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ORIGINAL REPORT

T-Cell Therapy Using Interleukin-21–Primed Cytotoxic T-Cell Lymphocytes Combined With Cytotoxic T-Cell Lymphocyte Antigen-4 Blockade Results in Long-Term Cell Persistence and Durable Tumor Regression

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ABSTRACT

Purpose

Peripheral blood–derived antigen-specific cytotoxic T cells (CTLs) provide a readily available source of effector cells that can be administered with minimal toxicity in an outpatient setting. In metastatic melanoma, this approach results in measurable albeit modest clinical responses in patients resistant to conventional therapy. We reasoned that concurrent cytotoxic T-cell lymphocyte antigen-4 (CTLA-4) checkpoint blockade might enhance the antitumor activity of adoptively transferred CTLs.

Patients and Methods

Autologous MART1-specific CTLs were generated by priming with peptide-pulsed dendritic cells in the presence of interleukin-21 and enriched by peptide-major histocompatibility complex multimer-guided cell sorting. This expeditiously yielded polyclonal CTL lines uniformly expressing markers associated with an enhanced survival potential. In this first-in-human strategy, 10 patients with stage IV melanoma received the MART1-specific CTLs followed by a standard course of anti–CTLA-4 (ipilimumab).

Results

The toxicity profile of the combined treatment was comparable to that of ipilimumab monotherapy. Evaluation of best responses at 12 weeks yielded two continuous complete remissions, one partial response (PR) using RECIST criteria (two PRs using immune-related response criteria), and three instances of stable disease. Infused CTLs persisted with frequencies up to 2.9% of $CD8^+$ T cells for as long as the patients were monitored (up to 40 weeks). In patients who experienced complete remissions, PRs, or stable disease, the persisting CTLs acquired phenotypic and functional characteristics of long-lived memory cells. Moreover, these patients also developed responses to nontargeted tumor antigens (epitope spreading).

Conclusion

We demonstrate that combining antigen-specific CTLs with CTLA-4 blockade is safe and produces durable clinical responses, likely reflecting both enhanced activity of transferred cells and improved recruitment of new responses, highlighting the promise of this strategy.

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INTRODUCTION

Adoptive immunotherapy involving the ex vivo expansion and reinfusion of tumor-reactive T cells is an emerging treatment modality, especially in patients for whom conventional therapy fails.[1] Consequential responses have been achieved in metastatic melanoma using tumor-reactive T cells expanded from a tumor site.[2] However, successful tumor-infiltrating lymphocyte therapies require sufficient accessible tumor for adequate sampling and have been confined to specialized centers by toxicities associated with high-dose preinfusion conditioning and postinfusion interleukin-2 (IL-2).[3] Endogenous antigen-specific CTLs can also be obtained and expanded from peripheral blood (PB) and infused with lower-dose conditioning and a tolerable safety profile, but they have effectively reduced tumor burdens in only a limited number of patients, in part because of the short persistence of the transferred

cells.[4-7] In our prior studies, infused CTLs persisted beyond 42 days in 11% to 15% of patients. Median CTL persistence in vivo was fewer than 14 days, and the overall response rate (inclusive of patients achieving complete remissions [CRs] and partial responses [PRs]) was only 7%.[4-7]

When transferred T cells have persisted and mediated antitumor responses, the PB-derived CTLs showed characteristics associated with survival after the in vitro culture period, including CD28 expression, or exhibited or acquired these characteristics in vivo post-transfer.[4,8] We hypothesized that ex vivo generation of antigen-specific CTLs with characteristics of long-lived memory may enhance cell survival after adoptive transfer, thus enhancing sustained antitumor activity.[9] Therefore, we made two modifications to the methods used for T-cell generation. First, antigen-specific T cells were primed in vitro in the presence of the cytokine IL-21,[10] which promotes expansion of CTLs that, in comparison with cells that have been generated in the absence of IL-21 priming, phenotypically exhibit a less terminally differentiated phenotype, with a majority of cells expressing CD28 after ex vivo culture[11,12] and have been shown to exhibit enhanced persistence in murine models and humans after adoptive transfer.[8,13] Second, peptide-major histocompatibility complex (pMHC) multimers and clinical-grade sorting were used to select polyclonal melanoma-specific CTLs early from in vitro–generated $CD8^+$ T-cell lines, limiting the time required to achieve therapeutic cell numbers.[14]

