concentrations of SLL peptide. Although all clones were able to recognize the SLL peptide, the peptide concentration needed for 50% of maximum IFN- $\gamma$  production differed significantly between the self- and allorestricted clones. As shown in Fig. 1E, HSS1 and HSS3 required only 1 to 2 nmol/L of peptide for half-maximal cytokine production, whereas AUP4, AUP6, and AUP10 needed approximately 300 nmol/L of peptide. These results show that in contrast to the allorestricted PRAME-specific T-cell clones, the self-restricted PRAME-specific T-cell clones exerted low-avidity recognition.

To investigate whether this large difference in avidity has consequences for reactivity of the clones against endogenously processed PRAME, the self- and allorestricted clones were tested against COS + A2 transfected with PRAME, K562 + A2, and 2 melanomas (Fig. 1F). As a positive control for the HLA-A2 expression and stimulatory capacity of the targets, the allo-HLA-A2-restricted control clone HSS12 specific for *USP11* was also tested against all targets. Clone HSS1 efficiently recognized all PRAME-expressing cell lines. Although the self-restricted clones intermediately recognized PRAME transfected COS + A2 as well as K562 + A2, the melanomas known to express approximately a 5- to 10-fold lower amount of PRAME than K562 were not recognized. The melanomas were strongly recognized by all 4 clones when exogenously loaded with SLL peptide. These results show that high-avidity allorestricted PRAME-specific clones efficiently recognize melanoma, whereas the low-avidity self-restricted PRAME clones exerted no reactivity against melanoma.

#### High-avidity allo-HLA-restricted PRAME-specific T cells effectively recognized a large proportion of tumors and a limited number of nonmalignant cells

The *PRAME* gene is expressed at a high level in a large proportion of tumors, including melanomas, non-small-cell lung carcinomas, renal cell carcinoma (RCC), breast carcinoma, cervix carcinoma, colon carcinoma, as well as

several types of leukemia (11, 15–17). We therefore analyzed whether clone HSS1 was able to recognize HLA-A2<sup>+</sup> tumor cell lines and primary tumor cells. Clone HSS12 was used as a control for the HLA-A2 expression of the targets and the ability of the targets to stimulate T cells. Figure 2A



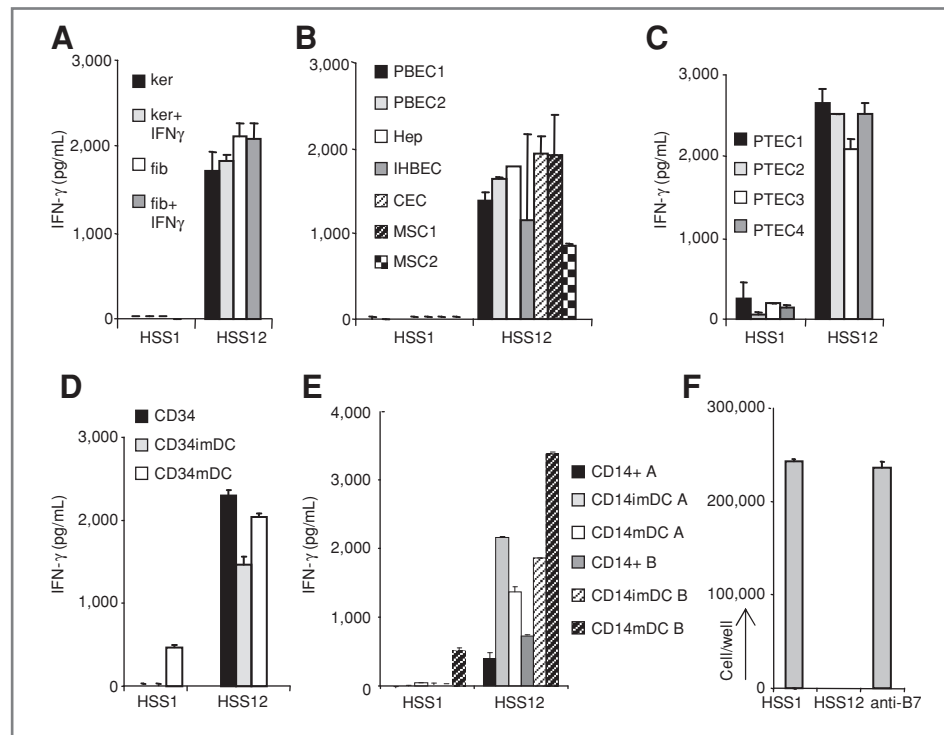
**Figure 2.** Allo-HLA-restricted clone HSS1 specific for PRAME is able to recognize different tumor cell lines and primary malignancies. HSS1 and control HSS12 were tested for IFN- $\gamma$  production against HLA-A2<sup>+</sup> (A) melanomas (518A2, FM3, FM6, SK2.3, MI-3046, BML, and 1.14); (B) breast carcinomas (MCF-7, BT549, and MDA231), colon carcinomas (SW480, HCT116, LS411, and LS180), and cervix carcinomas (SIHA + A2, HELA + A2, and CASKI); (C) RCCs (RCC1257, RCC1774, and RCC1851) and lung carcinomas (A549 + A2 and H292 + A2); and (D) 9 primary AMLs of different classification (as indicated).

