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ORIGINAL ARTICLE

MART-1 TCR gene-modified peripheral blood T cells for the treatment of metastatic melanoma: a phase I/IIa clinical trial

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Available online 18 June 2022

Background: Adoptive cell therapy with peripheral blood T cells expressing transgenic T-cell receptors (TCRs) is an innovative therapeutic approach for solid malignancies. We investigated the safety and feasibility of adoptive transfer of autologous T cells expressing melanoma antigen recognized by T cells 1 (MART-1)-specific TCR, cultured to have less differentiated phenotypes, in patients with metastatic melanoma.

Materials and methods: In this phase I/IIa trial, peripheral blood T cells from HLA-A2*02:01-positive patients with unresectable stage IIIC/IV melanoma expressing MART-1 were selected and stimulated with anti-CD3/CD28 beads, transduced with a modified MART-$1_{(26-35)}$-specific 1D3 TCR (1D3HMCys) and expanded in interleukin (IL)-7 and IL-15. Patients received a single infusion of transgenic T cells in a dose-escalating manner. Feasibility, safety and objective response rate were assessed.

Results: Twelve pretreated metastatic cutaneous ($n = 7$) and uveal ($n = 5$) melanoma patients were included. Patient 1 received 4.6×10^9 1D3HMCys T cells and experienced grade 5 toxicity after 9 days. Subsequent patients received 5.0×10^7 [$n = 3$; cohort (c) 2], 2.5×10^8 ($n = 2$; c3) and 1.0×10^8 ($n = 6$; c4) 1D3HMCys T cells. The study was prematurely terminated because of dose-dependent toxicity, concerning skin (10/12), eyes (3/12), ears (4/12) and cytokine release syndrome (5/12), with 7 patients experiencing grade 3-5 toxicity. Partial responses were seen in 2/11 (18%) assessable patients and persistence of 1D3HMCys T cells corresponded to infused cell dose.

Conclusions: Production of TCR-modified cells as described leads to highly potent T cells. Partial responses were seen in 18% of patients with dose-dependent 'on-target, off-tumor' toxicity and a maximum tolerated dose of 1.0×10^8 cells.

Key words: adoptive cell therapy, immunotherapy, MART-1, melanoma, T-cell receptor gene therapy, uveal

INTRODUCTION

Although the prognosis of patients with advanced melanoma has dramatically improved, there is still a need for novel treatment options as half of the patients obtain no durable clinical benefit from currently approved therapies.[1] This unmet need is even higher in patients with uveal melanoma, with no viable treatment options until recently.[2,3]

A novel treatment modality is adoptive cell therapy (ACT) with *in vitro*-expanded tumor-infiltrating lymphocytes (TILs), T-cell receptor (TCR)-modified peripheral blood T cells or chimeric antigen receptor-modified peripheral blood T cells.[4-7] Generation of autologous T cells expressing a TCR reactive to a particular shared tumor antigen (lineage-specific antigens, cancer/testis antigens, viral antigens and overexpressed antigens) is an attractive option to transfer cells with a highly defined antitumor reactivity. Adoptive transfer of such gene-modified T cells targeting melanoma antigen gene (MAGE)-A3,[8] New York esophageal squamous cell carcinoma-1 (NY-ESO-1),[9] human papillomavirus (HPV)[10] and melanoma antigen recognized by T cells 1 (MART-1)[11,12] is feasible and has resulted in clinical responses.

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The melanoma differentiation antigen (MDA) MART-1 is expressed in melanocytes and 80%-95% of melanomas.[13,14] MART-1-specific TCR gene therapy has already been explored utilizing the DMF4 TCR[11] derived from a patient with a near-complete response after treatment with TIL[15] and in a subsequent trial using the MART-1 DMF5 TCR, derived from the same patient but showing a higher affinity for cognate antigen.[12] In these earlier trials, peripheral blood T cells were activated and expanded with anti-CD3 monoclonal antibodies in the presence of interleukin 2 (IL-2), playing an important role in the differentiation of naïve $CD8^+$ T cells to effector (memory) cells,[16] and retrovirally transduced with the specific TCR. Efficacy of the DMF4 and DMF5 trials was modest, with an acceptable toxicity profile, leaving room for improvement. Preclinical models have shown improved function and engraftment potential of T cells activated and expanded through CD3/CD28 stimulation in the presence of IL-7 and IL-15,[17] in generating T cells with a less differentiated phenotype, as these cytokines are instrumental in the generation and maintenance of central memory T cells.[18] Adoptive transfer of low numbers of T cells with a less differentiated phenotype improved long-term immune responses[19] and may thus improve the potency of ACT.[20]

Based on these potential improvements for ACT, we developed a novel, robust good manufacturing practice (GMP) production process to generate autologous peripheral blood-derived T cells transduced with a MART-1-specific TCR (gene optimized, not affinity-enhanced), known as 1D3HMCys, expanded in IL-7 and IL-15 with anti-CD3/CD28 selection and activation to investigate the impact for TCR gene therapy, as described previously.[21-23] These cells harbored the potential for better engraftment[17] and improvement of long-term antitumor responses due to the less differentiated phenotype. As the affinity of the 1D3HMCys TCR was not higher than the earlier described MART-1 DMF5 TCR,[12] safety was hypothesized to be comparable. Between 2012 and 2018, we conducted an early phase clinical trial in which advanced melanoma patients were treated with a single infusion of 1D3HMCys T cells. Here, we report the results of this clinical trial.

MATERIALS AND METHODS

Study design and patients

This is a phase I/IIa, single-center, single-arm, dose-finding trial conducted at the Netherlands Cancer Institute (NKI, Amsterdam, The Netherlands) following the Declaration of Helsinki. This trial was approved by the Central Committee on Research Involving Human Subjects (NCT02654821). All patients gave written informed consent. The primary endpoints were safety, feasibility and objective response rate (ORR). Secondary endpoints were 1-year progression-free survival (PFS), median overall survival (OS), and evaluation of the induction of tumor-specific T-cell responses.

HLA-A*02:01-positive patients with treatment-refractory unresectable stage IIIC/IV melanoma with >10% MART-1 expression in tumors were eligible. All eligibility criteria are presented in Supplementary Table S1, available at https://doi.org/10.1016/j.iotech.2022.100089.

Study procedures

Patients underwent baseline apheresis to acquire $\geq 1 \times 10^9$ mononuclear cells, which were cryopreserved until start of culture. Before the adoptive transfer of 1D3HMCys TCR-transduced peripheral blood T cells, patients were preconditioned with non-myeloablative chemotherapy [cyclophosphamide 60 mg/kg/day intravenously (i.v.) for 2 days and fludarabine 25 mg/m^2/day i.v. for 5 days]. Subsequently, patients received subcutaneous injections of low-dose IL-2 (2×10^6 IU) once daily up to 2 weeks.

Safety was monitored according to the National Cancer Institute's Common Terminology Criteria for Adverse Events version 4.03 and evaluated by a Data Safety Monitoring Board (DSMB) to assess subsequent cell dose escalation. In accordance, the study protocol was amended seven times, as further described in the Results section. Patients' hearing level was evaluated by standard audiometry before treatment and 4 weeks post-treatment. Patients 7-12 received intratympanic dexamethasone injections (4.0 mg/ml, 0.5-1 ml per ear) following severe ototoxicity in patient 6. During hospitalization, standard supportive care was provided. Skin biopsies were taken from (un)affected areas of patients who developed dermatitis. Blood serum samples, peripheral blood mononuclear cells and tumor biopsies were collected for immunological monitoring and translational research. Tumor response was evaluated by computed tomography (CT) according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1[24] every 4 weeks. See Figure 1 for the detailed treatment schedule.

Production of MART-1 1D3HMCys TCR-modified T cells

The 1D3HMCys TCR, recognizing the HLA-A*02:01-restricted MART-$1_{(26-35)}$ epitope, was derived from a melanoma patient vaccinated with the MART-$1_{(26-35)}$ peptide[25] and selected for its high affinity.[21,22] To enhance TCR expression and minimize mixed dimer formation with endogenous TCR chains, the 1D3 TCR was optimized by replacing the human constant domains with murine constant domains[26] and by inclusion of a non-native cysteine pair.[27,28] Additionally, a self-cleaving P2A peptide was used to link the α- and β-chains to achieve equal expression of both chains.[29] Autologous peripheral blood $CD3^+$ T cells from the apheresis product were isolated and stimulated using anti-CD3/CD28 beads and retrovirally transduced with an MP71 vector encoding the 1D3HMCys TCR. Transduced T cells were subsequently expanded *ex vivo* in the presence of IL-7 and IL-15. TCR optimization and GMP production protocols have been described previously.[21,23]

Immunological monitoring

Flow cytometry analysis of infusion products and post-infusion peripheral blood lymphocytes. Expression of the 1D3HMCys TCR, composition and phenotypical