Cytotoxic T-cell lymphocyte antigen-4 (CTLA-4) is an inducible T-cell surface protein that binds to CD80 and CD86 with a higher affinity than the costimulatory receptor CD28, intercepting the binding of the latter and providing an inhibitory signal to T cells. Thus, anti–CTLA-4 can release the brake on antigen-specific T-cell activation.[15,16] Monotherapy with anti–CTLA-4 achieves disease control (CRs, PRs, and stable disease [SD] at least 12 weeks in duration) in 20% to 28% of patients with advanced melanoma and increases overall survival rates.[17-20] However, the magnitude, breadth, and/or maintenance of T-cell responses triggered by anti–CTLA-4 alone is in most cases insufficient to eradicate tumors, and long-term CRs are seen in a minority of patients (range, 0% to 7%).[17,21,22]

We hypothesized that the adoptive transfer of melanoma-reactive $CD28^+$ CTLs with enhanced survival properties would directly benefit from CTLA-4 blockade, promoting their antitumor reactivity in vivo. In turn, the transferred cells could facilitate the release of tumor antigens from lysed tumor cells in the proimmunogenic context fostered by anti–CTLA-4. Thus, the combination would have the potential to boost tumor-specific responses to nontargeted antigens (antigen spreading), extending the breadth

Table 1. Patient Clinical Characteristics

Patient No.	Age (years)	Sex	Previous Treatments	Therapy Between Leukapheresis Collection and Treatment	Disease Site	Stage IV Classification[25]
1	59	M	Surgery, IFN-α, HD IL-2, T-cell clones (#2179), ipilimumab × four cycles*	Two (T-cell clones, ipilimumab × four cycles)	Lung: subcarinal, hilar, paratracheal lesions	M1b
2	66	F	Surgery, HD IL-2, cisplatin + ALT 801 (anti–p53 antibody linked to IL-2), ipilimumab × four cycles†	One (ipilimumab × four cycles)	Lung: pretracheal, subcarinal, bilateral hilar LN, parenchymal lesions	M1b
3	33	M	Surgery, HD IL-2 × two cycles, ipilimumab × four cycles × two cycles‡	Two (HD IL-2 × one cycle, ipilimumab × four cycles)	Axillary, pretracheal, bilateral hilar, pelvic LN; pulmonary, liver, pancreatic lesions; high LDH	M1c
4	39	M	Surgery, HD IL-2 × two cycles	One (HD IL-2 × one cycle)	Parenchymal lung, gluteal, lower extremity subcutaneous and bone lesions; bilateral inguinal LN	M1c
5	46	M	Surgery × two	One (ipilimumab × one cycle)§	Lower-extremity superficially spreading cutaneous melanoma, in-transit lesions; high LDH	M1c
6	63	F	Surgery × two	One (ipilimumab × one cycle)§	Parenchymal lung, adrenal gland, subcutaneous gluteal lesions; high LDH	M1c
7	68	F	Surgery × three	None‖	Inguinal LN, multiple subcutaneous lesions in lower abdomen and chest wall	M1a
8	43	M	Surgery, irradiation, Gamma knife	One (Gamma knife)	Brain, sacral and costal bone lesions, axillary LN	M1c
9	61	F	Surgery × three, irradiation	None¶	Superficially spreading cutaneous melanoma, in-transit lesions, parenchymal lung, liver lesions; femoral and external iliac LN; high LDH	M1c
10	53	M	Surgery × three, IFN-α, Melacine vaccine	None‖	Unilateral lesions at base of neck	M1a

Abbreviations: HD, high dose; IFN-α, interferon alpha; IL-2, interleukin-2; LDH, lactate dehydrogenase; LN, lymph node.
*Tumor size by modified WHO in response to ipilimumab +8% at 12 weeks and +63% at 28 weeks.
†Tumor size by modified WHO in response to ipilimumab +43% at 12 weeks.
‡Received a first course of ipilimumab with no evaluable disease in the neoadjuvant setting immediately after first surgery; second course: tumor size by modified WHO in response to ipilimumab +56% at 12 weeks.
§Received one dose of ipilimumab 4 weeks before the start of the trial while the cytotoxic T-cell products were being generated in the laboratory. No documented response to one dose of ipilimumab.
‖Leukapheresis performed 3 or 4 weeks after last surgery, and cytotoxic T-cell product generated and frozen in anticipation of progressive disease.
¶No treatment for 6 weeks between leukapheresis and the start of experimental therapy because of local wound infection.