shows that in addition to the 2 previously tested melanomas, 4 different melanomas were also efficiently recognized by clone HSS1. In addition, from an HLA-A2<sup>+</sup> patient with lymph node metastatic melanoma we freshly isolated melanoma cells by ficolling the minced tumor cells and subsequently sorting the CD45, CD3, CD19, CD14, CD56 negative cells. HSS1 as well as the control HSS12 highly recognized the freshly isolated tumor cells, whereas a CMV-A2 control clone did not recognize the metastatic melanoma (Supplementary Fig. S2A). As shown in Fig. 2B and C, clone HSS1 intermediately recognized 1 of 3 breast carcinomas and 1 of 4 colon carcinomas, and efficiently recognized 1 of 3 cervix carcinomas, 2 of 3 RCCs, and 1 of 2 lung carcinomas. Three HLA-A2<sup>+</sup> acute lymphoblastic leukemias (ALL) were not recognized, but 2 of these 3 ALLs were recognized after activation with CD40L and CpG (Supplementary Fig. S2B). In addition, 4 of 9 primary AMLs were recognized by HSS1, of which 3 were strongly recognized (Fig. 2D). Also, the patient AML was tested for recognition, however no recognition was seen. As no material of the patient was available anymore, we determined using 4 primary AML samples from other patients with similar classification (M5) whether activated AML could be recognized. Supplementary Fig. S2C shows that without activation 2 of 4 AML samples were recognized, after activation all 4 tested AML samples were recognized.

Because PRAME has been described to be expressed at low and intermediate levels in certain normal tissues, we tested HSS1 against different HLA-A2<sup>+</sup> nonmalignant cell types. HSS1 showed no reactivity against fibroblasts and keratinocytes with or without pretreatment with IFN- $\gamma$  (Fig. 3A), primary bronchus epithelial cells, hepatocytes, intrahepatic biliary epithelial cells, colon epithelial cells, or mesenchymal stromal cells (Fig. 3B). In contrast, the clone showed low but significant recognition of all 12 tested HLA-A2<sup>+</sup> PTEC derived from kidney tubules, of which 4 are shown in Fig. 3C. Testing the clone against HLA-A2<sup>+</sup> nonmalignant cells of hematopoietic origin showed that HSS1 did not recognize B cells, CD40L-activated B cells or *in vivo* activated B cells derived from inflamed tonsils (Supplementary Fig. S3A and B). In addition, T cells and activated T-cell blasts were not recognized (data not shown). M $\phi$ 1 and M $\phi$ 2 macrophages (Supplementary Fig. S3C), CD34 cells or immature CD34DCs (Fig. 3D), monocytes (CD14), or immature CD14-derived DCs (CD14DC; Fig. 3E) were also not recognized by the PRAME-specific T-cell clone. However, clone HSS1 showed low reactivity against mDCs derived from CD34 and CD14 cells (Fig. 3D and E, respectively). To investigate whether the PRAME-specific T cells were also reactive against blood-derived myeloid and plasmacytoid DCs (MDC and PDC, respectively), HSS1 was tested against MDCs and PDCs. No recognition of immature MDCs and PDCs and mature PDCs was observed. However, low reactivity against mature MDCs was observed (data not shown).

To determine whether allorestricted PRAME-specific T cells were also reactive against other myeloid cell lineages, HSS1 was tested against proliferating and differentiating

**Figure 3.** Recognition of nonmalignant cells by the allo-HLA-restricted PRAME T cells. HSS1 and control HSS12 were tested for IFN- $\gamma$  production against HLA-A2<sup>+</sup> (A) fibroblasts (fib) and keratinocytes (ker) treated with (+ IFN- $\gamma$ ) or without IFN- $\gamma$ ; (B) primary bronchus epithelial cells (PBEC1 and PBEC2), hepatocytes (Hep), intrahepatic biliary epithelial cells (IHBEc), colon epithelial cells (CEC), and mesenchymal stromal cells (MSC1 and MSC2); (C) 12 different PTECs, of which 4 are shown; (D) CD34 cells, CD34imDC, CD34mDC; (E) CD14 cells, CD14imDCs, CD14mDCs; and (F) HSS1, HSS12, and an HLA-B7-restricted T-cell clone (negative control) were tested in a carboxyfluorescein succinimidyl ester proliferation inhibition assay against HLA-A2<sup>+</sup>, HLA-B7<sup>-</sup> CD34 cells. The number of viable cells differentiated from CD34 cells was measured at 144 hours by FACS analysis.