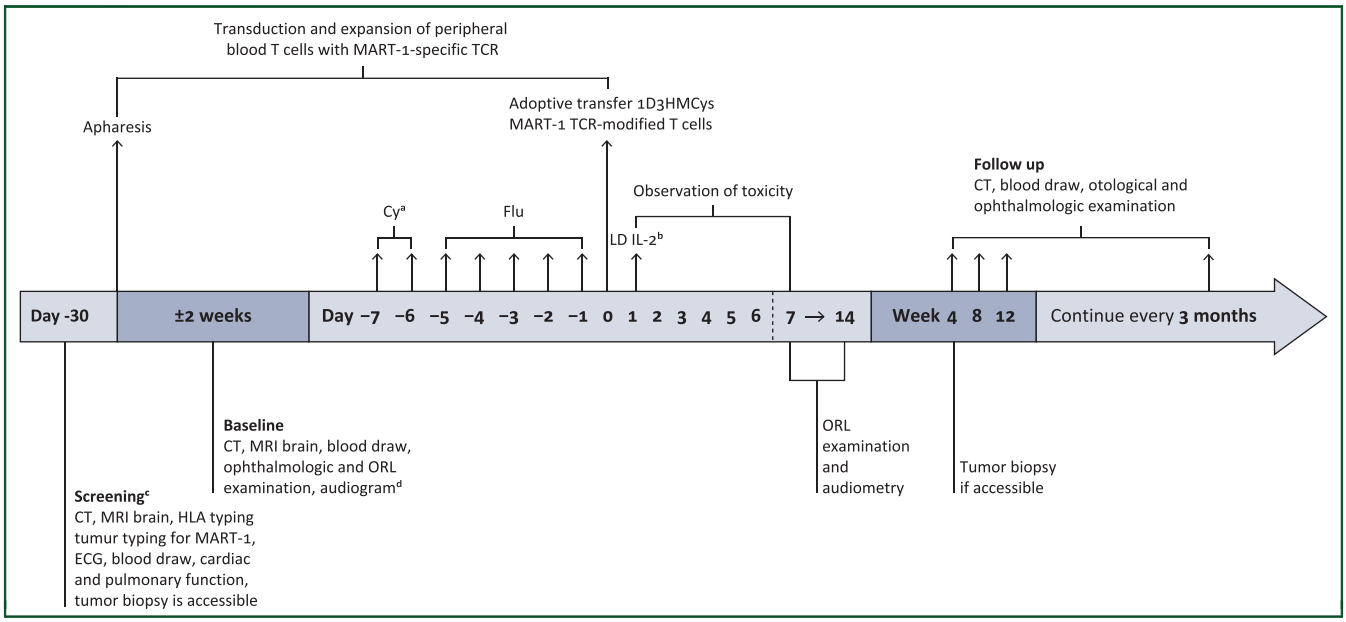

Figure 1. Treatment and monitoring schedule. Blood draw for serum and PBMCs was carried out 2 weeks before cell infusion, 24 h after cell infusion, days 7 and 14 and at follow-up at 1, 2, 3, 6, 9, 12, 18 and 24 months after infusion. Blood draw for serum alone was carried out pre-infusion on day 0 and daily thereafter during hospitalization and at follow-up at 1.5, 2.5, 4.5, 7.5 and 15 months post-infusion and continued every 3 months thereafter. If accessible, tumor biopsies were taken before and after treatment and at the first time point of documented response and/or at the first time of (proven) disease progression. Because of the observed toxicity during the trial, the following amendments to the protocol were made: [a]Cyclophosphamide dose was reduced from 60 to 30 mg/kg/day i.v. after patient 6. [b]Subcutaneous injections of LD IL-2 (2×10^6 IU/once daily up to 2 weeks) following cell infusion were omitted from patient 6 onward. [c]After the first treated patient suffered a fatal serious adverse event, subsequent patients with high disease burden (>2 × ULN LDH), brain metastases and/or pre-existing cardiac dysfunction were not eligible for participation in the trial. Serum IL-6 would be monitored in all patients and administration of tocilizumab would be considered when IL-6 levels exceed 200 pg/ml and/or the patient shows signs of clinical deterioration. In case of severe toxicity, high doses of corticosteroids in addition to anti-CD52 antibody could be administered to eliminate T cells. [d]Intratympanic dexamethasone injections (4.0 mg/ml, 0.5-1.0 ml per ear) starting 2 days before the transfer of the T cells and five times in the following 10 days was initiated from patient 7 due to severe ototoxicity in patient 6. In the last six patients, follow-up audiometry was carried out more frequently and up to 12 weeks post-treatment.
CT, computed tomography; Cy, cyclophosphamide; ECG, electrocardiogram; Flu, fludarabine; LDH, lactate dehydrogenase; LD IL-2; low-dose interleukin-2; MRI, magnetic resonance imaging; ORL, otorhinolaryngology; PBMC, peripheral blood mononuclear cells; TCR, T-cell receptor; ULN, upper limit of normal.

characteristics of the infusion products and patients' blood were analyzed by flow cytometry. Blood samples collected at indicated time points after infusion were cryopreserved in cryopreservation medium containing fetal calf serum and 10% dimethyl sulfoxide for subsequent analysis.

MART-1-specific T cells were identified by staining with HLA-A*02:01 MART-$1_{(26\text{-}35\ A>L)}$ tetramers, generated through ultraviolet-induced peptide exchange.[30,31] In addition, cells were stained with antibodies for phenotypic markers and anti-mouse TCRβ. See Supplementary Table S2, available at https://doi.org/10.1016/j.iotech.2022.100089, for the full list of antibodies, clones and manufacturers. Cell viability was quantified using LIVE/DEAD™ Fixable Near-IR Dead Cell Stain (Invitrogen — Thermo Fischer Scientific, Whalthman, MA Cat#L10119). Extracellular stains were carried out for 30 min at 4°C in Brilliant Stain Buffer (BD, Franklin Lakes, NJ Cat#563794). To allow intracellular staining of T-cell factor 1 (TCF1), cells were fixed for 30 min at 4°C with eBioscience Intracellular Fixation & Permeabilization Buffer (Invitrogen — Thermo Fischer Scientific Cat#88-8824-00, as per manufacturer's recommendation). Intracellular staining was carried out in 1X Permeabilization Buffer (Invitrogen — Thermo Fischer Scientific Cat#00-8333) for 30 min at 4°C. All samples were acquired using a BD LSR Fortessa, and data were analyzed using FlowJo 10 software (BD).

Analysis of tumor and skin biopsies. Biopsies were stained with hematoxylin—eosin. Immunohistochemistry was carried out using an automated Ventana Benchmark Ultra staining system (Ventana Medical Systems, Tucson, AZ) using antibodies against MART-1 (clone A103, Dako/Agilent, Santa Clara, CA), CD3 (clone 2GV6, Ventana/Roche, Oro Valley, AZ) and HLA-A (clone EP1395Y, Abcam, Cambridge, UK). Central review by an expert pathologist was carried out on all evaluable biopsies, blinded for clinical outcome. Immune infiltration was scored in a semi-quantitative manner according to a four-step grading system.[32]

For assessment of living skin-infiltrating lymphocytes (SILs) and TILs, biopsy sections were fragmented and cultured in Roswell Park Memorial Institute (RPMI) medium for 3-5 days supplemented with 10% human serum and 6000 IU/ml IL-2 in a 24-well plate. Cells were stained with anti-CD3, anti-CD8, HLA-A*02:01 MART-$1_{(26\text{-}35\ A>L)}$ tetramers and anti-mouse TCRβ (see Supplementary Table S2, available at https://doi.org/10.1016/j.iotech.2022.100089) in flow cytometry buffer for 20-30 min at 4°C and analyzed on a BD FACSCalibur and FlowJo 10 software (BD).

Cytokine measurements. Serum samples were stored at −80°C. Levels of IL-2, IL-4, IL-6, IL-10, interferon-γ, tumor necrosis factor-α, IL-17A, sFas, sFasL, granzyme A, granzyme B, perforin and granulysin were measured simultaneously

by multiplex bead-based assay using the LEGENDplex Human CD8/NK Panel, according to the manufacturer's protocol (Biolegend, San Diego, CA: 740267; Lot: B263848). Samples were mixed, centrifuged and diluted twofold with assay buffer and were run in duplicate.

Statistical analyses

An optimal Simon's two-stage design was used to test the null hypothesis that the ORR was $\leq$10% versus the alternative hypothesis that the ORR was $\geq$30%. Using an α error of 0.10 and a β error of 0.10, an objective response in at least 2 of the first 16 patients was needed to continue the study and recruit 25 patients in total. If $>$5 patients achieved an objective response in the total study population, adoptive transfer of autologous T cells expressing 1D3HMCys would be considered worthy of further investigation. Data are presented in a descriptive manner, and curves for PFS and OS were computed using R (version 3.6.1; Boston, MA), according to the Kaplan–Meier method. Other graphs were plotted using GraphPad Prism (version 9.0; GraphPad Software, San Diego, CA).

RESULTS

Patients

Between October 2012 and October 2017, 12 treatment-refractory advanced cutaneous ($n = 7$) and uveal ($n = 5$) melanoma patients were treated with 1D3HMCys T cells. The trial was stopped prematurely because of treatment-related toxicity. Patient baseline characteristics are summarized in Table 1.

Study conduct and protocol amendments

Enrolled patients were treated in four dose cohorts defined in agreement with the DSMB (Table 1). As described previously, patient 1 received 4.56×10^9 1D3HMCys T cells and died 9 days post-infusion, presumably due to grade 4 cytokine release syndrome (CRS)/sepsis, which in combination with other clinical factors led to multiple organ failure grade 5.[33] The trial was initially designed as phase Ib/IIa trial, however, based on this fatal event, the trial was heavily amended and resulted to be more a dose-finding phase Ia/IIa trial. Cell dose was subsequently lowered by 100-fold (5.0×10^7 cells) and was tolerated well by the next three patients. Two subsequent patients received 2.5×10^8 cells, and severe dose-limiting on-target, off-tumor and cytokine-associated toxicities were observed. The DSMB recommended lowering the cell dose by 2.5-fold, omitting the administration of low-dose IL-2 (from patient 6) and lowering the lymphodepleting chemotherapy regimen to cyclophosphamide 30 mg/kg/day (the fludarabine dose remained unchanged) after patient 6. The last six patients received 1.0×10^8 cells with acceptable toxicity, and this dose was thus defined as the maximum tolerated cell dose.