of antitumor responses and reducing the outgrowth of antigen-loss tumor variants.[23,24]

PATIENTS AND METHODS

Clinical Protocol, Patient Characteristics, and Generation of Melanoma-Specific CTL Products

Between August 2011 and April 2013, 10 patients with progressive metastatic melanoma (Table 1) received treatment according to protocol No. 2225, approved by the Fred Hutchinson Cancer Research Center Institutional Review Board and the US Food and Drug Administration. All treated patients provided written informed consent. Eligibility required HLA-A*0201 and tumor expression of MART1.[26]

A total of 14 patients underwent leukapheresis in anticipation of entering this trial. Of the four who underwent leukapheresis and did not receive treatment as part of the trial, all started an alternate treatment while waiting for the cells to be generated: two received a BRAF inhibitor, one started a trial of IL-21 and ipilimumab, and one received high-dose IL-2. One patient remains in CR after high-dose IL-2. By the time the three remaining patients had developed progressive disease (PD), the trial was closed to accrual.

Treatment Plan

Patients received a single outpatient infusion of cyclophosphamide 300 mg/m^2 48 hours before the infusion of 10^{10} melanoma-specific CTL/m^2 (determined safe from previous studies),[6] followed by low-dose subcutaneous IL-2 (250,000 IU/m^2 twice daily for 14 days) to enhance the survival of transferred T cells.[7] On day 1 after CTL infusion, patients received ipilimumab (anti–CTLA-4; Yervoy; Bristol-Myers Squibb, New York, NY) 3 mg/kg every 3 weeks for a total of four doses (Data Supplement). Patients were monitored for toxicities based on Common Toxicity Criteria (version 4.0).[27] Staging studies were obtained 6 and 12 weeks after the infusion and then as clinically indicated.

Assessment of Clinical Responses

Both immune-related response criteria (irRC) and RECIST criteria were used to assess clinical responses.[28,29] Discrepancies (Table 2) were the result of differences in the specific measures of tumor size and classification of new lesions,[29] with the latter being incorporated into the global tumor burden in irRC but classified as PD in RECIST criteria. A detailed description of the materials and methods used is provided in the Data Supplement.

RESULTS

Polyclonal IL-21–Primed MART1-Specific CTLs Demonstrate Ex Vivo Antitumor Activity and Express Phenotypic Characteristics Associated With In Vivo Survival

All polyclonal CTL products demonstrated intracellular production of interferon gamma (IFN-γ) in response to antigen-presenting targets (HLA-A*0201-positive, transporter for antigen presentation–deficient, B lymphoblastoid cell lines [ie, T2 B-LCLs]), pulsed with the HLA-A*0201–restricted MART1$_{26-35}$ peptide (Data Supplement), as well as specific lysis (median, 54%; range, 27% to 66%) of the HLA A*0201-positive, MART1-positive MEL-526 cell line[30] (Data Supplement). Immediately before infusion, multimer-binding CTLs expressed CD45RO (median,

Table 2. Assessment of Clinical Response

Patient No.	No. of Ipilimumab Infusions Received After CTL Infusion	During Study (weeks)		Follow-Up (weeks)					
		6	12	16	19	28	40 to 46	52 to 65	104 to 107
1	4 of 4	SD* (−2%) SD† (−10%)	SD (−34%) SD (−22%)			PR (−80%) PR (−59%)	PR (−90%) PR (−90%)	PR (−97%) PR (−90%)	CR (−100%) CR (−100%)
2	4 of 4	SD* (−2%) SD† (+4%)	SD (−6%) SD (+1%)		SD (−7%)‡ SD (−5%)				
3	2 of 4‡	PD* (+69%)§ PD† (+36%)	X						
4	4 of 4	SD* (0%) SD† (0%)	SD (+19%) SD (+7%)			PD (+25%)§ SD (+10%)			
5	2 of 4‡	PD* (+30%)§ PD† (+30%)							
6	4 of 4	SD* (+24%) SD† (+19%)	SD (+17%)§ SD (+17%)						
7	4 of 4	SD* (−45%) SD† (−41%)	PR (−90%) SD (−85%)			CR (−100%) CR (−100%)		CR (−100%) CR (−100%)	CR (−100%) CR (−100%)
8	3 of 4‡	SD* (+8%) SD† (+2%)	PD (+27%) PD (metastasis)		PD (+27%)§				
9	3 of 4‖	SD* (−41%) SD† (−42%)	ND¶ PD¶ (metastasis)	PR (−71%)		X			
10	4 of 4	PR* (−76%) PR† (−59%)	PR (−79%) PR (−65%)			PR (−79%) PR (−65%)	PR (−58%) PD (metastasis)		