CD34 cells in a proliferation inhibition assay (for detailed information see Supplementary Data). As is shown in Fig. 3F, HSS1 was not able to inhibit the proliferation of CD34 cells, and progeny of CD34 cells.

To investigate whether the high-avidity allorestricted PRAME T cells exerted antigen-specific cytolytic activity, HSS1 and control HSS12 were tested at different E/T ratio against HLA-A2<sup>+</sup> targets including 2 melanomas, 3 RCCs, CD14mDCs, and 2 PTECs. Clone HSS1 exerted highly cytolytic activity against the previously recognized HLA-A2<sup>+</sup> malignant cell lines, whereas only low reactivity against mature mDCs and PTECs was observed (Fig. 4A). The HSS12 control clone was highly reactive against all HLA-A2<sup>+</sup> targets, including mDCs and PTECs (Fig. 4B). These results show that the allorestricted PRAME T cells are able to exert efficient reactivity against a large proportion of tumors. In addition, the results indicate that the PRAME T cells exhibited low reactivity against nonmalignant mDCs and PTECs.

#### Expression of PRAME gene, determined by quantitative RT-PCR, strictly correlates with recognition of PRAME-specific T-cell clone

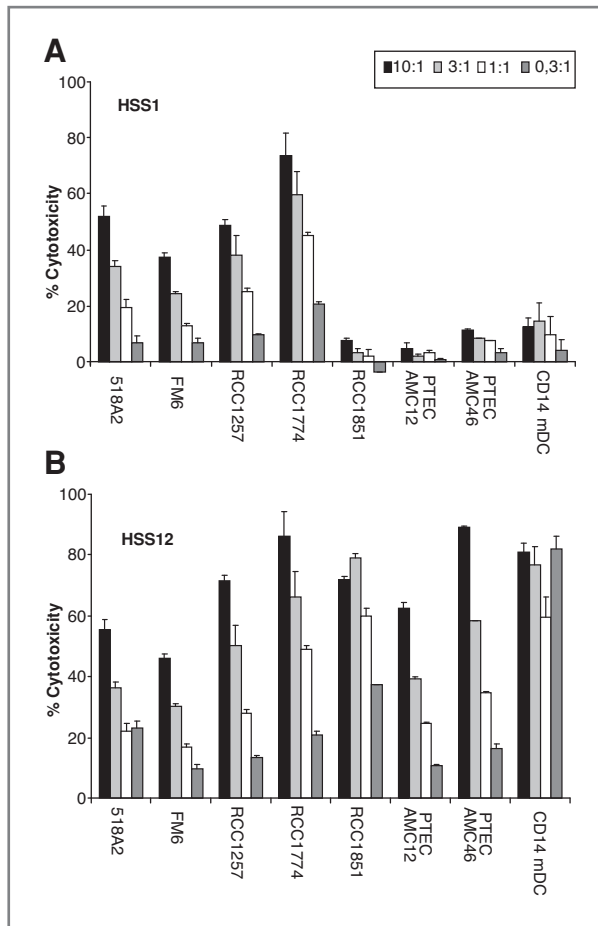
To establish whether the reactivity of the allo-HLA-restricted PRAME T cells correlated with expression of PRAME, we determined the mRNA expression level of PRAME by quantitative RT-PCR in the different cells. Figure 5 illustrates that reactivity of HSS1 strictly correlated with PRAME expression. Cells with high or intermediate PRAME expression were effectively or intermediately recognized by the T cells. Targets that did not

express PRAME were not recognized. Targets expressing low levels of PRAME were also not recognized, with the exception of CD40L-activated ALL, EBV-LCLs, and nonmalignant PTECs and mDCs.

To confirm the strict correlation between PRAME expression and recognition, and to exclude that the reactivity was because of off-target toxicity mediated by cross-reactivity against other peptide-HLA complexes, we transduced CD34mDCs, PTECs, and RCC1851 with PRAME silencing RNA (shRNA). The PRAME-specific shRNA almost completely blocked the recognition of CD34mDCs, PTECs, and RCC by HSS1, whereas the recognition of the targets by control HSS12 was unaltered (Fig. 6A). These results show that the reactivity against PTECs and mDCs by the allo-HLA-restricted PRAME T cells was based on PRAME recognition.

