

Adoptive Transfer of MART-1 T-Cell Receptor Transgenic Lymphocytes and Dendritic Cell Vaccination in Patients with Metastatic Melanoma

Thinle Chodon^{1,18}, Begoña Comin-Anduix^{2,6}, Bartosz Chmielowski^{1,6}, Richard C. Koya^{2,6,18}, Zhongqi Wu¹, Martin Auerbach⁵, Charles Ng¹, Earl Avramis¹, Elizabeth Seja¹, Arturo Villanueva¹, Tara A. McCanne⁷, Akira Ishiyama², Johannes Czernin^{5,6}, Caius G. Radu^{5,6}, Xiaoyan Wang¹, David W. Gjertson³, Alistair J. Cochran³, Kenneth Cornetta¹⁴, Deborah J.L. Wong¹, Paula Kaplan-Lefko¹, Omid Hamid¹⁰, Wolfram Samlowski¹⁵, Peter A. Cohen¹⁶, Gregory A. Daniels¹¹, Bijay Mukherji¹⁷, Lili Yang^{4,6,8}, Jerome A. Zack^{1,6,8}, Donald B. Kohn^{4,6,8}, James R. Heath¹², John A. Glaspy^{1,6}, Owen N. Witte^{4,5,8,9}, David Baltimore¹³, James S. Economou^{2,4,5,6}, and Antoni Ribas^{1,2,5,6,8}

Abstract

Purpose: It has been demonstrated that large numbers of tumor-specific T cells for adoptive cell transfer (ACT) can be manufactured by retroviral genetic engineering of autologous peripheral blood lymphocytes and expanding them over several weeks. In mouse models, this therapy is optimized when administered with dendritic cell (DC) vaccination. We developed a short 1-week manufacture protocol to determine the feasibility, safety, and antitumor efficacy of this double cell therapy.

Experimental Design: A clinical trial (NCT00910650) adoptively transferring MART-1 T-cell receptor (TCR) transgenic lymphocytes together with MART-1 peptide-pulsed DC vaccination in HLA-A2.1 patients with metastatic melanoma. Autologous TCR transgenic cells were manufactured in 6 to 7 days using retroviral vector gene transfer, and reinfused with ($n = 10$) or without ($n = 3$) prior cryopreservation.

Results: A total of 14 patients with metastatic melanoma were enrolled and 9 of 13 treated patients (69%) showed evidence of tumor regression. Peripheral blood reconstitution with MART-1-specific T cells peaked within 2 weeks of ACT, indicating rapid *in vivo* expansion. Administration of freshly manufactured TCR transgenic T cells resulted in a higher persistence of MART-1-specific T cells in the blood as compared with cryopreserved. Evidence that DC vaccination could cause further *in vivo* expansion was only observed with ACT using noncryopreserved T cells.

Conclusion: Double cell therapy with ACT of TCR-engineered T cells with a very short *ex vivo* manipulation and DC vaccines is feasible and results in antitumor activity, but improvements are needed to maintain tumor responses. *Clin Cancer Res*; 20(9); 2457–65. ©2014 AACR.

Introduction

The genetic transfer of α and β chains of the T-cell receptor (TCR) endows recipient T cells with the specificity of donor T cells (1), allowing the generation of large numbers of T cells with uniform antigen. Initial clinical experiences testing adoptive cell transfer (ACT) of TCR-engineered T cells in humans have provided clear evi-

dence of antitumor efficacy in patients with metastatic melanoma and sarcoma (2–4). Pioneering TCR-engineering clinical trials by Steven A. Rosenberg and colleagues at the Surgery Branch, National Cancer Institute (NCI, Bethesda, MD; refs. 2, 3, 5) included a 2- to 4-week *ex vivo* T-cell culture. Preclinical models suggest that extended *ex vivo* expansion of lymphocytes before ACT

Authors' Affiliations: Departments of ¹Medicine, ²Surgery, ³Pathology and Laboratory Medicine, ⁴Microbiology, Immunology and Molecular Genetics, and ⁵Molecular and Medical Pharmacology; ⁶Jonsson Comprehensive Cancer Center; ⁷Department of Ophthalmology, Jules Stein Eye Institute; ⁸Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research; ⁹Howard Hughes Medical Institute, University of California, Los Angeles (UCLA); ¹⁰The Angeles Clinic Research Institute, Los Angeles; ¹¹Department of Medicine, University of California San Diego (UCSD) Moores Cancer Center, La Jolla; Divisions of ¹²Chemistry and ¹³Biology, California Institute of Technology, Pasadena, California; ¹⁴Department of Medical and Molecular Genetics, Indiana University, and the Indiana University Viral Production Facility (IU VPF), Indianapolis, Indiana; ¹⁵Comprehensive Cancer Centers of Nevada, Las Vegas, Nevada; ¹⁶Mayo Clinic Scottsdale, Scottsdale, Arizona; ¹⁷Department of Medicine,

University of Connecticut Health Center, Farmington, Connecticut; and ¹⁸Center for Immunology, Roswell Park Cancer Institute, Buffalo, New York

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

Corresponding Author: Antoni Ribas, Division of Hematology-Oncology, 11-934 Factor Building, UCLA, 10833 Le Conte Avenue, Los Angeles, CA 90095-1782. Phone: 310-206-3928; Fax: 310-825-2493. E-mail: aribas@mednet.ucla.edu

doi: 10.1158/1078-0432.CCR-13-3017

©2014 American Association for Cancer Research.