NOTE. X indicates patient died as a result of PD. Metastasis indicates development of brain metastasis.
Abbreviations: CR, complete remission; CTL, cytotoxic T cell; ND, not done; PD, progressive disease; PR, partial response; SD, stable disease.
*Immune-related response criteria.[29]
†RECIST.[28]
‡Received two or three of four planned ipilimumab doses because of PD.
§Patient initiated alternate treatment modality.
‖Received three of four planned ipilimumab doses because of ipilimumab-induced hypophysitis.
¶Patient underwent brain magnetic resonance imaging, but no restaging scans were performed.

98.9%), consistent with an antigen-experienced phenotype (Data Supplement). In accordance with our previous studies,[11,12] a subset of the expanded CTLs retained expression of CD27, CD28, and CD127 (with medians of 63.5%, 72.4%, and 36.2%, respectively), consistent with IL-21 exposure during priming.[8] Little or no expression of the lymph node homing markers CD62L and CCR7 or the activation/exhaustion markers CD57 and PD1 was detected.

Concurrent Transfer of MART1-Specific CTLs Does Not Alter Safety and Toxicity Profiles of Anti–CTLA-4

Ten patients with metastatic melanoma whose disease progressed after previous systemic or surgical therapy, including ipilimumab monotherapy in three patients (Table 1), received combined CTLs plus anti–CTLA-4 treatment (Data Supplement). Seven patients experienced transient (< 24 hours) sterile fevers (≥ 38.3°C) with or without chills, associated with a CTL infusion–induced cytokine-release syndrome (Data Supplement). Lymphopenia lasting 10 days or fewer[8] and self-limiting moderately erythematous maculopapular skin rashes were observed in nine of 10 patients. Ipilimumab therapy was associated with self-limited nausea/diarrhea (grade ≤ 1) in eight of 10 patients, and transient (< 14 days) moderately elevated (grade ≤ 2) liver enzymes were detected in five of 10 patients. Patient 9 developed pituitary insufficiency secondary to ipilimumab-induced hypophysitis,[31] and patient 5 experienced a drug fever attributed to the combined effect of ipilimumab plus vemurafenib introduced for PD.[32,33] Both patients received systemic steroids as treatment for immune-related adverse events.[31] Overall, the toxicities could be attributed to either CTL infusion or ipilimumab alone, but no unexpected toxicities were associated with the combination.

Adoptive Transfer of MART1-Specific CTLs With Concurrent Anti–CTLA-4 Can Produce Sustained Clinical Responses

Two of 10 patients achieved sustained CRs as defined by irRC and RECIST criteria[26,34] at 12 and 104 weeks after CTL infusion, respectively (Table 2; Figs 1A [green lines,] and 1B). Patient 1 had experienced PD after salvage ipilimumab monotherapy initiated 7 months earlier (Chapuis et al, manuscript submitted for publication). The time to response after ipilimumab alone was outside the expected range, and the appearance of new lesions during the interim precluded any beneficial effect of ipilimumab monotherapy.[29] Neither patient received additional antitumor therapy, and both were alive and disease free 220 and 169 weeks (as of November 1, 2015), respectively, after the start of treatment.