#### PRAME-TCR-transduced T cells exert clinically relevant PRAME-specific reactivity

On the basis of the high reactivity against a large proportion of tumors and limited on-target toxicity exerted by the high-avidity PRAME T cells, their high-affinity TCR may be effective tools for TCR gene therapeutic strategies (24, 26). Therefore, we investigated the functional activity of PRAME-TCR-transduced T cells. To prevent mixed TCR dimer formation and to optimize TCR expression, the TCR $\alpha$  and TCR $\beta$  chain of HSS1 were codon optimized and cysteine modified (24, 25). Figure 6B shows that the PRAME-TCR-transduced T cells exerted similar to the parental HSS1 high reactivity against PRAME peptide-loaded T2 cells and PRAME<sup>+</sup> tumor cells, whereas PTECs and

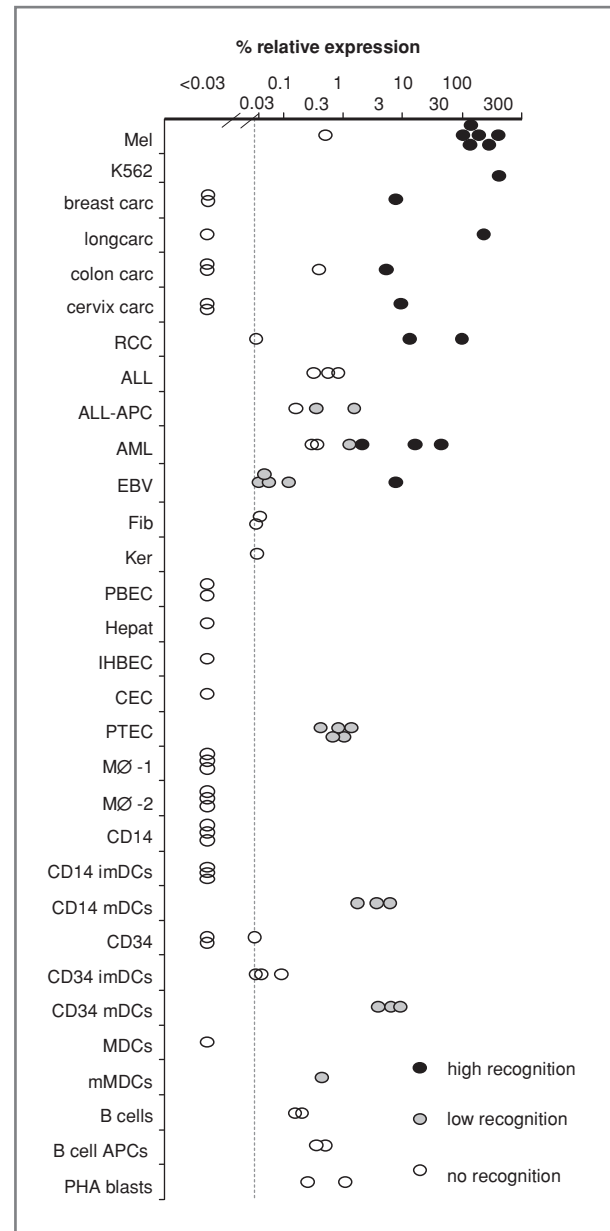


**Figure 4.** The allorestricted PRAME-specific T-cell clone HSS1 exerts specific cytotoxic activity. HSS1 (A) and control clone HSS12 (B) were tested for cytotoxicity against HLA-A2<sup>+</sup> melanomas (518A2 and FM6), RCCs (RCC1257, RCC1774, and RCC1851), PTECs, and CD14mDCs.

mDCs were only low recognized. To investigate the avidity of the PRAME-TCR-transduced T cells, these T-cells were compared with the parental high-avidity PRAME-specific T-cell clone HSS1 and to the low-avidity self-restricted T-cell clone AUP6 in a peptide titration experiment with the PRAME-SLL peptide loaded on T2 cells. As shown in Fig. 6C, PRAME-TCR-transduced T cells required comparable peptide concentration for 50% of maximum IFN- $\gamma$  production as the parental clone HSS1, showing that the avidity of PRAME-TCR-transduced T cells is similar to that of the parental high-avidity PRAME-specific T-cell clone. These results indicate that the PRAME-TCR of clone HSS1 can potentially be used for TCR gene therapy.

**Discussion**

In this study, we succeeded in isolating beneficial GVT-reactive T cells restricted by allo-HLA-A2 and specific for PRAME from a detrimental allo-HLA immune response after HLA-A2-mismatched SCT. We showed that the allo-restricted PRAME-specific T cells were single-peptide spe-