Infusion products show a large proportion of 1D3HMcys-edited T cells with a less differentiated phenotype

From all patients, sufficient mononuclear cells were harvested by apheresis for transduction with the 1D3HMCys TCR. Infusion products contained a median of 57.4% (range 38.4%-77.5%) of 1D3HMCys$^+$ CD8$^+$ cells and 51.3% (range 38.9%-63.4%) of 1D3HMCys$^+$ CD4$^+$ cells (Figure 2A and B).

The expression of phenotypical markers CD45RO, CD45RA, CCR7, CD27, CD28 and TCF1 on gene-modified cells in the infusion products is shown in Figure 2C. Expression of CD45RA was low amongst CD4$^+$ cells, but observed in around half of CD8$^+$ cells. Expression of CCR7 was low in all samples and did not correlate with the more substantial expression of CD62L which had been measured in the infusion product before cryopreservation (not shown). Both CD4$^+$ and CD8$^+$ T cells showed high expression of CD28 and, to a lesser extent, CD27, which has been associated with less terminally differentiated cells with a more central memory phenotype. The transcription factor TCF1 (encoded by the gene *TCF7*) was also quantified to identify cells with enhanced stemness and long-term persistence.[34] Due to the capacity of IL-15 to promote proliferation of natural killer (NK) and TCR$\gamma\delta$ lymphocytes, the presence of these cells in the final infusion products was evaluated. Flow cytometry analysis showed that the fraction of NK or TCR$\gamma\delta$ lymphocytes in the final infusion products was highly limited with a purity of CD3$^+$ cells of 99% (Supplementary Figure S1A, available at https://doi.org/10.1016/j.iotech.2022.100089).

The killing capacity of the CD8$^+$ 1D3HMCys$^+$ and CD4$^+$ 1D3HMCys$^+$ T cells was analyzed *in vitro*, and the ability to directly kill melanoma cells was almost exclusively observed for CD8$^+$ cells (Supplementary Figure S1B, available at https://doi.org/10.1016/j.iotech.2022.100089).

Treatment-related toxicity was highly 1D3HMCys T-cell dose-dependent

The most common adverse events (AEs) resulted from transient bone marrow depression secondary to chemotherapy, with a median duration of grade 3-4 neutropenia of 6.5 days (range 4-11 days). An overview of all observed AEs is presented in Supplementary Table S3, available at https://doi.org/10.1016/j.iotech.2022.100089. All enrolled patients developed grade $\geq$3 treatment-related toxicity (Figure 3A), but occurrence and severity of on-target, off-tumor toxicity was highly dependent on administered cell dose. Patients treated with 5.0×10^7 1D3HMCys T cells developed mild dermatitis with spontaneous full recovery within 2-19 days.

In the subsequent cohort of 2.5×10^8 1D3HMCys T cells, both patients experienced grade 3 dermatitis (Figure 3B), subsiding after 12-14 days upon topical and systemic steroid treatment. Skin biopsies showed increased lymphocyte infiltration and decreased MART-1 expression in melanocytes in the epidermis of affected skin compared to normal skin of the same individual (Figure 3C). Expression of the 1D3HMCys TCR on SIL derived from these biopsies was only

Table 1. Patient characteristics, dose cohort description and response

Patient	Age (years)/ sex	Primary melanoma	Disease stage (Sites)	Prior systemic therapy	ECOG score	Serum LDH level	Cell dose	Preparative lymphodepleting regimen	IL-2[a] (days)	BOR by RECIST 1.1 (DOR in months)	TTP[b] in months	Survival in months from time of infusion
1	43/F	Cutaneous	M1d (CNS, pan, lu, pl, li, ov, LN, SC, C, PRe, P, om)	MEK inhibitor, anti-CTLA-4	1	>2 × ULN	4.56×10^9	Cy 60 mg/kg for 2 days + Flu 25 mg/m^2 for 5 days	5	NA	NA[c]	0.3[d]
2	74/F	Cutaneous	M1c (lu, LN, spleen)	DTIC, DNA vaccination (trial), MEKinhibitor (trial), anti-CTLA-4	0	1-2 × ULN	5.0×10^7	Cy 45 mg/kg for 2 days + Flu 12.5 mg/m^2 for 5 days[e]	14	SD	2.1	70.2[d]
3	48/M	Cutaneous	M1c (C, LN)	T-cell therapy (trial), anti-CTLA-4, BRAF inhibitor	0	>2 × ULN	5.0×10^7	Cy 60 mg/kg for 2 days + Flu 25 mg/m^2 for 5 days	14	PD	1.2	8.1[d]
4	44/F	Cutaneous	M1d (IM, li, C, lung)	Anti-PD-1, anti-CTLA-4	0	>2 × ULN	5.0×10^7	Cy 60 mg/kg for 2 days + Flu 25 mg/m^2 for 5 days	14	SD	2.5	6.5[d]
5	49/M	Cutaneous (acral)	M1c (li, IM, C, SC)	MEK inhibitor, anti-PD-1, anti-CTLA-4, DITC, TIL (trial)	1	<ULN	2.5×10^8	Cy 60 mg/kg for 2 days + Flu 25 mg/m^2 for 5 days	3[f]	PR (7.1)	9.3	24.8[d]
6	59/F	Cutaneous	M1c (LN, IM, li)	Anti-CTLA-4, anti-PD-1	0	1-2 × ULN	2.5×10^8[g]	Cy 60 mg/kg for 2 days + Flu 25 mg/m^2 for 5 days	—	SD	2.7	3.5[d]
7	46/F	Cutaneous	M1c (li, LN, oss, P, ce, v, pa, IM)	Anti-PD-1, anti-CTLA-4	1	1-2 × ULN	1.0×10^8	Cy 30 mg/kg for 2 days + Flu 25 mg/m^2 for 5 days	—	SD	2.1[h]	2.3[d]
8	56/M	Uveal	M1c (oss, li, SC)	Anti-CTLA-4	0	1-2 × ULN	1.0×10^8	Cy 30 mg/kg for 2 days + Flu 25 mg/m^2 for 5 days	—	SD	6.3	14.0[d]
9	49/M	Uveal	M1c (pl, lu)	—	0	1-2 ×ULN	1.0×10^8	Cy 30 mg/kg for 2 days + Flu 25 mg/m^2 for 5 days	—	SD	4.1[h]	25.2[d]
10	69/M	Uveal	M1c (pl, lu, LN, pan, SC, IM)	—	0	<ULN	1.0×10^8	Cy 30 mg/kg for 2 days + Flu 25 mg/m^2 for 5 days	—	SD	2.7	6.2[d]
11	66/F	Uveal	M1c (SC, lu, li)	—	0	1-2 × ULN	1.0×10^8	Cy 30 mg/kg for 2 days + Flu 25 mg/m^2 for 5 days	—	PR (4.1)	5.1	5.2[d]
12	71/F	Uveal	M1c (SC, AG)	Anti-CTLA-4	0	<ULN	1.0×10^8	Cy 30 mg/kg for 2 days + Flu 25 mg/m^2 for 5 days	—	SD	12.2	37.6[i]

AG, adrenal gland; BOR, best overall response; C, cutaneous; ce, cervix; CNS, central nervous system; CRS, cytokine release syndrome; Cy, cyclophosphamide; DOR, duration of best response; DSMB, Data Safety Monitoring Board; ECOG, Eastern Cooperative Oncology Group performance score; F, female; Flu, fludarabine; IM, intramuscular; LDH, lactate dehydrogenase; li, liver; LN, lymph nodes; lu, lung; M, male; NA, not available; om, omental; oss, osseus; ov, ovary; P, peritoneal; pa, parametrium; pan, pancreas; pl, pleural; PD, progressive disease; PR; partial response; PRe, pararenal; SC, subcutaneous; SD, stable disease; ULN, upper limit of normal; v, vagina.

[a]Subcutaneous injections of low-dose interleukin-2 (2×10^6 IU/once daily up to 2 weeks) following cell infusion.

[b]Time to progression (TTP), defined as the length of time between moment of cell infusion and time of first documented disease progression.

[c]Patient 1 experienced a grade 5 serious adverse event and died 9 days after cell infusion and subsequent cell dose was drastically lowered.

[d]Deceased.

[e]Patient 2 received a dose reduction of the chemotherapy based on the observed toxicity of patient 1.

[f]Because of the occurring toxicity (grade 3 CRS), it was agreed with the DSMB to omit IL-2 support in subsequent patients.

[g]After the last patient treated with 2.5×10^8 cells, the DSMB recommended to lower the cell dose to 1.0×10^8 transduced cells with half the dose of cyclophosphamide (30 mg/kg/day i.v.). Patients in this last dose cohort were additionally treated with intratympanic dexamethasone injections.

[h]Clinical progression.