Two patients experienced PRs as best responses by irRC (Fig 1A [blue lines]). After demonstrating a 41% reduction in tumor burden at 6 weeks, patient 9 was diagnosed with new subcentimetric brain metastasis at 7 weeks, along with ipilimumab-induced hypophysitis treated with systemic steroids. Despite the new brain lesions, the patient exhibited a reduction in overall tumor burden of 71% at 16 weeks, associated first with flattening then central blanching of numerous cutaneous metastases (Data Supplement). However, as a possible consequence of systemic steroids, or because of the characteristics of the brain parenchyma in which CTLs may be excluded in the absence of inflammation,[35] the brain lesions progressed. The patient elected to receive comfort care and died 2 months later. Patient 10 experienced a PR at 6 weeks (by irRC), which was maintained at 28 weeks (−79%), but developed new brain metastases at 46 weeks. Patients 2, 4, and 6 exhibited SD at 12 weeks (Fig 1A [purple lines]); patients 2 and 6 elected to undergo alternate treatments because of persistent disease. Patients 3, 5, and 8 experienced PD at 6, 6, and 12 weeks, respectively (Fig 1A [red lines]).

Overall, by irRC, seven of 10 patients achieved best responses of CR, PR, or SD. Of three patients who experienced PD after four doses of ipilimumab monotherapy (patients 1, 2, and 3), one achieved a CR, one had SD, and one experienced PD. With a median follow-up of 187 weeks (range, 140 to 220 weeks), five of

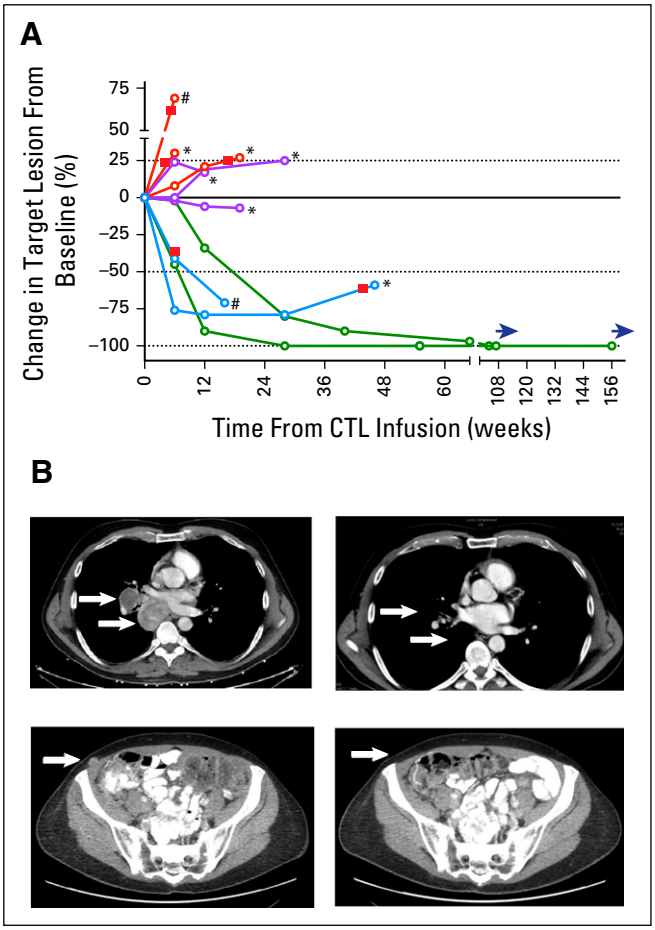

Fig 1. Tumor regressions after melanoma-reactive polyclonal cytotoxic T cells (CTLs) combined with anti–cytotoxic T-cell lymphocyte antigen-4. (A) Spider plot of all treated patients showing changes from baseline in the tumor burden (*y*-axis), measured as the products of the perpendicular diameters of all target lesions, assessed weeks after the CTL infusion (*x*-axis). The dashed line above the solid line indicates 25% progression (modified WHO progressive disease [PD]), and the dashed line below the solid line indicates 50% reduction (modified WHO partial response [PR]). Red lines indicate patients with PD, purple lines indicate patients with stable disease, blue lines indicate patients with PRs, and green lines indicate patients with complete remissions. Red squares indicate the occurrence of new lesions, asterisks indicate the start of an alternate treatment, pound signs indicate disease progression sufficient to transition to comfort care, and blue horizontal arrows indicate continued monitoring. (B) Serial images of computed tomography scans performed before infusion (left) and 64 and 55 weeks after treatment (right) for patients 1 (top) and 7 (bottom), respectively. Arrows indicate the location of the largest index lesions for each patient.