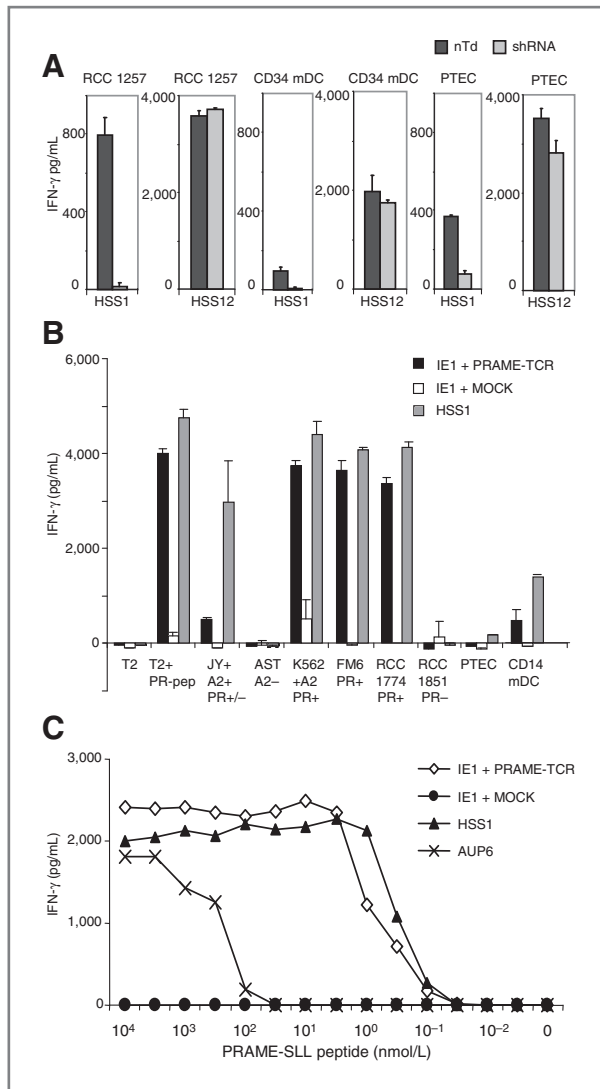


**Figure 5.** Correlation between functional reactivity of the PRAME-specific T-cell clone HSS1 and PRAME mRNA expression. The expression of PRAME mRNA in different cells was determined by quantitative RT-PCR, and referenced to the level of PRAME expression measured in Mel518. The limit of detection is indicated with the broken line. The black dots represent cells high or intermediate recognized by HSS1, gray dots represent cells low recognized by HSS1, and white dots represent cells not recognized by HSS1.

cific and, in contrast to self-restricted T cells, exerted high-avidity reactivity against PRAME-expressing tumor cells. The reactivity of the T cells strictly correlated with PRAME expression, indicating that the allorestricted PRAME T cells were not cross-reactive against other peptides presented in the context of HLA-A2. The clones showed high reactivity against a large panel of tumor cell lines, whereas a large

Downloaded from <http://aacrjournals.org/clinccancerres/article-pdf/17/17/5615/1999178/5615.pdf> by guest on 23 May 2024





**Figure 6.** PRAME-specific shRNA and PRAME-TCR transduction show that recognition of PTECs and mDCs is due to on-target toxicity, and tumor-specific reactivity of PRAME-TCR can be efficiently transferred. **A**, HSS1 and control HSS12 were tested against HLA-A2<sup>+</sup> RCC1257, CD34mDCs, and PTECs transduced with a retroviral vector encoding for a PRAME-specific shRNA and the puromycin selection gene. shRNA-transduced cells were cultured for 7 days with puromycin (0.4–4  $\mu$ g/mL) and used as stimulator cells. Nontransduced cells (nTd) were used as control stimulator cells. **B**, CMV-IE1-specific T cells transduced with PRAME-TCR or mock transduced, and HSS1 were tested against T2 cells, T2 cells loaded with PRAME peptide, HLA-A2<sup>+</sup> LCL-JY, K562 + A2, FM6, RCC1774, RCC1851, PTECs, CD14mDCs, and HLA-A2<sup>-</sup> LCL-AST. The expression of PRAME in the melanomas and RCCs is indicated. **C**, CMV-IE1-specific T cells transduced with the PRAME-TCR, mock-transduced CMV-IE1-specific T cells, HSS1 and AUP6 were tested against T2 cells loaded with titrated concentrations of the PRAME-SLL peptide.

panel of nonmalignant cells was not recognized. The allorestricted PRAME T cells exerted limited on-target toxicity against mDCs and PTECs. Finally, we showed high PRAME-specific tumor reactivity of PRAME-TCR-transduced T cells, indicating that the PRAME-TCR can potentially be used in TCR gene therapeutic strategies.

In the patient, at the time of severe GVHD approximately 85% of donor CD8 T cells were highly activated. By isolating these activated polyclonal CD8 T cells, we illustrated that 0.6% of T cells were PRAME-SLL specific, indicating that at the time of GVHD approximately 0.5% of the donor CD8 T cells were directed against PRAME. On the basis of our results we speculate that the PRAME-specific allorestricted T cells have likely been induced *in vivo* by activated patient-derived HLA-A2<sup>+</sup> mDCs or by the AML-DC. Although AML was not recognized in nonactivated state, 4 primary AML samples with similar classification were recognized after activation (Supplementary Fig. S2C), and may indicate that the AML-DC have induced the PRAME-specific T-cell response. On the basis of the limited reactivity against PTECs even after IFN- $\gamma$  activation, and the lack of expression of costimulatory molecules on these cells, we assume that it is unlikely that PTECs were the mediators of the PRAME-specific allorestricted T-cell response. Although the patient died of severe GVHD, at the time of the profound allo-HLA immune response in which a large variety of allo-HLA-reactive T cells including the PRAME-specific T cells were present, no clinical signs of nephrotoxicity were observed.

