Generation and administration of HA-1-specific T-cell lines for the treatment of patients with relapsed leukemia after allogeneic stem cell transplantation: a pilot study

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ABSTRACT

Since HA-1-specific T cells have been shown to make a significant contribution to the clinical responses in patients with relapsed leukemia, we investigated the feasibility of adoptive transfer of *in vitro* induced HA-1-specific CD8 positive T cells to patients with relapsed leukemia after allogeneic stem cell transplantation. The *in vitro* generation of clinical grade HA-1-specific T-cell lines from HA-1 negative donors was seen to be feasible and 3 patients were treated with HA-1-specific T-cell lines. No toxicity after infusion was observed. Although in one patient, during a period of stable disease, HA-1-specific T cells could be detected in the peripheral blood and bone marrow, these patients had no clear clinical response.

Key words: allogeneic stem cell transplantation, adoptive cellular immunotherapy, minor histocompatibility antigen, HA-1, CTL

Citation: Meij P, Jedema I, van der Hoorn MAWG, Bongaerts R, Cox L, Wafelman AR, Marijt EWA, Willemze R, and Falkenburg JHF. Generation and administration of HA-1-specific T-cell lines for the treatment of patients with relapsed leukemia after allogeneic stem cell transplantation: a pilot study. Haematologica 2012;97(8):1205-1208. doi:10.3324/haematol.2011.053371

Introduction

Allogeneic stem cell transplantation (alloSCT) is successfully used in the treatment of patients with hematologic malignancies, including acute myeloid leukemia (AML), chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL). Treatment of relapsed leukemia after alloSCT with donor lymphocyte infusion (DLI) can induce long-lasting complete remissions. T cells recognizing minor histocompatibility antigens (mHags) on recipient hematopoietic cells are thought to play an important role in the anti-leukemic effect observed after DLI, as demonstrated by the detection of mHag-specific donor T cells in patients responding to DLI.[1,2] Complete remissions after DLI have been achieved in 80-90% of patients with CML in chronic phase, but only in 10-30% of patients with relapsed AML and ALL.[3,4] Patients not responding to DLI may lack the appropriate *in vivo* environment for an efficient induction of mHag-specific T-cell responses. This may be bypassed by the *in vitro* generation of mHag-specific T cells that can be adoptively transferred to patients with relapsed leukemia after transplantation. In a previous phase I/II feasibility study, we treated patients with relapsed leukemia with *in vitro* generated leukemia-reactive T cells. In some patients, a clinical effect was observed, demonstrating a potential efficacy of the adoptive transfer of *in vitro* generated leukemia-reactive T cells.[5,6] The adoptive transfer of mHag-specific T-cell clones has been described by Warren *et al.*,[7] providing evidence that transferred T cells can exert anti-leukemic activity, although no long-term persistence of the T-cell clones was observed and all patients eventually relapsed. In our previous report we showed that T cells specific for the mHag HA-1 appeared to make a significant contribution to clinical responses after DLI and it has been shown that mHag-specific CD8 positive ($CD8^+$) T cells can have anti-leukemic activity.[1,7] We, therefore, investigated the feasibility of the adoptive transfer of *in vitro* induced HA-1-specific $CD8^+$ T-cell lines in patients with relapsed leukemia after alloSCT.

Design and Methods

Generation of dendritic cells

Clinical grade immature dendritic cells (DC) were generated by either: 1) culturing $CD34^+$ positive cells derived from the donor peripheral blood stem cell graft with 100 ng/mL GM-SCF (Novartis, Basel, Switzerland), 20 ng/mL human stem cell factor (huSCF) (a kind gift of Amgen, Thousand Oaks, CA, USA) and 2 ng/mL TNFα (Cellgro 1006, Cellgenix, Freiburg, Germany), and from Day 4,500 IU/m IL-4 (kindly provided by Schering Plough, Innishammon, Cork, Ireland); or 2) by