[i]Ongoing at the time of data close-out.

https://doi.org/10.1016/j.iotech.2022.100089

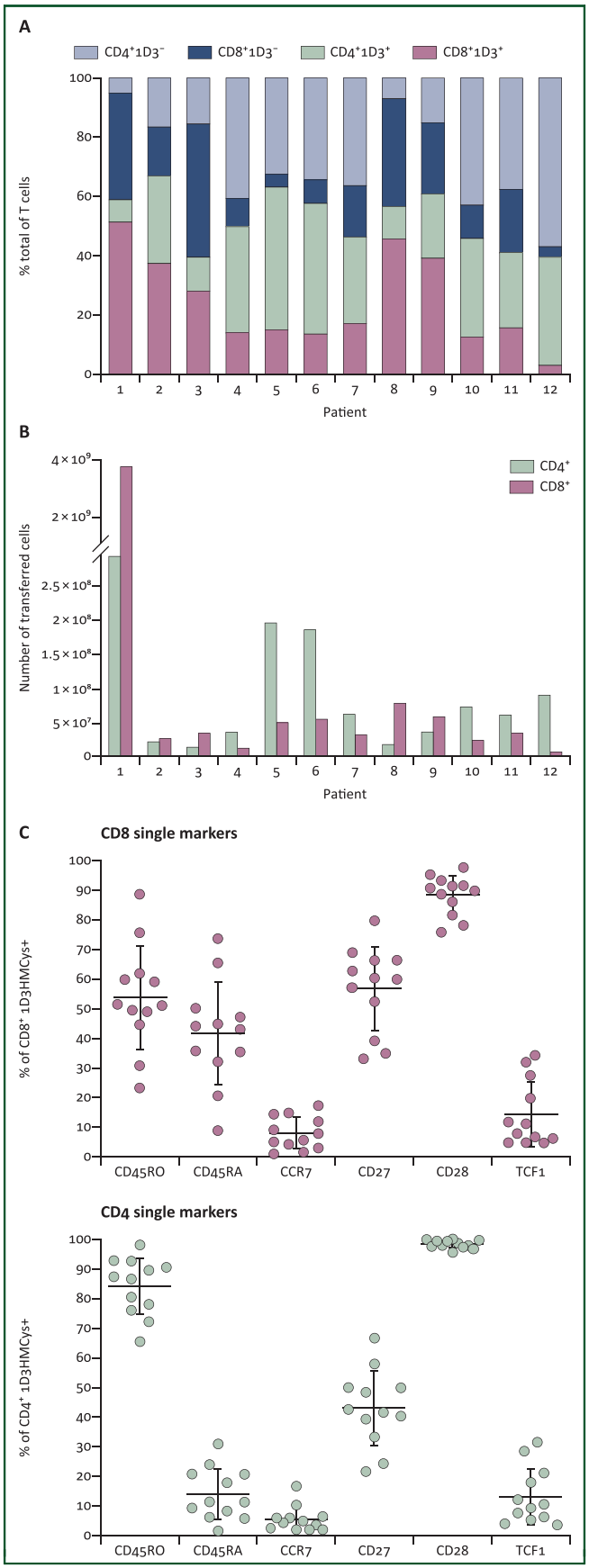

Figure 2. Characteristics of infusion products. Samples from the infusion products after 11 days of culture (end of production) were stained with HLA-A*02:01 MART-$1_{(26\text{-}35\ A>L)}$ tetramers to identify cells expressing the 1D3HMCys TCR. (A) Composition of infusion products. The median percentage of 1D3HMCys expression within $CD3^+$ T cells in the infusion products was 56.4% (range 41.9%-75.5%) with a median viability of 95.8% (range 92.9%-98.5%). (B) Number of $1D3HMCys^+$ transferred T cells. Total number of $CD4^+$ and $CD8^+$ cells expressing the 1D3HMCys TCR transferred per patient. (C) Phenotypical analysis of transgenic T cells. Differentiation marker expression of CD45RO, CD45RA, CCR7, CD27, CD28 and TCF1 was measured on infusion products. Expression in $CD8^+$ T cells (top) and $CD4^+$ T cells (bottom), gated on $1D3HMCys^+$ cells (as shown in Supplementary Figure S4A, available at https://doi.org/10.1016/j.iotech.2022.100089).
MART-1, melanoma antigen recognized by T cells 1; TCF1, T-cell factor 1; TCR, T-cell receptor.

seen in affected skin areas, suggesting that the skin toxicity was due to recognition of MART-1 on healthy melanocytes by the infused 1D3HMCys T cells (Figure 3D). Notably, most 1D3HMCys T cells cultured from the affected skin were $CD4^+$, possibly indicating a key role for these cells in the development of on-target, off-tumor toxicity at this site (Figure 3E). Uveitis and hearing impairment occurred in both patients. Patient 6 developed permanent sensorineural hearing loss in one ear, as previously described.[35] Both patients experienced CRS (grade 2 and 3), requiring tocilizumab (8 mg/kg with a maximum of 800 mg), an anti-IL-6 receptor antibody, and local and systemic steroids.

All patients treated in the final dose cohort of 1.0×10^8 1D3HMCys T cells developed acceptable toxicity, with grade 2 (two patients) and grade 3 (three patients) dermatitis occurring in five out of six patients. One patient experienced grade 2 conjunctivitis and two patients, treated with intratympanic dexamethasone injections, developed grade 1 hearing loss.

In the total cohort, the occurrence of CRS was dependent on the T-cell dose and was associated with elevated IL-6 serum levels (Figure 4). However, occurrence of toxicities did not seem to correspond to other measured serum cytokine levels in peripheral blood upon cell infusion (Supplementary Figure S2, available at https://doi.org/10.1016/j.iotech.2022.100089).

Clinical benefit after treatment with 1D3HMCys T cells was not clearly associated with administered cell dose

Two of 11 assessable patients (18%) reached an objective partial response (PR) (Figure 5A and B) at 9.5 weeks [duration of best response (DOR) 7.1 months after 2.5×10^8 cells] and 4 weeks (DOR 4.1 months after 1.0×10^8 cells), respectively. In five additional patients, minor tumor shrinkage (not qualifying for response as per RECIST 1.1) was observed.

The percentage of MART-1 expression in tumors before therapy did not correspond with response and sequential pre- and post-treatment tumor biopsies from most patients showed no significant changes in MART-1, HLA or CD3 expression (Supplementary Table S4, available at https://doi.org/10.1016/j.iotech.2022.100089). However, a 40% decrease in MART-1 expression and HLA-A expression relative to a pre-treatment biopsy of the same lesion was observed upon progression in patient 4 (Figure 5C). Flow cytometry analysis of expanded TIL from biopsies of the same subcutaneous metastasis from patient 5 at 7 and 9 months (time of progression) after treatment showed

A

Patient	Cell dose	Hospitalization (days)	T-cell related on-target AEs, all grades (day of onset after cell infusion)[a]	Cytokine-mediated AEs, all grades[b]	≥ Grade 3 AEs related to chemotherapy/IL-2		
					Grade 3	Grade 4	Grade 5
1	4.56×10^9	16	Multiple organ failure due to CRS/ sepsis, (G5; +9)[c]	G4	Anemia, febrile neutropenia, AST, fever, AF, catheter-related infection, GGT, hypoxia, nervous system disorder	Neutropenia, lymphopenia, thrombocytopenia, cardiac arrest, depressed level of consciousness	Sepsis
2	5.0×10^7	18	Dermatitis, G1 (4) Skin hypopigmentation, G1 (74)	..	Febrile neutropenia, hypophosphatemia, hypertension, vasovagal reaction	Neutropenia, lymphopenia	
3	5.0×10^7	21	Dermatitis, G2 (4) Skin hypopigmentation, G1 (18)[d]	..	Anemia, febrile neutropenia, thrombocytopenia, catheter-related infection	Neutropenia, lymphopenia	
4	5.0×10^7	24	Dermatitis, G3 (6)	..	Febrile neutropenia, thrombocytopenia, hypophosphatemia, AST, ALT, GGT	Neutropenia, lymphopenia, fever	
5	2.5×10^8	21	Dermatitis, G3 (2) Uveitis, G2 (10) Hearing impairment, G3 (14)[e]	G3[f]	Anemia, febrile neutropenia, thrombocytopenia, hypophosphatemia, fever, hypotension, dyspnea	Neutropenia, lymphopenia, hepatic hemorrhage	
6	2.5×10^8	21	Dermatitis, G3 (2) Uveitis, G2 (10) Hearing impairment, G3 (11)[g]	G2[f]	Anemia, febrile neutropenia, hypophosphatemia, hearing impairment, hypoxia	Neutropenia, lymphopenia, thrombocytopenia	
7	1.0×10^8	20	Dermatitis, G3 (5) Conjunctivitis, G2 (6)	G1	Thrombocytopenia, AF	Neutropenia, lymphopenia	
8	1.0×10^8	16	Dermatitis, G2 (5)	..	Febrile neutropenia	Neutropenia, lymphopenia	
9	1.0×10^8	16	Dermatitis, G3 (2) Skin hypopigmentation, G2 (18)[h]	..	Anemia, febrile neutropenia	Neutropenia, lymphopenia	
10	1.0×10^8	18	Dermatitis, G2[i]	..	Febrile neutropenia, hypophosphatemia	Neutropenia, lymphopenia	
11	1.0×10^8	24	Hearing impairment, G1 (15)[j]	G2[f]	Febrile neutropenia, thrombocytopenia, hypophosphatemia, fever, blood bilirubin, thromboembolic event	Neutropenia, lymphopenia	
12	1.0×10^8	22	Dermatitis, G3 (4) Hearing impairment, G1 (8)	..	Febrile neutropenia, ALT	Neutropenia, lymphopenia	