Translational Relevance

This article describes an investigator-initiated clinical trial using two cell therapies, one genetically modified and one pulsed with peptides, for the treatment of melanoma. We report on a high response rate in patients with melanoma, attesting to the high antitumor activity of adoptively transferred T-cell receptor–engineered lymphocytes administered with dendritic cell (DC) vaccination and high-dose interleukin 2. However, it also points out to the need to improve the durability of the tumor responses, which is the basis of further research in this protocol. With the evidence that adoptive cell transfer (ACT) therapy is a viable option for patients with advanced cancers, there is an increasing need in establishing ACT programs at multiple institutions. We think that this work demonstrating the feasibility of a short 1-week manufacture protocol and detailed clinical observations will help other groups in establishing similar programs.

results in more terminally differentiated cells with limited proliferation ability *in vivo* and lower antitumor activity (6, 7). Provision of antigen in the form of a vaccine is required in some animal models to support the antitumor activity of adoptively transferred T cells (8–10). This may be because exposure to antigen while undergoing homeostatic proliferation can stimulate further T-cell expansion (11, 12).

To test this combined cell therapy approach in the clinic, the University of California, Los Angeles (UCLA)/Caltech F5 clinical trial was designed with a short, 1-week, cell manipulation that included initial lymphocyte activation followed by retroviral transduction and limited further *ex vivo* cell expansion. We also provided autologous MART-1_{26–35} peptide-loaded DC, a vaccine that in our prior experience had resulted *per se* in two complete responders out of 25 patients with metastatic melanoma. These responses are durable over 10 years later (13, 14).

Patients and Methods

Study design and conduct

A Simon optimal two-stage phase II clinical trial design (15) was used to allow for the simultaneous testing of three coprimary endpoints, safety, feasibility, and objective tumor response. Patients were enrolled in the clinical trial after signing a written informed consent approved by the UCLA Institutional Review Board (#08-02-020 and #10-001212) under an investigational new drug (IND) filed with the U.S. Food and Drug Administration (IND# 13859). The study was conducted in accordance with local regulations, the guidelines for Good Clinical Practice, and the principles of the current version of the Declaration of Helsinki. The study had the clinical trial registration number NCT00910650.

Trial eligibility and screening procedures

Eligible patients were HLA-A*0201 by molecular subtyping, had progressive locally advanced (stage IIIc) or metastatic melanoma (stage IV) with either no available standard therapeutic options with a curative intent, or who had progressed on standard options like chemotherapy, high-dose interleukin (IL)-2, interferon, and experimental therapies as listed in Table 1; the melanoma was MART-1–positive by immunohistochemistry, age greater than or equal to 18, Eastern Cooperative Oncology Group performance status 0 or 1, life expectancy greater than 3 months, adequate organ function as routinely required to receive high-dose IL-2 (16), and seronegative for HIV, Hepatitis B and C. Patients with clinically active brain metastases were excluded. Baseline radiological documentation of absence of active brain metastases was required for all patients, but previously treated brain metastases were acceptable. All patients underwent formal ophthalmologic and otologic exams at baseline and periodically after TCR-engineered ACT.

Study outline

The study outline is included in Fig. 1. Patients underwent baseline leukapheresis for the manufacture of the two cell therapies, the F5 TCR gene–modified T cells, and the MART-1 peptide-pulsed DC (see Supplementary Online Methods). Patients received conditioning chemotherapy as inpatients consisting of cyclophosphamide 60 mg/kg/day × 2 days i.v. and fludarabine 25 mg/m²/day i.v. daily for 4 or 5 days as previously described (2, 3, 5, 17). On day 0, patients received the ACT of TCR-engineered lymphocytes as an i.v. infusion with the cells thawed at bedside under the initial study protocol. Patients were transferred for monitoring to the intensive care unit to start high-dose IL-2 on the next morning, and then they received the first intradermal administration of MART-1_{26–35} peptide-pulsed DC. IL-2 (Proleukin; Prometheus) was given at 600,000 IU/kg i.v. every 8 hours for up to 14 (amendments 1–7) or 9 (amendments 8–10) doses, as tolerated, following the standard high-dose IL-2 UCLA protocol (16). Standard supportive care was provided including filgrastim (Neupogen; Amgen), antibiotics for neutropenic fever, and red blood cell and platelet transfusions. When patients had recovered peripheral blood cell counts and were transfusion-free, they were discharged from the hospital. Patients received two more doses of DC vaccines at 2 weeks intervals. Patients were eligible to receive 3 more booster DC vaccines after study day 90 if the level of MART-1 TCR transgenic cells was below 5% of total lymphocytes by MHC tetramer or dextramer assay. A research [¹⁸F]FDG positron emission tomography (PET) scan and biopsy was planned at around day 30, and formal restaging PET computed tomography (CT) scans were performed on day 90.

Safety assessments

Safety was determined in stage I, and if 3 of 8 patients have MART-1 F5 TCR-induced dose-limiting toxicities (DLT), then further accrual would not be warranted. Adverse events were analyzed following NCI CTCAE v3.0.