Acknowledgments: HuSCF for clinical use was kindly provided by Amgen Inc., Thousand Oaks, CA, USA.
Funding: Allostem (European Union grant n. 503319), KWF (2007-3937).
Manuscript received on August 12, 2011. Revised version arrived on March 9, 2012. Manuscript accepted on April 4, 2012.
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culturing CD14+ cells derived from donor peripheral blood with 100 ng/mL GM-CSF, 500 IU/ml IL-4 and 100 IU/mL IFNα-2a (Roferon-A, Roche, Basel, Switzerland) for five days. Immature CD34 or CD14 derived DC were maturated using DKTP (diphtheria, whooping cough, tetanus and polio) vaccine and diluted 1:1000 (RIVM, Bilthoven, The Netherlands).

Generation of HA-1-specific T-cell lines

After obtaining informed consent, HA-1-specific donor T-cell lines were generated by culturing donor peripheral blood mononuclear cells (PBMC) with donor-derived dendritic cells (DC) pulsed with the specific HA-1 peptide (VLHDDLLEA, synthesized at the Department of Immunohematology, LUMC, Leiden, The Netherlands) at a responder:stimulator (R/S) ratio of 10:1. DC (5-10x10^6/mL) were pulsed by adding 1 μg/mL HA-1 peptide for 2 h at 37°C followed by extensive washing. Cells were cultured in IMDM containing penicillin and streptomycin, L-glutamin, 10% heat inactivated human serum and 6 IU/mL IL-2 (Proleukin (Aldesleukin) Novartis Pharmaceuticals, Horskam, UK). At Day 5 of the immune response, 120 IU/mL IL-2 was added. Until a maximum of five weeks, T-cell cultures were restimulated once a week with HA1 peptide pulsed-DC, at an R/S ratio of 10:1 in T-cell medium containing 120 U/mL IL-2.[8] Cultures were evaluated for the presence of HA-1-specific T cells by tetramer staining as previously described.[1,9] HA-1-specific T-cell lines had to meet the following specifications before being released for administration: i) no microbiological contamination; ii) donor origin of the HA-1-specific CTL line established by chimerism analysis as previously described;[1] iii) more than 80% CD3+ T cells; iv) more than 5% HA-1-specific CD8+ T cells within the CD3 population; iv) less than 10% CD19+ B cells; and v) absence of anti-donor reactivity as measured by reactivity against donor-derived PHA blasts and/or EBV-LCL in a ^{51}Cr release assay.[10]

Study design

HLA-A*0201 positive and HA-1 positive patients with relapsed AML, CML or ALL after alloSCT with an HLA-A*0201 positive and HA-1 negative donor were eligible. CTL treatment was initiated not earlier than two months after transplantation by which time donor cell engraftment had been established. Patients with grade 3 or 4 GvHD, or patients who were treated with chemotherapy or high-dose immunosuppressive drugs at the time of CTL infusion, were ineligible. Toxicity was scored according to WHO criteria and GvHD according to the Glucksberg criteria. Response criteria were the anti-leukemic effect and presence or increase of HA-1-specific T cells in the peripheral blood and/or bone marrow following infusion. Patients gave written informed consent and the study was performed in accordance with the regulations of the institutional ethics committee.

Results and Discussion

A total of nine HA-1-specific CD8+ T-cell lines were generated after stimulation with HA-1 peptide-loaded CD34-derived or CD14-derived DC. All T-cell lines met the quality criteria as specifically established for the HA-1-specific CD8+ T-cell lines. The percentage of HA-1-specific T cells in the T-cell lines varied between 6-36%, corresponding to a total number of 11-372x10^6 HA-1-specific CD8+ T cells per T-cell line (Table 1). Most HA-1-specific T cells in the T-cell lines expressed CD45RO and had a memory T-cell phenotype (Table 1). Furthermore, the absence of CCR7 staining and the decrease in the percentage of CD27 and/or CD28 positive T cells indicate an effector memory phenotype of the HA-1-specific T cells. Functionality of the T-cell lines was tested in a ^{51}Cr release assay (Table 1). All HA-1 specific CD8+ T-cell lines recognized HA-1 peptide-loaded donor PHA blasts, patient PHA blasts and leukemic cells, while no recognition of non-peptide loaded donor PHA blasts was shown.

