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

Thinle Chodon1,18, Begoña Comin-Anduix2,6, Bartosz Chmielowski1,6, Richard C. Koya2,6,18, Zhongqi Wu1, Martin Auerbach5, Charles Ng1, Earl Avramis1, Elizabeth Seja1, Arturo Villanueva1, Tara A. McCannel7, Akira Ishiyama2, Johannes Czernin6,6, Calus G. Radu5,6, Xiaoyan Wang1, David W. Gjertson3, Jerome A. Zack1,6,8, Donald B. Kohn4,6,8, James R. Heath12, Owen N. Witte4,5,8,9, David Baltimore13, James S. Economou2,4,5,6, and Antoni Ribas1,2,5,6,8

Martin Auerbach5, Charles Ng1, Earl Avramis1, Elizabeth Seja1, Arturo Villanueva1, Tara A. McCannel7, Akira Ishiyama2, Johannes Czernin6, Calus G. Radu5,6, Xiaoyan Wang1, David W. Gjertson3, Jerome A. Zack1,6, Donald B. Kohn4,6, James R. Heath12, Owen N. Witte4,5,8,9, David Baltimore13, James S. Economou2,4,5,6, and Antoni Ribas1,2,5,6,8

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 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.
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.

Trial eligibility and screening procedures

Eligible patients were HLA-A∗02:01 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-126–35 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

<table>
<thead>
<tr>
<th>Patient study number</th>
<th>Sex (M/F)</th>
<th>Age (y)</th>
<th>Prior treatments for stages III–IV</th>
<th>Active metastasis sites</th>
<th>Stage</th>
<th>Protocol amendment</th>
<th>Number of F5 TCR transgenic cells</th>
<th>Cryo or fresh</th>
<th>Number of doses of DCs</th>
<th>Number of doses of IL-2</th>
<th>Evidence of transient tumor response</th>
<th>Response at EOS (day 90)</th>
<th>PFS (mo)</th>
<th>OS (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F5-1</td>
<td>M</td>
<td>60</td>
<td>—</td>
<td>Lung, stomach, liver, pancreas, peritoneum, soft tissues, bone, lymph nodes</td>
<td>M1c</td>
<td>1–52</td>
<td>$1 \times 10^9$</td>
<td>Cryo</td>
<td>12/14</td>
<td>3/3</td>
<td>Yes by PET/CT</td>
<td>Progression</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>F5-2</td>
<td>F</td>
<td>46</td>
<td>HD IL-2</td>
<td>Skin, LN, bone</td>
<td>M1c</td>
<td>1–52</td>
<td>$1 \times 10^9$</td>
<td>6/14</td>
<td>3/3</td>
<td>Yes by PET/CT</td>
<td>Stable disease</td>
<td>6</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>F5-3</td>
<td>M</td>
<td>55</td>
<td>HD IL-2</td>
<td>Lung, LN, SC</td>
<td>M1c</td>
<td>1–52</td>
<td>$1 \times 10^9$</td>
<td>NA</td>
<td>14/14</td>
<td>3/3</td>
<td>No</td>
<td>Progression</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td>F5-4</td>
<td>M</td>
<td>53</td>
<td>HD IL-2</td>
<td>Lung, LN</td>
<td>M1b</td>
<td>4–51</td>
<td>$0.6 \times 10^9$</td>
<td>NA</td>
<td>13/14</td>
<td>3/3</td>
<td>No</td>
<td>Progression</td>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>F5-5</td>
<td>M</td>
<td>50</td>
<td>HD IL-2</td>
<td>Lung, LN</td>
<td>M1c</td>
<td>1–52</td>
<td>$1 \times 10^9$</td>
<td>NA</td>
<td>13/14</td>
<td>3/3</td>
<td>No</td>
<td>Progression</td>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>F5-6</td>
<td>M</td>
<td>48</td>
<td>HD IL-2</td>
<td>SC, bone</td>
<td>M1c</td>
<td>1–52</td>
<td>$1 \times 10^9$</td>
<td>NA</td>
<td>9/14</td>
<td>3/3</td>
<td>No</td>
<td>Progression</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>F5-7</td>
<td>F</td>
<td>44</td>
<td>HD IL-2</td>
<td>SC, bone</td>
<td>M1c</td>
<td>1–52</td>
<td>$1 \times 10^9$</td>
<td>11/14</td>
<td>11/14</td>
<td>3/3</td>
<td>No</td>
<td>Progression</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>F5-8</td>
<td>F</td>
<td>46</td>
<td>6</td>
<td>Skin, LN</td>
<td>M1c</td>
<td>1–52</td>
<td>$1 \times 10^9$</td>
<td>14/14</td>
<td>6/14</td>
<td>3/3</td>
<td>Yes by PET/CT</td>
<td>Stable disease</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>F5-9</td>
<td>F</td>
<td>46</td>
<td>6</td>
<td>Skin, LN</td>
<td>M1c</td>
<td>1–52</td>
<td>$1 \times 3 \times 10^9$</td>
<td>6/14</td>
<td>3/3</td>
<td>Yes by PET/CT</td>
<td>Stable disease</td>
<td>6</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>F5-10</td>
<td>F</td>
<td>56</td>
<td>6</td>
<td>Skin, LN</td>
<td>M1b</td>
<td>6–7</td>
<td>$1.8 \times 10^9$</td>
<td>Fresh</td>
<td>6/9</td>
<td>3/3</td>
<td>Yes by PET/CT</td>
<td>Progression</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>F5-11</td>
<td>F</td>
<td>49</td>
<td>6</td>
<td>Skin, LN</td>
<td>M1b</td>
<td>8–9</td>
<td>$3.9 \times 10^9$</td>
<td>3/9</td>
<td>1/3</td>
<td>Yes by PET/CT</td>
<td>Stable disease</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>F5-12</td>
<td>M</td>
<td>50</td>
<td>6</td>
<td>Skin, LN</td>
<td>M1b</td>
<td>8–9</td>
<td>$3.9 \times 10^9$</td>
<td>3/9</td>
<td>1/3</td>
<td>Yes by PET/CT</td>
<td>Stable disease</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>F5-13</td>
<td>M</td>
<td>56</td>
<td>6</td>
<td>Skin, LN</td>
<td>M1b</td>
<td>8–9</td>
<td>$3.9 \times 10^9$</td>
<td>3/9</td>
<td>1/3</td>
<td>Yes by PET/CT</td>
<td>Stable disease</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>F5-14</td>
<td>F</td>
<td>49</td>
<td>6</td>
<td>Skin, LN</td>
<td>M1b</td>
<td>8–9</td>
<td>$3.9 \times 10^9$</td>
<td>3/9</td>
<td>1/3</td>
<td>Yes by PET/CT</td>
<td>Stable disease</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