Table 1. Patient demographics and outcomes

Patient study number	Sex (M/F)	Age	Prior Treatments for stages III-IV	Active metastasis sites	Stage	Protocol amendment	Number of F5 TCR transgenic cells	Cryo or fresh	Number of doses of IL-2	Number of doses of DCs	Evidence of transient tumor response	Response at EOS (day 90)	PFS (mo)	OS (mo)
F5-1	M	60	—	Lung, stomach, liver, pancreas, peritoneum, soft tissues, bone	M1c	1-5	1×10^9	Cryo	12/14	3/3	Yes by PET/CT	Progression	3	5
F5-2	F	46	HD IFN- α 2b MKC prime-boost vaccine, HD IL-2	Skin, LN, bone	M1c		1×10^9		6/14	3/3	Yes by PET/CT	Stable disease	6	10
F5-3	M	61	—	Lung, liver	M1c		1×10^9		13/14	3/3	Yes by PET/CT	Stable disease	5	46+
F5-4	M	50	HD IL-2	Lung, LN, SC	M1c		0.6×10^9		14/14	3/3	No	Progression	2	22
F5-5	M	53	HD IFN	Lung	M1c		NA		NA	NA	NA	NA	NA	NA
F5-6	M	59	—	Lung, LN	M1b		1×10^9		13/14	3/3	Yes by PE	Stable disease	3	4
F5-7	M	48	HD IL-2	SC, bone	M1c		1×10^9		9/14	3/3	Yes by CT	Stable disease	4	11
F5-8	M	44	—	LN, liver, lung	M1c		1×10^9		11/14	3/3	Yes by PET/CT	Stable disease	4	11
F5-9	F	46	—	Skin, LN	M1a		1×10^9		11/14	3/3	No	Progression	3	16
F5-10	F	47	—	Liver, adrenal, SC, LN, orbit	M1c	6-7	4.8×10^9		14/14	2/3	Yes by PET/CT	Progression	2	8
F5-11	F	56	—	Lung, LN	M1b		1.8×10^9		8/14	3/3	Yes by PET/CT	Stable disease	4	4
F5-12	M	40	HD IFN- α 2b, temozolomide, ipilimumab, CR-011, paclitaxel, MLN-4924, AMG-337	Lung, LN	M1b	8-9	3.9×10^9	Fresh	6/9	3/3	Yes by PET/CT	Stable disease	5	6
F5-13	M	60	HD IFN, HD IL-2, ipilimumab	Lung, abdomen, SC	M1b		4.41×10^9		4/9	3/3	Yes by PET/CT	Progression	3	6
F5-14	F	50	Ipilimumab	Lung, liver, adrenal gland, LN, bone	M1c		3.93×10^9		3/9	1/3	Yes by CXR	Progression	0	1

Abbreviations: Cryo, cryopreserved TCR transgenic cells; CXR, chest X-ray; F, female; HD, high dose; LN, lymph nodes; M, male; N, normal; NA, not available; Neg, negative; SC, subcutaneous; PE, physical exam; PFS, progression free survival; EOS, end of study; DC, dendritic cells; TCR, T cell receptor; IL-2, interleukin-2; mo, months.

Results

Patient characteristics

Between April 2009 and September 2011, 14 HLA-A*0201-positive patients with MART-1-positive metastatic melanoma were accrued. Patients had a median age of 50 years. Nine patients had M1c metastatic melanoma with visceral and/or bone metastases, 4 had lung metastases (M1b), and 1 had skin, nodal, and subcutaneous-only metastases (M1a). Half of the patients had received prior therapy for metastatic disease, including high-dose IL-2 in 4 patients and ipilimumab in 3 patients (Table 1).

Study conduct and protocol changes

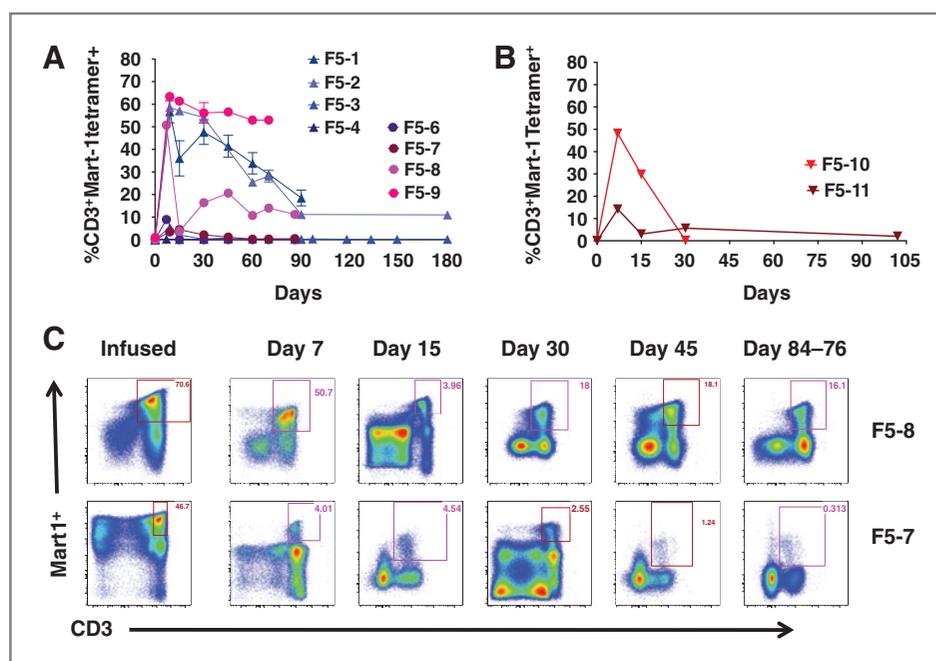
This study underwent nine protocol amendments during its conduct, with some significantly changing the delivered cell therapies; therefore, we analyzed patients in subgroups based on the protocol amendments. The first 9 patients (F5-1 to F5-9) were treated under the original protocol (amendments 1–5) administering up to 1×10^9 previously cryopreserved TCR transgenic lymphocytes following the full cyclophosphamide–fludarabine conditioning regimen and up to 14 infusions of high-dose IL-2. One patient (F5-5) had brain metastases at the baseline MRI screening exam and did not receive the TCR transgenic infusion. Therefore, this patient is not accounted in the safety, feasibility, or efficacy evaluations. After the planned study endpoint assessment based on the first 8 patients receiving the full protocol, it was deemed that the study was safe and feasible, but the antitumor activity assessed on day +90 was suboptimal. Therefore, patients F5-10 and F5-11 were treated under amendments 6–7 that allowed an increase in the cell number of previously cryopreserved TCR transgenic lymphocytes up to 1×10^{10} . Because of an event of delayed pancytopenia in patient F5-10 who had continu-