A phase I clinical study examined the toxicity and the potential anti-leukemic effect of treatment with HA-1-specific CTL lines for leukemic relapse following allogeneic SCT. Three patients who relapsed after alloSCT received an HA-1-specific T-cell line, containing 10-170x10^6 HA-1-specific T cells per administration. None of the 3 patients developed severe complications during infusion of the T-cell line. Patient KHP showed a temporary rise in temperature during administration of the T-cell line. None of the patients developed GvHD. One month after administration of the HA-1-specific T-cell line, patient AHP developed a pericarditis. Since a diagnostic aspirate consisted mainly of macrophages and the microbiological culture was positive

Table 1. Characteristics of the HA-1-specific CD8+ T-cell lines generated for *in vivo* administration.

CTL Line	Culture period (days)	Cells (x 10^6)	CD8+HA-1+ T cells (x 10^6)	Composition T-cell line (% positive cells)			Phenotype HA-1+ T cells (% positive cells)					Cytotoxicity (% lysis)[1]			
				CD4	CD8	CD8+HA-1+	CD45RO	CD45RA	CCR7	CD27	CD28	PHA[2] donor - peptide	PHA[2] donor + peptide[3]	PHA patient	Leukemia
Administered															
NMJ - 1	30	869	243	12	84	27	84	51	1	93	30	8	72	56	n.a.
KHP	38	56	11	3	89	19	n.a.	n.a.	n.a.	n.a.	n.a.	5	57	30	61[4]
AHP - 2	35	325	20	1	61	6	n.a.	n.a.	n.a.	n.a.	n.a.	6[4]	63[4]	68[4]	13[4]
Not-administered															
NMJ - 2	34	30	11	20	80	36	98	2	5	85	11	1	58	43	n.a.
UBW	29	600	60	1	70	10	99	29	0	74	85	1	59	64	35
FPW - 3	28	6200	372	9	91	6	97	21	2	71	75	2	39	64[4]	60[4]
VWX	35	239	14	11	70	6	99	7	0	30	73	3	83	34	n.a.
AHP - 3	37	660	224	1	82	34	99	0	8	4	46	0	61	37	32
AHP - 4	36	3600	252	2	90	7	99	0	8	12	18	0	38	35	36[4]

[1]Percentage lysis after 4 h in a ^{51}Cr release assay, E:T ratio of 30:1; [2]PHA blasts; [3]Pulsed with HA-1 peptide; [4]lysis after 24 h and E:T ratio 10:1; n.a.: not available.

for streptococci, it was considered unlikely that the pericarditis was related to the infused T-cell line.

Patient AHP, who had been transplanted for CML in chronic phase, was treated for a molecular relapse (BCR-ABL/PBGD ratio 10^{-2}; chimerism status 99.5% donor) after alloSCT and received a single HA-1-specific T-cell line. After administration of the T-cell line, the BCR-ABL PCR remained stable for three months after infusion. However, thereafter a localized blast crisis developed in the left leg. Patient KHP was transplanted for a refractory AML and relapsed seven months after alloSCT. The patient received chemotherapy consisting of Ara-C and daunorubicin but had no response and no remission (leukocyte count $1.8x10^9$/L; 33% leukemic blasts in peripheral blood and 95% in bone marrow; chimerism status 5% donor). He received a single HA-1-specific T-cell line. Patient NMJ was transplanted for AML in remission and relapsed three months after alloSCT (leukocyte count $11.6x10^9$/L; 34% leukemic blasts in peripheral blood; chimerism status 64% donor). She received three HA-1-specific T-cell lines and no additional treatment. No anti-leukemic reactivity was observed in patients KHP and NMJ after administration of the HA-1-specific T-cell lines and rapidly progressive disease led to the death of both patients.