**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; OS, overall survival; EOS, end of study; DC, dendritic cells; TCR, T cell receptor; IL-2, interleukin-2.
The known toxicities and side effects of the chemotherapy preparative regimen, or from the administration of IL-2 or G-CSF, as listed in the protocol or package insert, were not considered for the assessment of DLTs. Analysis of patient blood samples for potential replication competent retrovirus and cytokine storm is described in the Supplementary Online Methods.

Assessment of feasibility
Feasibility was also determined in the first stage, and if 3 of 8 patients could not receive the intended cellular therapies, or if they resulted in suboptimal TCR transgenic cell in vivo persistence, further accrual would not be warranted to the protocol as originally designed. Feasibility was assessed after the first 8 patients were followed up for a minimum of 3 months after the last subject had received the infusion of the MART-1 F5 TCR transgenic cells.

Assessment of antitumor activity
Quantification of changes in PET imaging for the intra-tumoral accumulation of $[^{18}F]$FDG was performed by counting the total number of FDG avid lesions as well as the maximum standardized uptake value (SUVmax) averaged over up to 5 hottest lesions at baseline, at day 30, and day 90. Objective clinical response rate was assessed on study day 90 and recorded following a modified Response Evaluation Criteria in Solid Tumors (RECIST; ref. 18).

MHC tetramer immunologic monitoring
MHC tetramer analysis in cryopreserved peripheral blood mononuclear cell (PBMC) collected at different time points was performed as previously described (19, 20). Our previous definitions for a positive or negative immunologic response using standardized MHC tetramer assays were used, which are based on the assay performance specifications by defining changes that were beyond the assay variability with a 95% confidence level (19).

Statistical design and analysis
The Simon optimal two-stage design (15) was used to determine the sample size, using the coprimary endpoint of response rate as the criterion for the clinical trial statistical design. This clinical trial was set up to rule out the null hypothesis that $P_0 / C_20 = 0.10$ (i.e., to rule out that this combined therapy has a response beyond 10%, since several current treatment approaches achieve response rates of 10% in patients with advanced melanoma) versus the alternative that the effect size $\mu = P_1 - P_0 > 0.25 (\alpha \leq 0.05, \beta \leq 0.20)$. The alternative hypothesis of a response rate of 35% was chosen because it was felt to represent a clinically meaningful difference and results in a study sample size that is feasible to be conducted within a pilot single-institution study. Using this statistical design, if 2 or more of 8 patients in stage I had an objective response at day 90, the study would proceed to stage II and accrue a total of 22 patients. If 5 or more patients in the overall study had a complete response or a partial response, defined as the objective response rate at day 90, the study would be declared positive. Because of the small sample size, the statistical analyses are mostly descriptive. Descriptive statistics such as mean, SD, median, minimum, maximum, and frequency of variables of interest are calculated. Responses of individual patients are plotted over time to present the data.
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 $1 \times 10^9$ previously cryopreserved TCR transgenic lymphocytes following the full cyclophosphamide–fludarabine conditioning regimen and up to 14 infusions of high-dose IL-2. One patient (F5-3) 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 $1 \times 10^{10}$. Because of an event of delayed pancytopenia in patient F5-10 who had continuing 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 $\leq 5$ 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 $1 \times 10^9$ cells and the next 2 patients receiving up to $1 \times 10^{10}$ 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
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...
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...
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 temporally 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 transgenic 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


Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T. Chodon, Z. Wu, E. Avramis, F. Seja, A. Villanueva, T.A. McCannel, P. Kaplan-Jetko, O.N. Witte, D. Baltimore, 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 ICL 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. Glasper), US4 CA119347 (to J.R. Heath and A. Ribas), P50 CA086306 (to A. Ribas), and R01 CA129816 (to J.S. Economou), the California Institute for Regenerative Medicine New Facility 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 Monkash 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
MART-1 TCR Adoptive Cell Transfer

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

_References_


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

Thinle Chodon, Begoña Comín-Andúix, Bartosz Chmielowski, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-13-3017

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2014/05/01/1078-0432.CCR-13-3017.DC1

Cited articles
This article cites 34 articles, 17 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/20/9/2457.full.html#ref-list-1

Citing articles
This article has been cited by 10 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/20/9/2457.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.