The results showing that mDCs express PRAME at levels that can activate high-avidity PRAME-specific T cells, also explains why self-restricted T cells exhibiting high avidity for the self-antigen PRAME can be deleted by negative selection during thymic development. The observation that we failed to isolate self-restricted PRAME-specific T cells exerting high-avidity PRAME-specific reactivity against tumor cells is in agreement with this hypothesis. In addition, although self-restricted PRAME-specific T cells that were previously isolated exhibited high peptide affinity, these T cells exerted low antitumor reactivity (27–29). On the basis of these findings we consider it unlikely that transfer of self-restricted PRAME-specific T cells (28, 29) or vaccination with PRAME (28–32), even combined with optimal adjuvant, will induce high-avidity PRAME-specific T cells exerting potent antitumor reactivity. In contrast, we isolated high-avidity PRAME-specific T cells by circumventing immune tolerance, because negative selection is limited to self-HLA. The reactivity of the allorestricted PRAME clones against multiple different tumor cell lines and primary leukemia show that these PRAME-TCRs could potentially be useful in adoptive T-cell therapy with TCR engineered T cells for the treatment of patients with many different malignancies.

The allorestricted PRAME-specific T-cell clones unfortunately also showed reactivity against mDCs and PTECs, due to low expression of PRAME. We hypothesize that the PRAME-specific T cells were induced in our patient after HLA-mismatched SCT and subsequent DLI by either the mDCs or the AML-DCs, and therefore we speculate that PRAME-TCR engineered T cells may be reactive against autologous mDCs. The recognition and thereby eradication of mDCs by PRAME-specific T cells could lead to immune impairment, because mDCs play an important role in the initiation of new immune responses.

In addition, the reactivity directed against PTECs could possibly lead to renal failure. It has indeed been previously shown that recognition of specific nonmalignant cells by administered high-avidity TAA-specific T cells can result in toxicity. The infusion of high-avidity T cells directed against the RCC antigen carboxy anhydrase IX (CAIX) resulted in severe cholestasis, based on the CAIX expression by bile duct epithelial cells (33). In addition, patients who received high-avidity gp100 T cells developed uveitis and hearing loss due to expression of gp100 by melanocytes in eye and ear (15).

On the basis of the possible on-target toxicity induced by the PRAME-TCR a safety strategy may be necessary to cotransduce PRAME-TCR engineered T cells with a suicide gene, which enables *in vivo* elimination of the engineered T cells if serious adverse events occur (34, 35). Using this strategy, a temporary loss of mDCs could be resolved, because imDCs, CD14<sup>+</sup>, and CD34<sup>+</sup> progenitor cells as well as MDCs and PDCs from peripheral blood are not recognized by the PRAME T cells. In fact, recognition of mDCs might also be beneficial because it could potentially boost antitumor responses and enhance persistence of the infused T cells. In contrast, potential damage to the kidney powered by recognition of PTECs could be irreversible, and therefore cotransduction of a suicide gene might be necessary for protection against toxicity directed against the kidney. It is, however, also possible that the low reactivity observed against cultured PTECs even after IFN- $\gamma$  activation *in vitro* does not necessarily indicate nephrotoxicity *in vivo*, as indicated by absence of renal dysfunction in our patient.

On the basis of these results we conclude that potentially beneficial high-avidity TAA-specific T cells can be isolated from detrimental allo-HLA immune responses after HLA-

mismatched SCT. The high-affinity TCRs of these allorestricted PRAME-specific T cells highly reactive against a large panel of tumors, may be effective tools to engineer T cells with tumor-targeting TCRs that can be used for adoptive T-cell therapy of patients with metastasized cancer or relapsed leukemia.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Authors' Contributions

A.L. Amir designed and conducted research, and wrote the manuscript; D.M. van der Steen, M.M. van Loenen, R.S. Hagedoorn, R. de Boer, M.D.G. Kester, A.H. de Ru, G.-J. Lugthart, I. Jedema, M. Griffioen, and P.A. van Veelen conducted research; C. van Kooten and P.S. Hiemstra provided essential primary cell lines; J.H.F. Falkenburg wrote the manuscript; M.H. M. Heemskerk designed research and wrote the manuscript; and all authors reviewed and edited the manuscript.