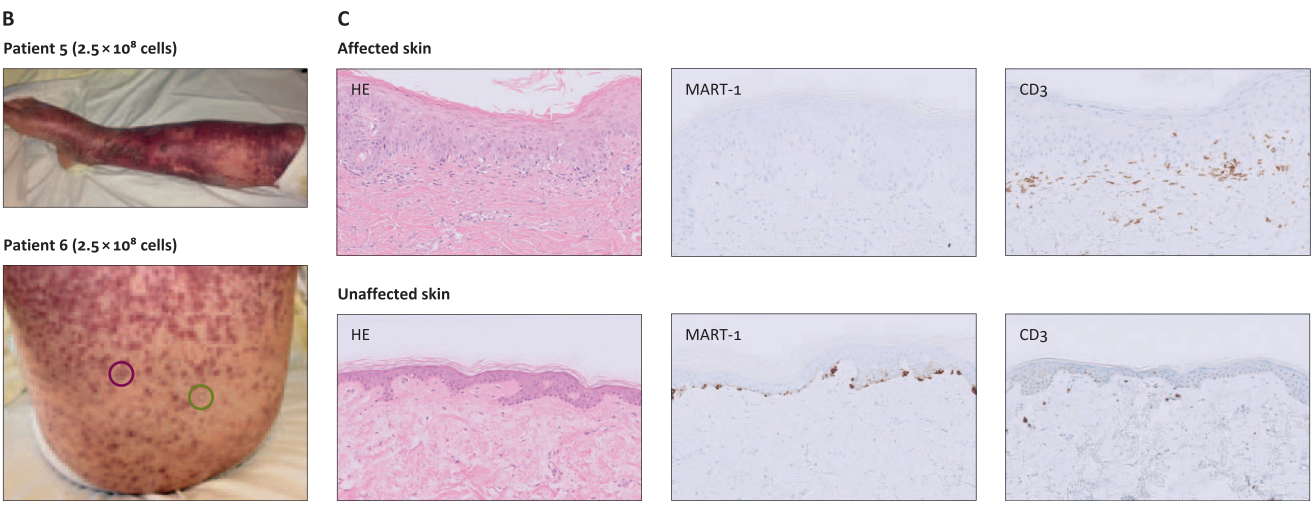

Figure 3. Treatment-related toxicity. (A) All T-cell product-related on-target, off-tumor, cytokine-mediated and ≥ grade 3 adverse events (AEs) related to the lymphodepleting chemotherapy and/or interleukin-2 (IL-2), per dose cohort. All presented AEs are the worst grade (G) occurring in the patient graded by Common Terminology Criteria for Adverse Events (CTCAE) v.4.03. The most common on-target, off-tumor toxicities due to the 1D3HMCys T cells presented as dermatitis in

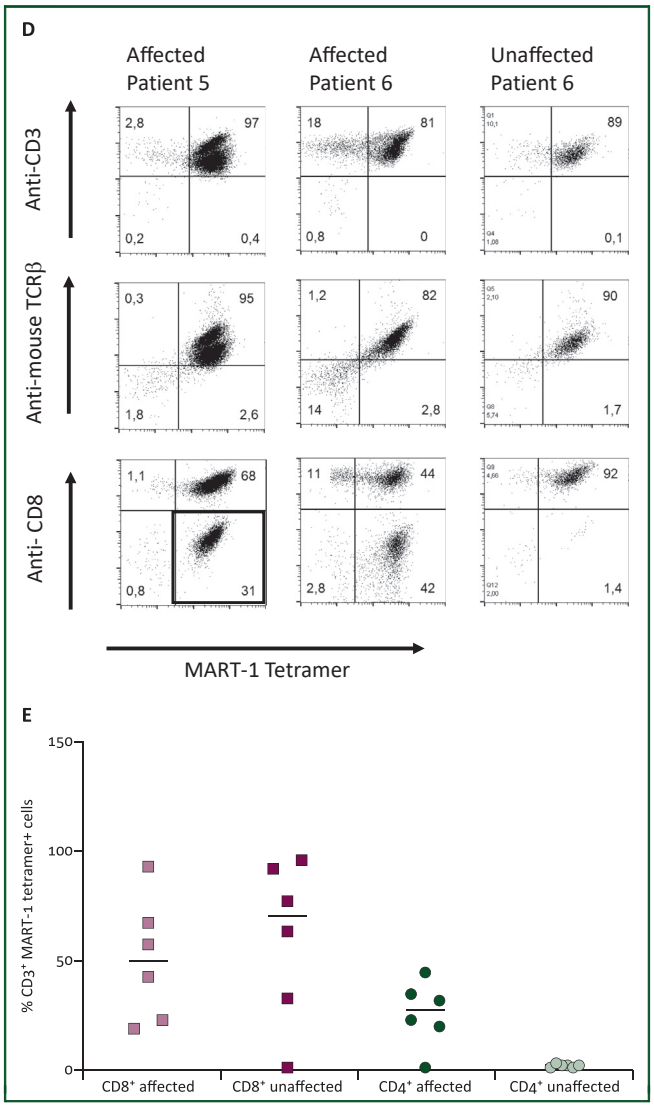

Figure 3. Continued.

persistence of $CD4^+$ and $CD8^+$ 1D3HMCys T cells (Figure 5D).

After a median follow-up of 37 months, the median PFS for the total cohort was 2.8 months [95% confidence interval (CI) 1.28-6.41 months] (Supplementary Figure S3A, available at https://doi.org/10.1016/j.iotech.2022.100089). Median OS for the total cohort was 7.3 months (95% CI 2.33-25.26 months) with a 1-year OS of 41.7% (95% CI 15.25% to 66.53%) (Supplementary Figure S3B, available at https://doi.org/10.1016/j.iotech.2022.100089).

Long-term in vivo *persistence of 1D3HMCys T cells in peripheral blood*

Persistence of 1D3HMCys T cells in peripheral blood was related to infused cell dose and 1D3HMCys T cells could be traced in peripheral blood up to 9 months post-infusion in the patient with the most durable response. Peak frequencies of 1D3HMCys T cells in the peripheral blood were observed within 20 days of infusion, and 1D3HMCys T-cell numbers dropped less quickly in patients treated with the highest cell dose (Figure 6A). A large variation in the percentage of 1D3HMCys $CD4^+$ and $CD8^+$ T cells in peripheral blood was observed over time, with a trend toward longer persistence of 1D3HMCys $CD8^+$ T cells compared to 1D3HMCys $CD4^+$ T cells (Figure 6B), possibly reflecting distinct tissue distributions.

Expression of CD4, CD8, LAG-3, TIM-3, CCR7, CD45RA, CD137, OX40 and programmed cell death protein 1 (PD-1) on 1D3HMCys T cells was evaluated in infusion products and on peripheral blood T cells at multiple time points after infusion. Supplementary Figure S4A, available at https://doi.org/10.1016/j.iotech.2022.100089, shows the gating strategy and Supplementary Figure S4B, available at https://doi.org/10.1016/j.iotech.2022.100089, illustrates results for patient 5, with a PR, showing long-term persistence of 1D3HMCys T cells. Infusion products from all patients

10/12 (83%) (max grade 3, median duration of worst-grade dermatitis of 12 days), uveitis/conjunctivitis in 3/12 (25%) (max grade 2, median duration of 26 days) and ototoxicity in 4/12 (33%) patients (max grade 3, median duration of worst-grade hearing impairment of 86.5 days in assessable patients). [a]Indicates the time of onset of first symptoms. [b]Cytokine-mediated AEs, with a heterogeneous presentation characterized by fever, tachycardia, hypotension, edema and increased oxygen need [clinically referred to as cytokine release syndrome (CRS)]. [c]The first patient experienced a grade 5 serious AE and died 9 days after cell infusion. [d]In patients 2 and 3, development of vitiligo was seen following the dermatitis. [e]Patient 5 recovered to grade 2 hearing impairment 16 days after onset of symptoms. Further evaluation was not possible as no subsequent audiograms were carried out and the patient was thus lost to follow-up for this AE. [f]Tocilizumab (8 mg/kg with a maximum of 800 mg) was administered in patients 5, 6 and 11 with resolution of symptoms thereafter. Tocilizumab administration for the treatment of CRS was added to the study protocol after patient 1 experienced a grade 5 AE. [g]Patient 6 suffered from permanent unilateral hearing loss, as described earlier.[35] [h]Patient 9 was already known with grade 1 skin hypopigmentation, but worsened to grade 2 after treatment. [i]In patient 10, the dermatitis could have been an exacerbation of previously diagnosed Darier's disease. [j]Grade 1 hearing impairment in patient 11 was still present at the time of death. (B) Clinical representations of grade 3 dermatitis as an 'on-target, off-tumor' AE in patients 5 and 6. Development of dermatitis occurred at day 2 after cell infusion for patients 5 and 6. Both patients required treatment with topical steroids and patient 5 also received systemic steroid therapy due to progressive skin rash and persisting CRS despite tocilizumab administration. In patient 6, skin biopsies from affected and unaffected skin (locations of biopsies outlined in purple and green, respectively) were taken for histopathological evaluation (see Figure 3C and D). (C) Histopathological features of dermatitis from patient 6. Hematoxylin—eosin (HE) and immunohistochemical stainings of MART-1 and CD3 were carried out on skin biopsies from the back of affected and unaffected skin, taken 4 days after development of dermatitis (6 days after cell infusion). Loss of melanocytes and MART-1 expression and infiltration of $CD3^+$ T cells along the dermoepidermal junction can be seen in the affected skin compared to the unaffected skin (magnification ×200). (D) Flow cytometry analysis of skin-infiltrating lymphocytes (SILs) in patients with dermatitis. Needle biopsies were taken 4 days post-infusion from the affected and unaffected skin of patient 5, who developed dermatitis 2 days after infusion of 2.5×10^8 1D3HMCys T cells. After 8 days, SILs expanded from the affected skin but not from the unaffected skin. Flow cytometry analysis of the affected skin showed that skin-infiltrating cells were 95% double positive for the 1D3HMCys TCR and anti-mouse TCRβ indicating that all cells were derived from the infusion product. From patient 6, needle biopsies were taken from the affected and unaffected skin 6 days post-infusion (see Figure 3B for the exact location). After 7 days, SIL expanded from the affected and unaffected skin. FACS analysis showed that infiltrating cells were >80% double positive for the 1D3HMCys TCR and anti-mouse TCRβ both for the unaffected and affected skin. (E) Presence of 1D3HMCys T cells in the affected skin of patients with dermatitis. From a total of six patients, biopsies were obtained from both the affected and unaffected skin. In five out of six patients, an increase was seen in $CD4^+$ 1D3HMCys T cells in the affected skin compared to the unaffected skin.