We analyzed whether HA-1-specific $CD8^+$ T cells could be detected in peripheral blood and bone marrow at varying time points (up to 8 weeks) after infusion of the HA-1 specific T-cell line. In patient AHP, a low percentage of HA-1-specific $CD8^+$ T cells (0.2%) could be detected in the peripheral blood and bone marrow after one week of *in vitro* culture with the HA-1 peptide. This low percentage of HA-1-specific T cells could be detected up to eight weeks after infusion in both peripheral blood and bone marrow. This may indicate survival of the infused T-cell line at low frequencies, although, very low frequencies of HA-1-specific $CD8^+$ T cells could also be detected prior to infusion of the HA-1-specific $CD8^+$ T-cell line. For patient NMJ, peripheral blood samples and bone marrow samples up to two weeks after infusion were available, and for patient KHP peripheral blood samples up to three weeks after infusion were available. No HA-1-specific $CD8^+$ T cells could be detected in these samples from either patient.

In this study, we have shown that *in vitro* generation of clinical grade HA-1-specific T-cell lines is feasible. However, at least four weeks and in most cases five weeks of culture were necessary to obtain HA-1-specific T-cell lines containing significant numbers of HA-1-specific T cells. This culture period was too long, and obtained cell numbers that may have been too low for patients with rapidly growing leukemia (patients KHP and NMJ). In patients with persistent CMV of EBV reactivation after transplantation, infusion of low numbers of antigen specific T cells has resulted in *in vivo* expansion and sustained immune responses.[11,12] However, our culture protocol led to the infusion of T-cell lines with an effector memory phenotype that might have had a limited capacity to survive and expand *in vivo*.[7,13] In addition, the high dose of IL-2 used during the culture period might have led to IL-2 dependency of the HA-1-specific T cells which could also have contributed to their lack of *in vivo* persistence.[13,14] Additionally, the lack of HA-1 specific $CD4^+$ T cells may have contributed to the lack of *in vivo* persistence, since it has been shown that virus-specific $CD8^+$ T cells may show better persistence in the presence of virus-specific $CD4^+$ T cells.[15] The relapsed tumor cells may also have had a reduced susceptibility to killing by the antigen specific T cells, e.g. by downregulation of HLA molecules, leading to impaired presentation of HA-1 on the tumor cells. However, since the leukemic blasts were susceptible to lysis by HA-1-specific $CD8^+$ T cells in our *in vitro* ^{51}Cr-release assay (Table 1), we do not expect that escape of the tumor cells significantly contributed to the lack of anti-leukemic reactivity of the HA-1-specific T cells.

Three patients were treated with HA-1-specific T-cell lines. After infusion, no toxicity was observed, showing that administration of these T-cell lines was safe. Although in one patient, during a period of stable disease, HA-1-specific T cells could be detected in the peripheral blood and bone marrow, no clear clinical response occurred in these patients. In the study by Warren *et al.*, in which high numbers of mHag-specific T-cell clones were infused in patients with relapsed leukemia, transient remissions were observed after adoptive transfer, which might be related to the difference in cell numbers infused.[7] However, also in their study, no durable *in vivo* persistence of the mHag-specific T cells could be demonstrated.

To improve the anti-leukemic effect of mHag-specific T-cell lines, a strategy has to be developed for the early isolation of mHag-specific $CD4^+$ and $CD8^+$ T cells.[16] Alternatively, TCR gene transfer may also be an attractive strategy for rapid *in vitro* generation of high-affinity mHag-specific T cells.[17,18] The *in vitro* culture period should probably be limited, and more physiological culture conditions are necessary to enhance the *in vivo* potential of the mHag-specific T cells.[13] This may result in a better anti-leukemic effect and in the development of a population of central memory type T cells which may be necessary for the long-term control of the leukemia.

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

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