ing evidence of durable antitumor activity at that time, and fludarabine being the most likely cause of marrow toxicity, the study was further amended (amendments 8–9, patients F5-12 to F5-14) to decrease the conditioning regimen (one fewer day of fludarabine). Because this patient also had received 14 doses of IL-2, a potential adverse contributor to the marrow aplasia, which was more than what the other patients tolerated except for F5-4, the number of potential IL-2 doses was also limited to a maximum of 9. Studies in murine model of ACT showed that cryopreservation has a profound detrimental effect on the *in vivo* long-term survival of the adoptively transferred T cells and their ability to have a secondary response to antigen exposure. Therefore, we amended to administer noncryopreserved, freshly produced TCR transgenic lymphocytes (Fig. 1B).

Peripheral blood reconstitution with cryopreserved TCR transgenic cells

TCR transgenic cell preparations were manufactured for all patients that met the lot release criteria of viability >70%, negative for mycoplasma, Gram and fungal stain, endotoxin level of ≤ 5 EU/kg body weight, and >10% MART-1 tetramer-positive CD3 lymphocytes (Supplementary Fig. S1). On average, transduction efficiency was 64% (range, 42%–84%; Supplementary Figs. S2 and S3). The first 10 patients received cryopreserved TCR-engineered cells, with the first 8 receiving up to 1×10^9 cells and the next 2 patients receiving up to 1×10^{10} cells. However, there was not much difference in the absolute number of delivered MART-1 TCR transgenic cells (Supplementary Fig. S3). Despite administering a similar number of cells to these patients, peripheral cell reconstitution varied widely (Fig. 2). In this group of patients, the mean and median peak blood levels of TCR transgenic cells were 31%, with a maximum of 63% and a

Figure 2. Postinfusion peripheral blood levels of MART-1 TCR transgenic cells at various time points in patients receiving cryopreserved transgenic cells. A, F5-1 to F5-9 receiving up to 10^9 cryopreserved transgenic cells. B, F5-10 and F5-11 receiving up to 10^{10} cryopreserved transgenic cells. C, representative dot plots of MART-1 MHC tetramer analysis of infused cells and postinfusion peripheral blood PBMC in F5-7 and F5-8.



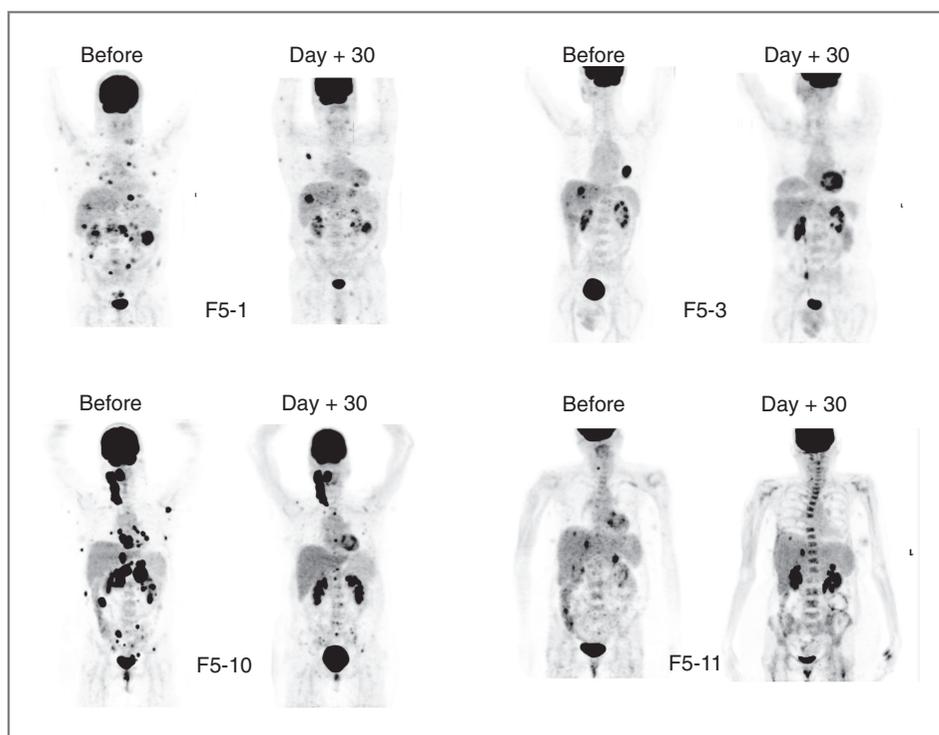


Figure 3. Pre- and posttreatment day 30 PET scans indicating initial antitumor activity. Representative scans of F5-1 and F5-3 receiving up to 10^9 cryopreserved transgenic cells, F5-10 and F5-11 receiving up to 10^{10} cryopreserved transgenic cells.

minimum of 0.67% of total $CD3^+$ T lymphocytes. The peak of TCR transgenic cell frequency was early, within the first 2 weeks after ACT, and the percentage and absolute number of TCR transgenic cells in peripheral blood decreased over time in all patients. There was no clear evidence of enhancing number or function of peripheral blood TCR transgenic cells with the delivery of MART-1 peptide-pulsed DC vaccines on study days 14 and 28.