AF, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; FACS, fluorescence-activated cell sorting; GGT, γ-glutamyl transferase; MART-1, melanoma antigen recognized by T cells 1; TCR, T-cell receptor.

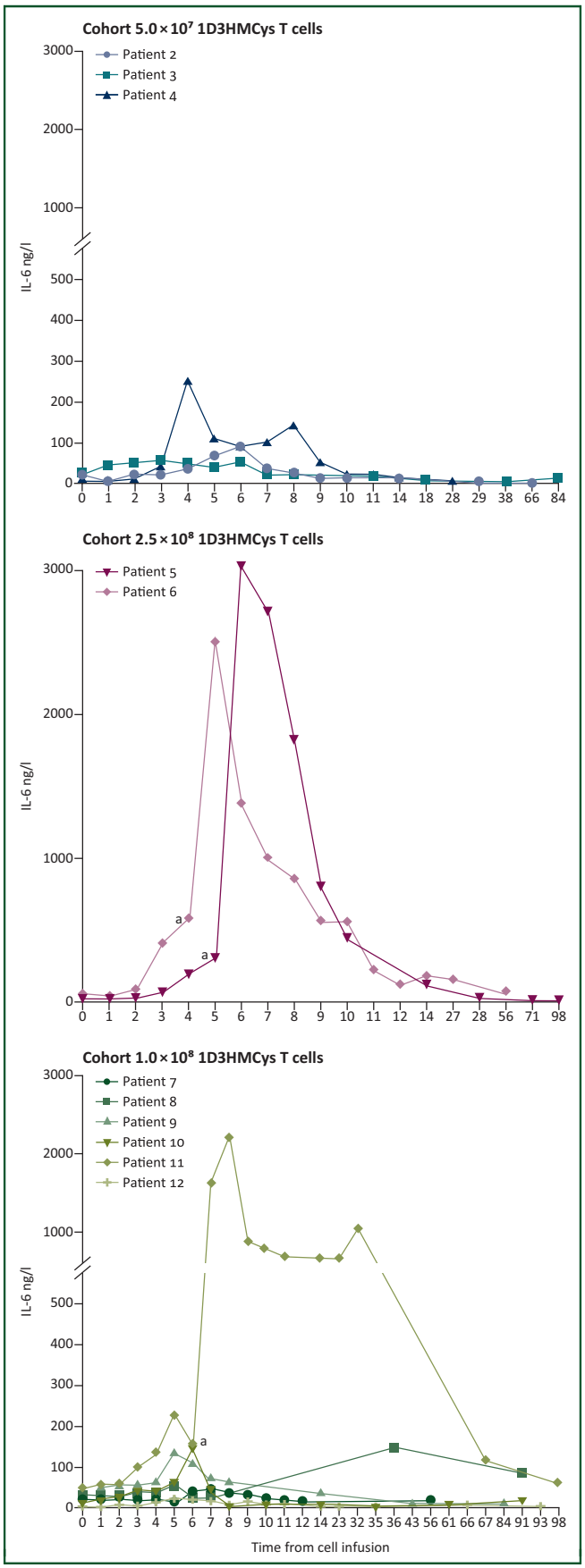

Figure 4. Course of serum IL-6 levels. Serum levels of IL-6 after infusion of 1D3HMCys T cells represented from day of cell infusion (day 0) and per cell dose cohort. Reference value IL-6 <7.0 ng/l. In all patients, a marked increase was seen in IL-6 at a median of 5 days (range 3-7 days) after cell infusion, with a median peak value of 141.4 ng/l (range 26.1-588.8 ng/l). A clear association was seen between the value of IL-6 and the severity of toxicity, as the three patients with the highest IL-6 peaks developed CRS and required tocilizumab administration. [a]Patients 5, 6 and 11 received a single dose of tocilizumab (8 mg/kg with a maximum of 800 mg) on days 5, 4 and 6, respectively, due to persisting symptoms despite supportive measures.
CRS, cytokine release syndrome; IL-6, interleukin-6.

showed low expression of LAG-3, CD137, OX40 and PD-1, but relatively high expression of TIM-3 both on $CD4^+$ and $CD8^+$ cells, consistent with a non-exhausted T-cell phenotype.

DISCUSSION

This phase I/IIa, single-center, single-arm, dose-escalation trial, investigating adoptive transfer of autologous peripheral blood T cells transduced with the MART-1-specific 1D3HMCys TCR in patients with metastatic melanoma, was stopped prematurely because of severe dose-dependent toxicity and modest clinical activity at tolerated cell doses. Production of 1D3HMCys T cells expanded in the presence of IL-7 and IL-15 was feasible for all patients and *in vitro* killing capacity of transduced $CD8^+$ T cells was demonstrated.

The first patient in this study received 4.56×10^9 1D3HMCys T cells, based on previous MART-1-specific TCR gene therapy studies,[11,12] and experienced grade 5 toxicity 9 days after cell infusion because of CRS/sepsis and multiple organ failure.[33] In subsequent patients, cell dose was reduced by 100-fold. In these cohorts with 5.0×10^7, 2.5×10^8 and 1.0×10^8 cells, respectively, severe dose-dependent toxicity was observed, comprising dermatitis, uveitis/conjunctivitis, ototoxicity and CRS (in 5/12 patients), with grade 3-5 toxicity in 7/12 patients.

Before this study, three clinical trials with MART-1-specific TCR therapy in melanoma patients have been reported. Morgan et al. (2006)[11] treated patients with autologous peripheral blood T cells retrovirally transduced with the DMF4 TCR recognizing the MART-$1_{(26-35)}$ epitope. Infused transduced cells ranged from 0.5 to 34.4×10^9 and 13% (2/15) of patients showed objective tumor regression without development of treatment-related toxicities. In a subsequent trial by Johnson et al. (2009),[12] T cells expressing a high-avidity MART-1-specific TCR (DMF5) were generated and patients received between 0.5 and 97.4×10^9 transgenic cells, resulting in an ORR of 30% (6/20 patients). However, these patients experienced widespread on-target, off-tumor toxicity, ascribed to the on-target recognition of the MART-1 antigen in normal melanocytes. Biopsies of affected skin showed a dense infiltrate of T cells (mainly $CD8^+$ T cells) with concurrent destruction of melanocytes, comparable with the observations in our clinical trial. A third clinical trial by Chodon et al. (2014)[36] combined ACT of $0.6\text{-}4.8 \times 10^9$ MART-1-specific T cells, produced using a shorter (1 week) manufacturing protocol, and dendritic cell vaccination treating 13 patients with metastatic melanoma. Severe toxicity (rash and acute respiratory distress) and