## Acknowledgments

The authors thank Menno van der Hoorn, Guido de Roo, and Patrick van der Holst for expert technical assistance.

## Grant Support

This work was supported by grant LSBR0611 from the Landsteiner Foundation for Blood Transfusion Research and grant NKB 2005-3251 from the Dutch Cancer Society.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received April 21, 2011; revised June 28, 2011; accepted June 30, 2011; published OnlineFirst July 19, 2011.

## References

- Falkenburg JH, Wafelman AR, Joosten P, Smit WM, van Bergen CA, Bongaerts R, et al. Complete remission of accelerated phase chronic myeloid leukemia by treatment with leukemia-reactive cytotoxic T lymphocytes. *Blood* 1999;94:1201-8.
- Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. *Lancet* 2009;373:1550-61.
- Weiden PL, Storb R, Deeg HJ, Graham TC, Thomas ED. Prolonged disease-free survival in dogs with lymphoma after total-body irradiation and autologous marrow transplantation consolidation of combination-chemotherapy-induced remissions. *Blood* 1979;54:1039-49.
- Goulmy E, Schipper R, Pool J, Blokland E, Falkenburg JH, Vossen J, et al. Mismatches of minor histocompatibility antigens between HLA-identical donors and recipients and the development of graft-versus-host disease after bone marrow transplantation [see comments]. *N Engl J Med* 1996;334:281-5.
- Marijt EA, Heemskerk MH, Kloosterboer FM, Goulmy E, Kester MG, van der Hoorn MAWG, et al. Hematopoiesis-restricted minor histocompatibility antigens HA-1- or HA-2-specific T cells can induce complete remissions of relapsed leukemia. *Proc Natl Acad Sci U S A* 2003;100:2742-7.
- den Haan JM, Meadows LM, Wang W, Pool J, Blokland E, Bishop TL, et al. The minor histocompatibility antigen HA-1: a diallelic gene with a single amino acid polymorphism. *Science* 1998;279:1054-7.
- Kawase T, Matsuo K, Kashiwase K, Inoko H, Saji H, Ogawa S, et al. HLA mismatch combinations associated with decreased risk of relapse: implications for the molecular mechanism. *Blood* 2009;113:2851-8.
- Wilde S, Sommermeyer D, Frankenberger B, Schiemann M, Milosevic S, Spranger S, et al. Dendritic cells pulsed with RNA encoding allogeneic MHC and antigen induce T cells with superior antitumor activity and higher TCR functional avidity. *Blood* 2009;114:2131-9.
- Stronen E, Abrahamsen IW, Gaudernack G, Walchli S, Munthe E, Buus S, et al. Dendritic cells engineered to express defined allo-HLA peptide complexes induce antigen-specific cytotoxic T cells efficiently killing tumour cells. *Scand J Immunol* 2009;69:319-28.
- Savage P, Gao L, Vento K, Cowburn P, Man S, Steven N, et al. Use of B cell-bound HLA-A2 class I monomers to generate high-avidity, allo-restricted CTLs against the leukemia-associated protein Wilms tumor antigen. *Blood* 2004;103:4613-5.
- Stanislowski T, Voss RH, Lotz C, Sadovnikova E, Willemsen RA, Kuball J, et al. Circumventing tolerance to a human MDM2-derived tumor antigen by TCR gene transfer. *Nat Immunol* 2001;2:962-70.
- Li LP, Lampert JC, Chen X, Leitao C, Popovic J, Muller W, et al. Transgenic mice with a diverse human T cell antigen receptor repertoire. *Nat Med* 2010;16:1029-34.
- van Baren N, Chambost H, Ferrant A, Michaux L, Ikeda H, Millard I, et al. PRAME, a gene encoding an antigen recognized on a human melanoma by cytolytic T cells, is expressed in acute leukaemia cells. *Br J Haematol* 1998;102:1376-9.
- Ikeda H, Lethe B, Lehmann F, van Baren N, Baurain JF, de Smet C, et al. Characterization of an antigen that is recognized on a melanoma showing partial HLA loss by CTL expressing an NK inhibitory receptor. *Immunity* 1997;6:199-208.