Antitumor activity with cryopreserved TCR transgenic cells

There was evidence of initial antitumor activity in 8 of 10 patients with metastatic melanoma regression detected at day 30 PET scans (examples in Figs. 3 and 4, and additional explanation in Supplementary Online Methods) or physical examination. However, the initial antitumor activity was incomplete and transient. At the formal restaging PET CT scans on day 90, none of the patients had evidence of a sustained tumor response by RECIST criteria. Therefore, after the first 8 patients had been enrolled, it was deemed that improvements in the protocol were needed. This led to the testing of a higher TCR transgenic cell dose, and then the testing of the infusion of freshly manufactured cells, without cryopreservation.

Safety evaluation of cryopreserved TCR transgenic cells

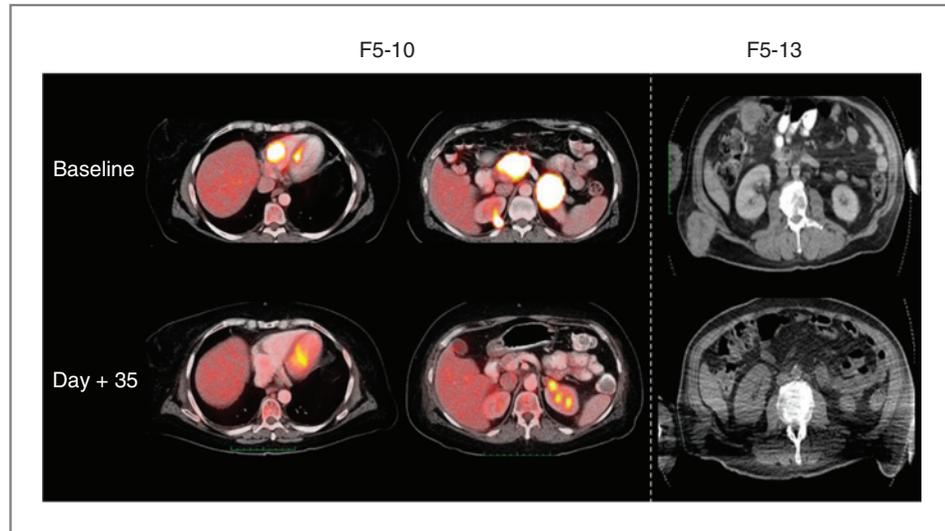
There were no unanticipated serious toxicities in the first 8 patients in the protocol beyond the ones that would be expected with the conditioning chemotherapy and high-dose IL-2 therapy. Of note, there were no findings of otologic, vestibular, or ophthalmologic toxicities after repeated specialist visits. Therefore, the protocol was deemed to meet

the coprimary endpoint of safety when administered as 1×10^9 cryopreserved cells. Because the antitumor activity did not meet the prespecified criteria to proceed to the second stage of study, the next 2 patients were treated with up to 1×10^{10} cryopreserved cells. However, the outcomes did not improve because one patient had a transient tumor response and the other had stable disease (Supplementary Figs. S4–S6).

Safety and antitumor activity with noncryopreserved TCR transgenic cells

A new protocol amendment was introduced to administer 1×10^{10} TCR-engineered cells to be infused fresh after harvesting from the *ex vivo* culture, without a cryopreservation step. The baseline leukapheresis was performed on the day of hospital admission, and cells were manufactured concurrent to patients receiving conditioning chemotherapy. The conditioning chemotherapy was shortened by one day and the number of potential IL-2 doses was limited at nine. Three patients were enrolled and had evidence of transient tumor responses by serial X-rays and PET scans, but also had a more pronounced whole body erythematous skin rash compared with the majority of prior patients in this protocol. Furthermore, 2 of the patients (F5-12 and F5-14) had serious adverse events (SAE) of acute respiratory distress requiring intubation associated with patchy pulmonary infiltrates within 1 week of cell infusion (Supplementary Fig. S7), resulting in the discontinuation of this cohort due to increased toxicities. Plasma from peripheral blood was analyzed for the production of multiple cytokines to study the potential development of a cytokine storm (Supplementary Fig. S8). Both patients received

Figure 4. Pre- and posttreatment day 35 PET/CT (F5-10) and CT (F5-13) showing evidence of initial antitumor activity. Representative scans of F5-10 receiving up to 10^{10} cryopreserved transgenic cells and F5-13 receiving up to 10^{10} freshly harvested transgenic cells.



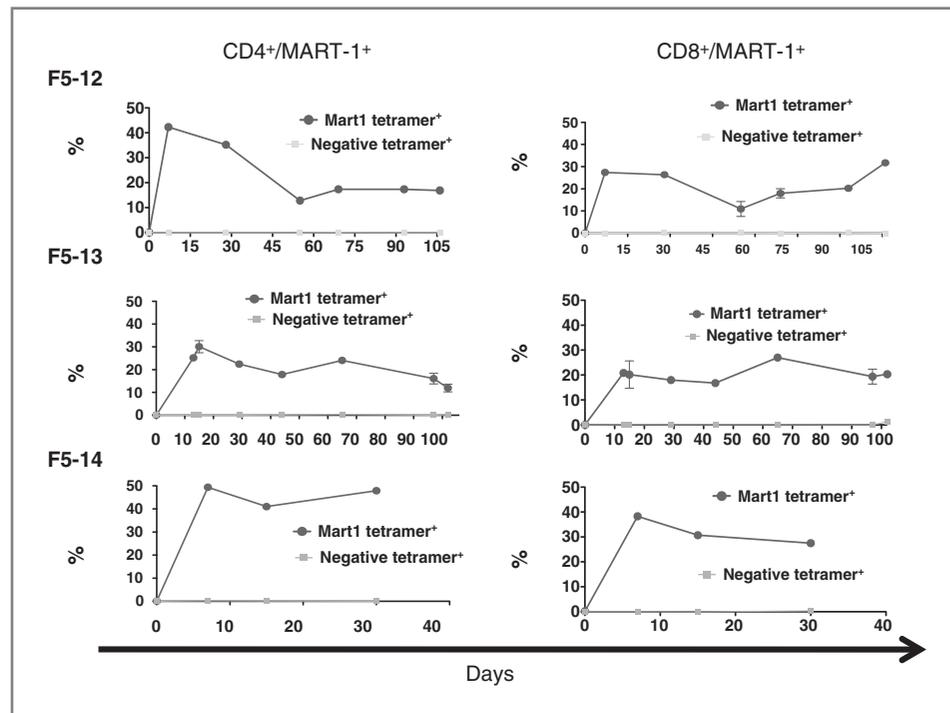
corticosteroid therapy and recovered their baseline respiratory function within 2 weeks.