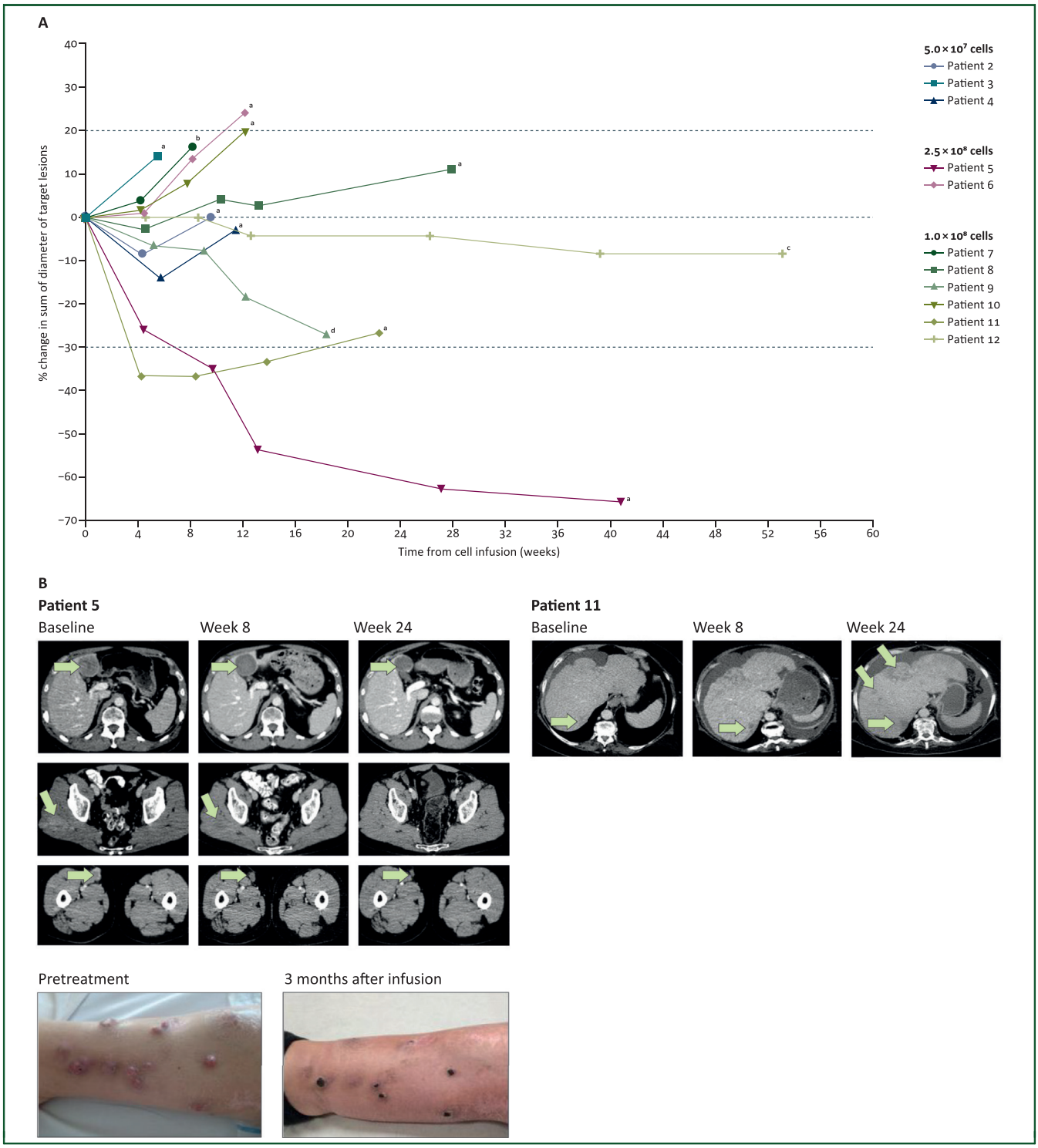

Figure 5. Clinical outcomes. (A) Clinical activity of 1D3HMCys T cells. Spider plot showing changes in size of target lesions according to RECIST 1.1 for all assessable patients ($n = 11$). Baseline computed tomography (CT) scans were carried out at t = 0 and changes in lesion size at each follow-up visit are presented at the subsequent time points. The dashed lines at 20% and −30% change in the sum of the diameter of the target lesions represent progressive disease and partial response (PR), respectively. Patients 5 and 11 reached a PR at 9.5 and 4 weeks after cell infusion, respectively. [a]New lesion. [b]Clinical progression and deceased before next tumor evaluation. [c]New lesion detected clinically and confirmed by pathology. [d]Clinical progression with palliative radiotherapy on target lesion. (B) Pre- and post-treatment CT scans of the two patients (5 and 11) with a PR according to RECIST 1.1. The duration of responses was 7.1 and 4.1 months, for patients 5 and 11, respectively. (C) Immunohistochemical changes in tumor sites upon infusion of 1D3HMCys T cells in patient 4. Hematoxylin—eosin (HE) and immunohistochemical stainings with melanoma antigen recognized by T cells 1 (MART-1), HLA-A and CD3 were carried out on pre- and post-treatment biopsies from the same subcutaneous metastasis from the right flank of patient 4 (magnification ×400). This patient showed progressive disease 88 days after cell infusion. A decrease can be seen in MART-1 and HLA-A expression at the time of progression compared to the pre-treatment biopsy, from 80% to 40% and 75% to 40%, respectively. Immune infiltration scored by CD3 expression remained grade 1. (D) Persistence of 1D3HMCys T cells in tumor sites in patient 5. Tumor biopsies were taken at 7 and 9 months after infusion of 2.5×10^8 1D3HMCys T cells from the same subcutaneous metastasis and were cultured in the presence of IL-2. Expanded T cells were analyzed by flow cytometry. Biopsy at 7 months: expanded TILs were analyzed after 5 days of culture. Nine percent of the $CD3^+$ cells stained with HLA-A*02:01 MART-$1_{(26\text{-}35\ A>L)}$ tetramers and anti-mouse TCRβ, indicating that they express the transgenic 1D3HMCys TCR. Of the $CD4^+$ cell population, 20% were 1D3HMCys$^+$, while for the $CD8^+$ cells this was 6%. Biopsy at 9 months (time of progression): expanded TILs were analyzed after 3 days of culture. Within the $CD3^+$ cells, 3% expressed the 1D3HMCys TCR. One percent of the $CD4^+$ and 5% of the $CD8^+$ T cells were 1D3HMCys$^+$.

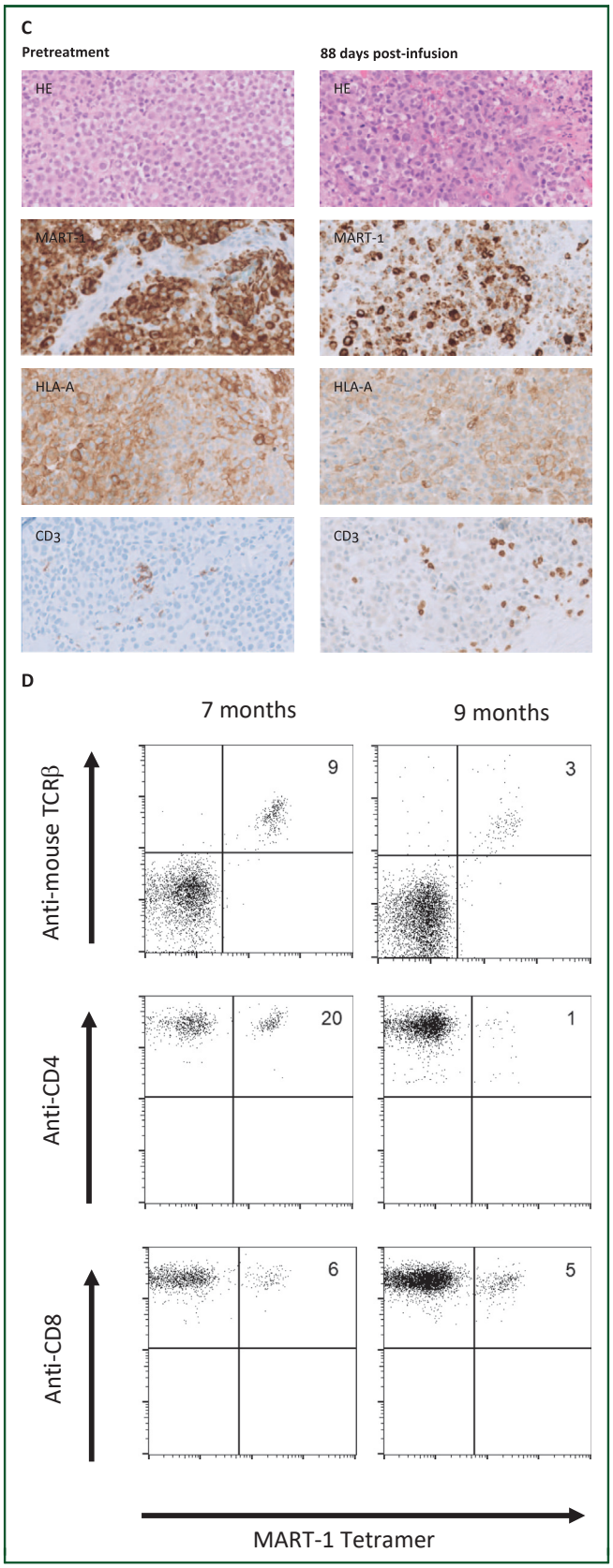

Figure 5. **Continued.**

short-lived antitumor activity were observed after the infusion of MART-1 transgenic T cells.

In this clinical trial, severe dose-dependent, on-target toxicity and CRS with a maximum tolerated dose of 1.0×10^8 gene-modified T cells were observed. This 10-fold or more lower maximum tolerated dose compared to the previous MART-1-specific TCR studies led to similar or even more severe on-target, off-tumor toxicity and CRS, which might very well be attributed to the production method of MART-1-engineered T cells. Although the same epitope of MART-1 was targeted, peripheral blood-derived T cells were isolated and activated using anti-CD3/CD28 beads instead of an anti-CD3 monoclonal antibody. Subsequently, isolated T cells were expanded and activated in the presence of IL-7 and IL-15 instead of the more commonly used IL-2, with the goal of stimulating the *in vitro* development and maintenance of less differentiated T cells with a memory stem/central memory phenotype.

A major fraction of engineered T cells in our infusion product expressed CD45RO, CD45RA, CD27 and CD28, indicating a less differentiated phenotype. Moreover, this expansion protocol yielded a relatively high percentage of $CD4^+$ cells in the infusion product. Both could be possible downstream effects of the novel production strategy, resulting in highly active cells. Patients 5 and 6 received the largest amount of $CD4^+$ 1D3HMCys T cells, possibly contributing to the severe toxicity in both patients. Whether this increased toxicity was due to the less differentiated phenotype or increased proportion of $CD4^+$ T cells or both remains unclear. Intriguingly, the two patients with a PR also had relatively high fractions of $CD4^+$ 1D3HMCys T cells in their infusion products (48% and 25% for patients 5 and 11, respectively). $CD4^+$ T cells have been shown to contribute importantly to antitumor activity[37]; however, their contribution to cytotoxicity in this study could not be addressed separately.

Besides exposure to 1D3HMCys T cells, patients received lymphodepleting chemotherapy and low-dose IL-2 to facilitate engraftment, as in previous trials.[11,12] Both can cause significant toxicity; however, there is no clear indication that either of these supporting treatments was a potential confounder affecting safety in this trial. After patient 1 experienced grade 5 toxicity, cell dose was drastically lowered in the following three patients, with unchanged chemotherapy and IL-2 dosing. Mild, transient toxicity was observed and cell dose was increased. After patient 5 experienced grade 3 CRS, IL-2 was omitted in the subsequent patients to reduce toxicity. Despite receiving the same cell dose as patient 5 without IL-2, patient 6 still developed severe on-target, off-tumor toxicity and grade 2 CRS. As the exact role of lymphodepletion next to cell numbers in the development of toxicities was unknown, the cyclophosphamide and cell dose were lowered. In retrospect, however, both protocol amendments and