15. Johnson LA, Morgan RA, Dudley ME, Cassard L, Yang JC, Hughes MS, et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. *Blood* 2009;114:535–46.
16. Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science* 2006;314:126–9.
17. Kessler JH, Beekman NJ, Bres-Vloemans SA, Verdijk P, van Veelen PA, Kloosterman-Joosten AM, et al. Efficient identification of novel HLA-A\*0201-presented cytotoxic T lymphocyte epitopes in the widely expressed tumor antigen PRAME by proteasome-mediated digestion analysis. *J Exp Med* 2001;193:73–88.
18. Mollndrem J, Dermime S, Parker K, Jiang YZ, Mavroudis D, Hensel N, et al. Targeted T-cell therapy for human leukemia: cytotoxic T lymphocytes specific for a peptide derived from proteinase 3 preferentially lyse human myeloid leukemia cells. *Blood* 1996;88:2450–7.
19. Inoue K, Ogawa H, Sonoda Y, Kimura T, Sakabe H, Oka Y, et al. Aberrant overexpression of the Wilms tumor gene (WT1) in human leukemia. *Blood* 1997;89:1405–12.
20. Gao L, Bellantuono I, Elsasser A, Marley SB, Gordon MY, Goldman JM, et al. Selective elimination of leukemic CD34(+) progenitor cells by cytotoxic T lymphocytes specific for WT1. *Blood* 2000;95:2198–203.
21. van Bergen CA, Kester MG, Jedema I, Heemskerk MH, Luxemburg-Heijs SA, Kloosterboer FM, et al. Multiple myeloma-reactive T cells recognize an activation-induced minor histocompatibility antigen encoded by the ATP-dependent interferon-responsive (ADIR) gene. *Blood* 2007;109:4089–96.
22. Epping MT, Wang L, Edel MJ, Carlee L, Hernandez M, Bernards R. The human tumor antigen PRAME is a dominant repressor of retinoic acid receptor signaling. *Cell* 2005;122:835–47.
23. Heemskerk MH, de Paus RA, Lurvink EG, Koning F, Mulder A, Willemze R, et al. Dual HLA class I and class II restricted recognition of alloreactive T lymphocytes mediated by a single T cell receptor complex. *Proc Natl Acad Sci U S A* 2001;98:6806–11.
24. van Loenen MM, de BR, Hagedoorn RS, van Egmond EH, Falkenburg JH, Heemskerk MH. Optimization of the HA-1-specific T-cell receptor for gene therapy of hematologic malignancies. *Haematologica* 2011;96:477–81.
25. van Loenen MM, de Boer R, Amir AL, Hagedoorn RS, Volbeda GL, Willemze R, et al. Mixed T cell receptor dimers harbor potentially harmful neoreactivity. *Proc Natl Acad Sci U S A* 2010;107:10972–7.
26. Heemskerk MH, Hoogeboom M, Hagedoorn R, Kester MG, Willemze R, Falkenburg JH. Reprogramming of virus-specific T cells into leukemia-reactive T cells using T cell receptor gene transfer. *J Exp Med* 2004;199:885–94.
27. Griffioen M, Kessler JH, Borghi M, van Soest RA, van der Minne CE, Nouta J, et al. Detection and functional analysis of CD8+ T cells specific for PRAME: a target for T-cell therapy. *Clin Cancer Res* 2006;12:3130–6.
28. Quintarelli C, Dotti G, De Angelis B, Hoyos V, Mims M, Luciano L, et al. Cytotoxic T lymphocytes directed to the preferentially expressed antigen of melanoma (PRAME) target chronic myeloid leukemia. *Blood* 2008;112:1876–85.
29. Quintarelli C, Dotti G, Hasan ST, De AB, Hoyos V, Errichiello S, et al. High-avidity cytotoxic T lymphocytes specific for a new PRAME-derived peptide can target leukemic and leukemic-precursor cells. *Blood* 2011;117:3353–62.
30. Rezvani K, Yong AS, Tawab A, Jafarpour B, Eniafe R, Mielke S, et al. Ex vivo characterization of polyclonal memory CD8+ T-cell responses to PRAME-specific peptides in patients with acute lymphoblastic leukemia and acute and chronic myeloid leukemia. *Blood* 2009;113:2245–55.
31. Ringhoffer M, Muller CR, Schenk A, Kirsche H, Schmitt M, Greiner J, et al. Simultaneous expression of T-cell activating antigens in renal cell carcinoma: implications for specific immunotherapy. *J Urol* 2004;171:2456–60.
32. Li L, Reinhardt P, Schmitt A, Barth TF, Greiner J, Ringhoffer M, et al. Dendritic cells generated from acute myeloid leukemia (AML) blasts maintain the expression of immunogenic leukemia associated antigens. *Cancer Immunol Immunother* 2005;54:685–93.
33. Lamers CH, Sleijfer S, Vulto AG, Kruit WH, Kliffen M, Debets R, et al. Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. *J Clin Oncol* 2006;24:e20–2.
34. Griffioen M, van Egmond EH, Kester MG, Willemze R, Falkenburg JH, Heemskerk MH. Retroviral transfer of human CD20 as a suicide gene for adoptive T-cell therapy. *Haematologica* 2009;94:1316–20.
35. Bonini C, Ferrari G, Verzeletti S, Servida P, Zappone E, Ruggieri L, et al. HSV-TK gene transfer into donor lymphocytes for control of allogeneic graft-versus-leukemia. *Science* 1997;276:1719–24.