Despite this increased toxicities with the infusion of fresh TCR transgenic cells and the administration of corticosteroids in these 2 patients and lack of administration of further MART-1/DC vaccinations, this group of 3 patients all had evidence of antitumor activity. The serial chest X-rays shown in Supplementary Fig. S7 document a time-course decrease in size of lung metastases in patients F5-12 and F5-14, and the CT scan images from patient F5-13 in Fig. 4 demonstrate the regression of large subcutaneous/muscle metastases. However, none of these responses were durable with all 3 patients having disease progression within 6 months from study start.

Peripheral blood reconstitution and DC boosting effect with fresh TCR transgenic cells

The infusion of fresh TCR transgenic lymphocytes resulted in a more prolonged persistence of circulating TCR transgenic cells in blood in the 3 patients from whom we had samples for analysis compared with the previous experience with cryopreserved cells (Fig. 5). It is remarkable that both $CD4^+$ and $CD8^+$ TCR transgenic cells were approximately 20% of the peripheral T lymphocytes at 3 months after ACT in F5-12 and F5-13. Furthermore, patient F5-13, who did not have a SAE and received the three scheduled MART-1/DC administrations, had evidence of recall whole body rash and reexpansion of the TCR

Figure 5. Postinfusion peripheral blood levels of MART-1 TCR transgenic cells at various time points in patients receiving freshly harvested transgenic cells. MART-1 tetramer-positive $CD4^+$ and $CD8^+$ levels in F5-12, F5-13, and F5-14 receiving up to 10^{10} freshly harvested transgenic cells.



transgenic cells in peripheral blood, demonstrating *in vivo* activation impact of MART-1/DC vaccination (Supplementary Fig. S9).

Discussion

The MART-1 TCR ACT protocol described herein resulted in a high rate of transient tumor responses. These results are not too different from the studies at the NCI Surgery Branch despite multiple differences in the cell manufacture protocol (see Supplementary Online Discussion), primarily our short 1-week *ex vivo* culture using basic equipment available in any facility, resulting in younger cells and the use of DC vaccination. The generation of transgenic T cells, TILs, and endogenous antigen-specific T cells that have been used in the trials so far involved complex processes and at least 4 to 8 weeks of culture, thereby feasible only in a few specialized centers, and many of these patients progressing rapidly do not have this long a time to wait. Tumor progression after an initial response may be due to loss of antitumor activity of the TCR transgenic cells given to patients or changes in tumor cells resulting in acquired resistance to this mode of immunotherapy. The decrease in frequency of TCR transgenic T cells after the initial brisk expansion is a logical evolution of an effective immune response, as is commonly noted with T-cell responses to viral infections (21). In other work (22), we analyzed TCR transgenic T cells administered and recovered from 3 of the patients in this series using new-generation microfluidics-based miniaturized assays able to simultaneously study multiple functional responses of T cells selected based on defined antigen specificities (23, 24). These studies showed that the initial polyfunctionality resulting in high antitumor activity of the administered TCR transgenic T cells is gradually lost over time *in vivo*, which is temporarily associated with the clinical course of initial tumor response followed by progression. Therefore, there is a need to better maintain the TCR transgenic cell polyfunctionality upon ACT. This could be achieved pharmacologically using monoclonal antibodies blocking negative immune checkpoints (CTLA4; PD-1; ref. 25), with small molecules fostering T memory cell function (26, 27), or by endogenously generating fully active TCR transgenic cells from stem cell precursors (28–31).

Patients who experienced respiratory distress with the administration of noncryopreserved TCR transgenic cells demonstrated an increase in circulating cytokines and chemokines, nevertheless lower than the levels noted in two clinical reported cases of life-threatening cytokine storm (32, 33). In fact, these cytokine levels are comparable with those observed in acute pneumonia (34). Despite these toxicities, this group of patients had favorable antitumor activity with evidence of longer persistence of circulating TCR transgenic cells and *in vivo* expansion following DC vaccination.

In conclusion, a short *ex vivo* manufacture protocol was able to generate large numbers of tumor-specific TCR trans-

genic T cells. Administration of these in combination with DC vaccination is feasible and has high initial antitumor activity. These two cell therapies can be concurrently manufactured while patients undergo conditioning with lymphodepleting chemotherapy over a period of 1 week. The administration of freshly manufactured cells without cryopreservation results in higher *in vivo* persistence, but also in a higher incidence of side effects when targeting the self-melanosomal antigen MART-1. However, improvements in the conditions to maintain TCR transgenic cell functionality, tumor responses, and to lower toxic side effects are needed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: T. Chodon, B. Chmielowski, R.C. Koya, D.W. Gjertson, B. Mukherji, L. Yang, J.A. Zack, O.N. Witte, D. Baltimore, J.S. Economou, A. Ribas

Development of methodology: T. Chodon, B. Chmielowski, R.C. Koya, C.G. Radu, D.W. Gjertson, A.J. Cochran, K. Cornetta, B. Mukherji, L. Yang, D. Baltimore, A. Ribas