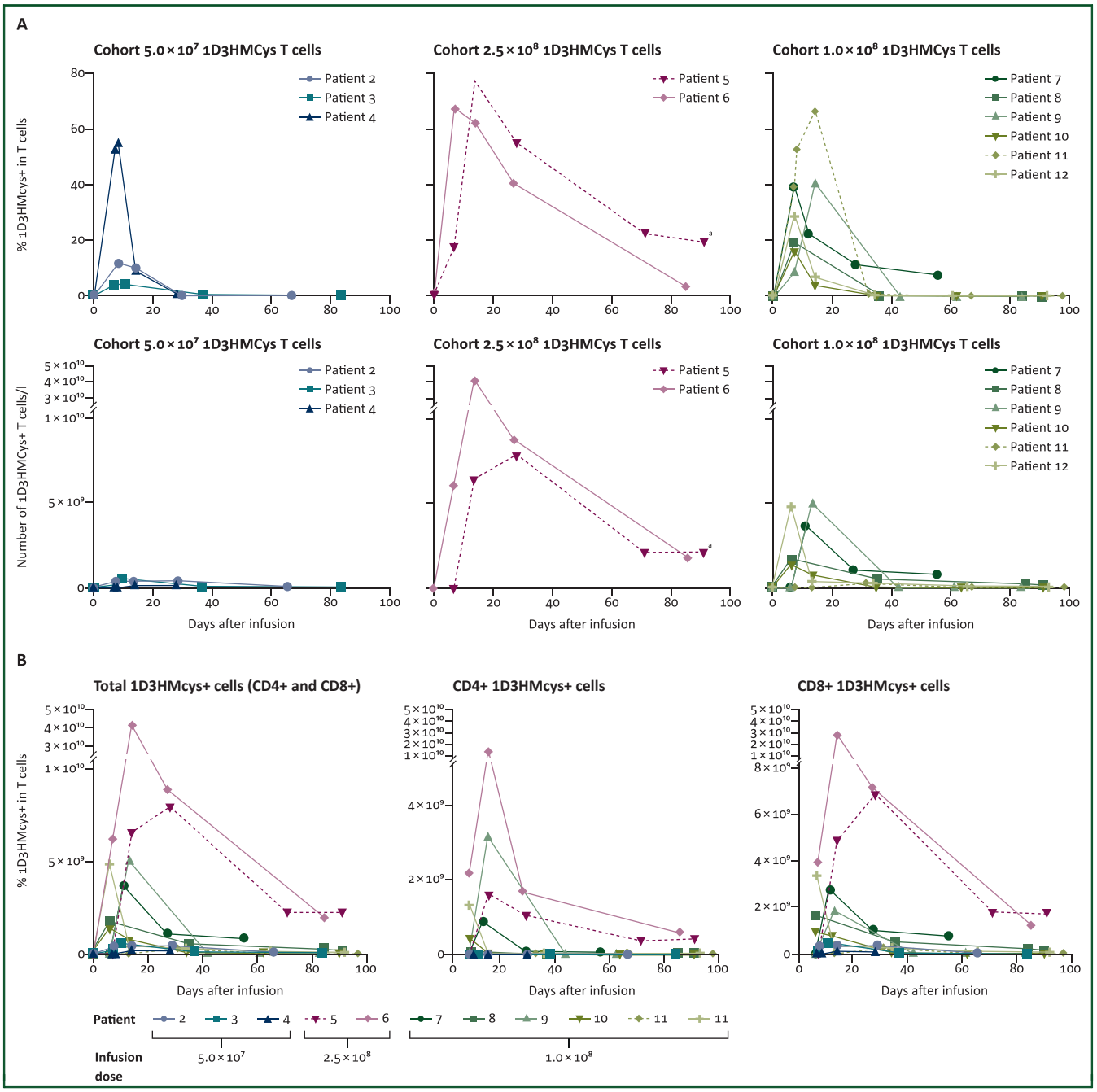

Figure 6. Persistence of 1D3HMCys T cells in peripheral blood after infusion. (A) The percentages (top row) and absolute numbers (bottom row) of 1D3HMcys$^+$ cells in the blood of patients were quantified after infusion and were correlated with infused cell dose. [a]Persistence of transgenic cells was confirmed at 9 months post-infusion in patient 5. (B) Distribution of 1D3HMcys$^+$ CD4$^+$ and CD8$^+$ T cells over time. Absolute numbers of total 1D3HMcys$^+$ cells and CD4$^+$ or CD8$^+$ 1D3HMcys$^+$ cells after infusion, quantified at different time points as indicated. Absolute numbers of transduced T cells were calculated by determining the percentage of 1D3HMCys TCR T cells within the flow cytometry lymphocyte gate, multiplied by the absolute number of lymphocytes per liter of blood.
TCR, T-cell receptor.

subsequent clinical course in patients clearly suggest that the toxicity was more related to the cell dose than the supporting treatments.

Objective antitumor activity was observed in two patients receiving 1.0×10^8 and 2.5×10^8 cells. For enrollment, >10% MART-1 expression in tumors was required; however, the percentage of cells expressing target antigen for optimal efficacy is unknown. As most melanomas express MART-1,[13,14] the arbitrary minimum 10% MART-1 expression was used.[38] The percentage of MART-1 expression before treatment did not clearly correspond with clinical efficacy. The patient with the most durable response received the highest cell dose and demonstrated the longest persistence of modified T cells in peripheral blood, in line with observations in earlier trials.[12,39,40] In contrast to previous trials, patients with cutaneous melanoma in our trial had failed prior immune checkpoint inhibition (ICI) and therefore response rates are difficult to compare, as these

patients (in the post-ICI era) are considered less susceptible to immunotherapy. Of note, one patient who had failed both anti-cytotoxic T-lymphocyte-associated protein-4 and anti-PD-1 therapy achieved a PR, adding to the evidence that adoptive cellular therapies can be efficacious after failure of prior ICI. This was also observed in a recent trial with lifileucel, a TIL therapy, that induced a response rate of 36% in ICI-refractory cutaneous melanoma patients.[41]

An objective response was also observed in a patient with uveal melanoma, a disease with, until recently, no viable treatment options and poor response to ICI.[2] Intriguingly, this patient received an attenuated dose of chemotherapy and no IL-2, supporting the fact that these cells are highly potent and that the supporting treatments had little influence on clinical efficacy. In a recent phase III trial, treatment with tebentafusp (a bispecific protein of a soluble TCR fused to an anti-CD3 single-chain variable fragment-activating domain targeting an MDA) resulted in prolonged OS,[3] further indicating that T-cell-directed killing targeting MDAs in uveal melanoma has merit. Promising antitumor activity has also been seen with adoptive transfer of TIL in these patients,[42] supporting further investigation of immune therapies including TCR-based therapies in this disease.

The observed strong on-target, off-tumor reactivity poses a major limitation for the further development of TCR gene therapy targeting tumor-associated antigens such as MART-1 that are also presented on normal tissue cells. To further improve the safety and efficacy of TCR gene therapy, target selection, ideally expressed exclusively on tumor cells, is of great importance. Attractive strategies currently under investigation showing high specificity are targeting neo-antigens solely expressed on tumors, the tumor microenvironment or engineering T cells solely triggered by multiple specific antigens (or absence thereof).[43,44]

In conclusion, this clinical trial demonstrated the feasibility to generate and to adoptively transfer TCR-engineered T cells grown in the presence of IL-7 and IL-15, with a less differentiated phenotype leading to a highly reactive cell population with clear dose-dependent toxicity and modest antitumor activity. This production method in combination with strictly tumor-specific TCR-engineered T cells could be an attractive strategy to improve ACT, leading to highly potent cells with high tumor reactivity and reduction of on-target, off-tumor toxicity.

ACKNOWLEDGEMENTS

The authors thank all co-workers from the Netherlands Cancer Institute for making this trial run successfully. We especially kindly acknowledge the nursing staff and medical team of the nursing ward for excellent patient care and crucial support. Furthermore, we thank the departments of surgery, radiology, pulmonology, dermatology, intensive care unit, clinical laboratory and ophthalmology (external centers) for their clinical expertise and patient care. In addition, the Quality Assurance and Quality Control departments of the Hospital Pharmacy, the Clinical Chemistry Laboratory and Pathology are kindly acknowledged for their generous support. We also thank the Flow Cytometry Facility and Genomic Core Facility for their contributions to this trial. Lastly, we express our appreciation to the blood bank Sanquin for working with us and preserving the material obtained from apheresis.

FUNDING

This work was supported by ZonMw [grant number 431-00-005] and the Dutch Cancer Society (project number AVLZ 2013-5924).

DISCLOSURE

RGE is stockholder in Neogene Therapeutics. JHvdB has received grants from NEON therapeutics, BMS and Medimmune. DvdB declares to have received compensation (paid to the institute) for advisory roles for Roche. CUB received compensation (all paid to the institute except TRV) for advisory roles for Bristol-Myers Squibb, Merck Sharp & Dohme, Roche, Novartis, GlaxoSmithKline, AstraZeneca, Pfizer, Lilly, GenMab, Pierre Fabre, Third Rock Ventures, received research funding (all paid to the institute) from Bristol-Myers Squibb, Novartis, NanoString and declares stockownership in Immagene BV, where he is co-founder. JHB is part-time employee and (in)direct stock holder of Modra Pharmaceuticals (small spin off company of the Netherlands Cancer Institute) and (partly) holds a patent on oral taxane formulations, which are clinically developed by Modra Pharmaceuticals. This is not related to the manuscript. TNS is advisor to and stockholder in Allogene Therapeutics, Asher Bio, Merus, Neogene Therapeutics and Scenic Biotech and consultant to Third Rock Ventures. JBAGH received compensation (all paid to the institute except for Neogene Therapeutics) for advisory roles for Achilles Therapeutics, BioNTech, BMS, Gadeta, Immunocore, Instil Bio, Iovance Biotherapeutics, Ipsen, MSD, Merck Serono, Molecular Partners, Neogene Therapeutics, Novartis, Pfizer, Roche/Genentech, Sanofi, Third Rock Ventures and T-knife, and has received grants (all paid to the institute) from Amgen, Asher Bio, BioNTech, BMS, MSD, Novartis and Neogene Therapeutics. All other authors have declared no conflicts of interest.

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