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T. Chodon, B. Comin-Anduix, B. Chmielowski, R.C. Koya, Z. Wu, M. Auerbach, C. Ng, E. Avramis, T.A. McCannel, A. Ishiyama, J. Czernin, K. Cornetta, D.J.L. Wong, P. Kaplan-lefko, O. Hamid, W. Samlowski, P.A. Cohen, G.A. Daniels, J.R. Heath, J.A. Glaspy, A. Ribas

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T. Chodon, B. Comin-Anduix, B. Chmielowski, R.C. Koya, M. Auerbach, E. Avramis, J. Czernin, X. Wang, D.W. Gjertson, A.J. Cochran, J.R. Heath, J.A. Glaspy, J.S. Economou, A. Ribas

Writing, review, and/or revision of the manuscript: T. Chodon, B. Chmielowski, R.C. Koya, M. Auerbach, E. Avramis, T.A. McCannel, J. Czernin, X. Wang, A.J. Cochran, K. Cornetta, W. Samlowski, G.A. Daniels, J.A. Zack, D.B. Kohn, D. Baltimore, A. Ribas

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T. Chodon, Z. Wu, E. Avramis, E. Seja, A. Villanueva, T.A. McCannel, P. Kaplan-lefko, O.N. Witte, D. Baltimore, A. Ribas

Study supervision: T. Chodon, B. Chmielowski, R.C. Koya, A.J. Cochran, J.S. Economou, A. Ribas

Acknowledgments

The authors thank Steven A. Rosenberg, Richard Morgan, Laura Johnson, and Mark Dudley (all from the NCI Surgery Branch, Bethesda, MD) for their guidance in establishing the TCR-engineered ACT protocol at UCLA and allowing access to their clinical grade retroviral vector master cell bank. They also acknowledge the contributions of Erika von Euw, Joanne Cox, and Narsis Attar in some of the cell therapy preparations.

Grant Support

This work was funded by the NCI grants P01 CA132681 (to D. Baltimore, J.S. Economou, O.N. Witte, A. Ribas, D.B. Kohn, and J.A. Glaspy), U54 CA119347 (to J.R. Heath and A. Ribas), P50 CA086306 (to A. Ribas), and RO1 CA129816 (to J.S. Economou), the California Institute for Regenerative Medicine New Faculty Award RN2-00902-1 (to A. Ribas), the Eli & Edythe Broad Center of Regenerative Medicine and Stem Cell Research at UCLA (to O.N. Witte and A. Ribas), the Samuel Waxman Foundation (to J.S. Economou, D. Baltimore, and O.N. Witte), the Keck Foundation (to J.S. Economou, D. Baltimore, and O.N. Witte), The Seaver Institute (to A. Ribas), the PhaseOne Foundation (to A. Ribas), the Louise Belley and Richard Schnarr Fund (to A. Ribas), the Wesley Coyle Memorial Fund (to A. Ribas), the Garcia-Corsini Family Fund (to A. Ribas), the Bila Alon Hacker Memorial Fund (to A. Ribas), the Fred L. Hartley Family Foundation (to A. Ribas), the Ruby Family Foundation (to A. Ribas), the Joy and Jerry Monkarsh Fund (to J.S. Economou), the Caltech/UCLA Joint Center for Translational Medicine (to D. Baltimore, O.N. Witte, and A. Ribas), and the Melanoma Research Alliance (to A. Ribas, D. Baltimore, and J.R. Heath). O.N. Witte is an investigator of the Howard Hughes Medical Institute. R.C. Koya was supported in part by the V Foundation-Gil Nickel Family Endowed

Fellowship in Melanoma Research. The UCLA Jonsson Comprehensive Cancer Center (JCCC) Flow Cytometry Core Facility is supported by NIH awards CA-16042 and AI-28697.

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 November 4, 2013; revised January 21, 2014; accepted February 17, 2014; published OnlineFirst March 14, 2014.

References

- Dembic Z, Haas W, Zamojska R, Parnes J, Steinmetz M, von Boehmer H. Transfection of the CD8 gene enhances T-cell recognition. *Nature* 1987;326:510-1.
- 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.
- 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.
- Robbins PF, Morgan RA, Feldman SA, Yang JC, Sherry RM, Dudley ME, et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. *Am J Clin Oncol* 2011;29:917-24.
- Johnson LA, Heemskerk B, Powell DJ Jr, Cohen CJ, Morgan RA, Dudley ME, et al. Gene transfer of tumor-reactive TCR confers both high avidity and tumor reactivity to nonreactive peripheral blood mononuclear cells and tumor-infiltrating lymphocytes. *J Immunol* 2006;177:6548-59.
- Klebanoff CA, Gattinoni L, Torabi-Parizi P, Kerstann K, Cardones AR, Finkelstein SE, et al. Central memory self/tumor-reactive CD8+ T cells confer superior antitumor immunity compared with effector memory T cells. *Proc Natl Acad Sci U S A* 2005;102:9571-6.
- Klebanoff CA, Gattinoni L, Restifo NP. CD8+ T-cell memory in tumor immunology and immunotherapy. *Immunol Rev* 2006;211:214-24.
- Overwijk WW, Theoret MR, Finkelstein SE, Surman DR, de Jong LA, Vyth-Dreese FA, et al. Tumor regression and autoimmunity after reversal of a functionally tolerant state of self-reactive CD8+ T cells. *J Exp Med* 2003;198:569-80.
- Lou Y, Wang G, Lizee G, Kim GJ, Finkelstein SE, Feng C, et al. Dendritic cells strongly boost the antitumor activity of adoptively transferred T cells in vivo. *Cancer Res* 2004;64:6783-90.
- Koya RC, Mok S, Comin-Anduix B, Chodon T, Radu CG, Nishimura MI, et al. Kinetic phases of distribution and tumor targeting by T cell receptor engineered lymphocytes inducing robust antitumor responses. *Proc Natl Acad Sci U S A* 2010;107:14286-91.
- Borrello I, Sotomayor EM, Rattis FM, Cooke SK, Gu L, Levitsky HI. Sustaining the graft-versus-tumor effect through posttransplant immunization with granulocyte-macrophage colony-stimulating factor (GM-CSF)-producing tumor vaccines. *Blood* 2000;95:3011-9.
- Cui Y, Kelleher E, Straley E, Fuchs E, Gorski K, Levitsky H, et al. Immunotherapy of established tumors using bone marrow transplantation with antigen gene-modified hematopoietic stem cells. *Nat Med* 2003;9:952-8.
- Butterfield LH, Ribas A, Dissette VB, Amarnani SN, Vu HT, Oseguera D, et al. Determinant spreading associated with clinical response in dendritic cell-based immunotherapy for malignant melanoma. *Clin Cancer Res* 2003;9:998-1008.
- Ribas A, Glaspy JA, Lee Y, Dissette VB, Seja E, Vu HT, et al. Role of dendritic cell phenotype, determinant spreading, and negative costimulatory blockade in dendritic cell-based melanoma immunotherapy. *J Immunother* 2004;27:354-67.
- Simon R. Optimal two-stage designs for phase II clinical trials. *Control Clin Trials* 1989;10:1-10.
- Figlin R, Gitlitz B, Franklin J, Dorey F, Moldawer N, Rausch J, et al. Interleukin-2-based immunotherapy for the treatment of metastatic renal cell carcinoma: an analysis of 203 consecutively treated patients. *Cancer J Sci Am* 1997;3 Suppl 1:S92-7.
- Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science* 2002;298:850-4.
- Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000;92:205-16.
- Comin-Anduix B, Gualberto A, Glaspy JA, Seja E, Ontiveros M, Reardon DL, et al. Definition of an immunologic response using the major histocompatibility complex tetramer and enzyme-linked immunospot assays. *Clin Cancer Res* 2006;12:107-16.
- Comin-Anduix B, Lee Y, Jalil J, Algazi A, de la Rocha P, Camacho LH, et al. Detailed analysis of immunologic effects of the cytotoxic T lymphocyte-associated antigen 4-blocking monoclonal antibody tremelimumab in peripheral blood of patients with melanoma. *J Transl Med* 2008;6:22.
- Murali-Krishna K, Altman JD, Suresh M, Sourdive DJ, Zajac AJ, Miller JD, et al. Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. *Immunity* 1998;8:177-87.
- Ma C, Cheung AF, Chodon T, Koya RC, Wu Z, Ng C, et al. Multifunctional T-cell analyses to study response and progression in adoptive cell transfer immunotherapy. *Cancer Discov* 2013;3:1-12.
- Kwong GA, Radu CG, Hwang K, Shu CJ, Ma C, Koya RC, et al. Modular nucleic acid assembled p/MHC microarrays for multiplexed sorting of antigen-specific T cells. *J Am Chem Soc* 2009;131:9695-703.
- Ma C, Fan R, Ahmad H, Shi Q, Comin-Anduix B, Chodon T, et al. A clinical microchip for evaluation of single immune cells reveals high functional heterogeneity in phenotypically similar T cells. *Nat Med* 2011;17:738-43.
- Quezada SA, Simpson TR, Peggs KS, Merghoub T, Vider J, Fan X, et al. Tumor-reactive CD4(+) T cells develop cytotoxic activity and eradicate large established melanoma after transfer into lymphopenic hosts. *Jpn J Exp Med* 2010;207:637-50.
- Araki K, Turner AP, Shaffer VO, Gangappa S, Keller SA, Bachmann MF, et al. mTOR regulates memory CD8 T-cell differentiation. *Nature* 2009;460:108-12.
- Li Q, Rao RR, Araki K, Pollizzi K, Odunsi K, Powell JD, et al. A central role for mTOR kinase in homeostatic proliferation induced CD8+ T cell memory and tumor immunity. *Immunity* 2011;34:541-53.
- Gattinoni L, Lugli E, Ji Y, Pos Z, Paulos CM, Quigley MF, et al. A human memory T cell subset with stem cell-like properties. *Nat Med* 2011;17:1290-7.
- Yang L, Qin XF, Baltimore D, Van Parijs L. Generation of functional antigen-specific T cells in defined genetic backgrounds by retrovirus-mediated expression of TCR cDNAs in hematopoietic precursor cells. *Proc Natl Acad Sci U S A* 2002;99:6204-9.
- Yang L, Baltimore D. Long-term in vivo provision of antigen-specific T cell immunity by programming hematopoietic stem cells. *Proc Natl Acad Sci U S A* 2005;102:4518-23.
- Vatakis DN, Koya RC, Nixon CC, Wei L, Kim SG, Avancena P, et al. Antitumor activity from antigen-specific CD8 T cells generated in vivo from genetically engineered human hematopoietic stem cells. *Proc Natl Acad Sci U S A* 2011;108:E1408-16.
- Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol Ther* 2010;18:843-51.
- Suntharalingam G, Perry MR, Ward S, Brett SJ, Castello-Cortes A, Brunner MD, et al. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *N Engl J Med* 2006;355:1018-28.
- Endeman H, Meijvis SC, Rijkers GT, van Velzen-Blad H, van Moorsel CH, Grutters JC, et al. Systemic cytokine response in patients with community-acquired pneumonia. *Eur Respir J* 2011;37:1431-8.