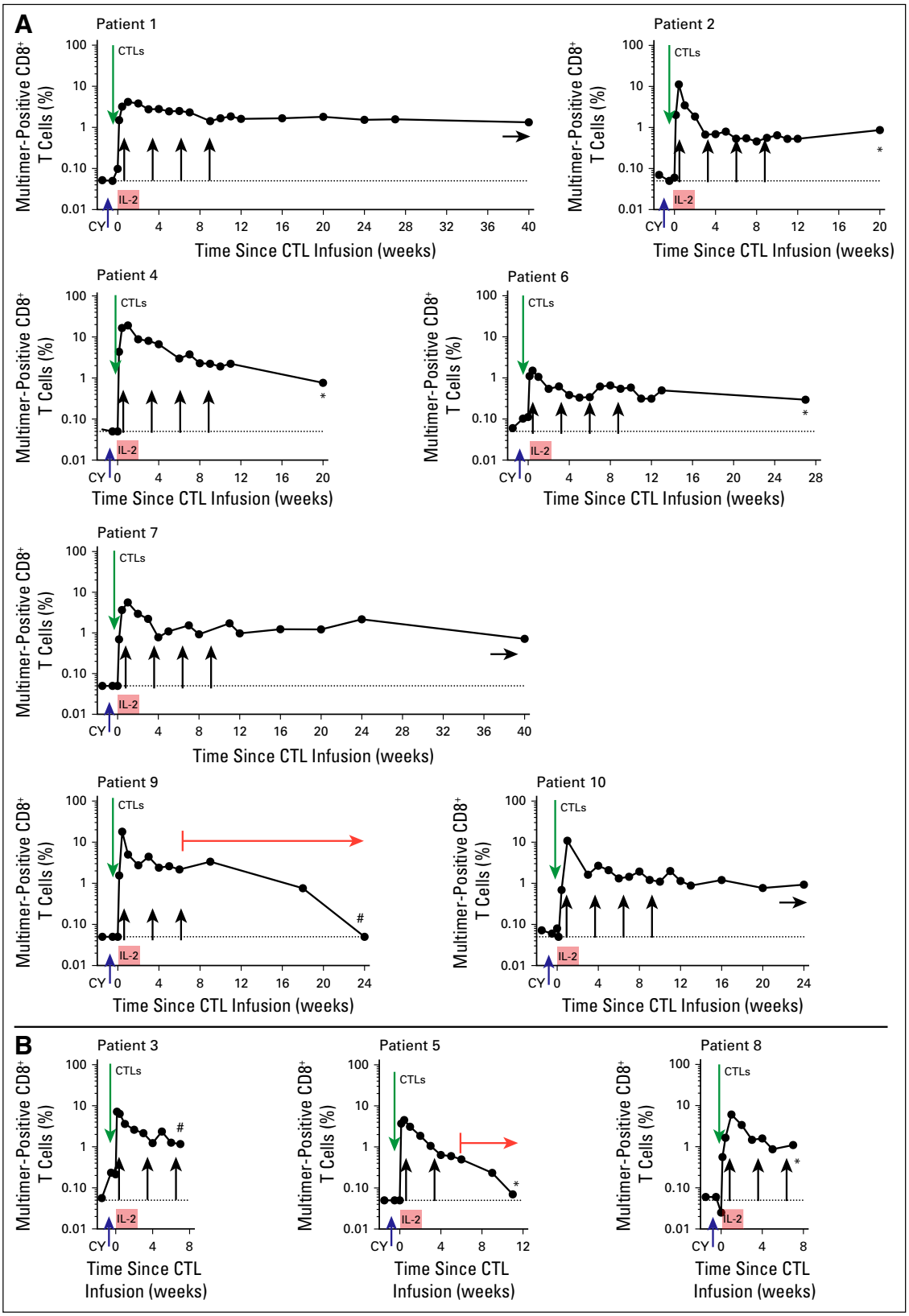

Fig 2. Kinetics of in vivo persistence of melanoma-reactive polyclonal cytotoxic T cells (CTLs). (A) Percent multimer-positive $CD8^+$ T cells (*y*-axis) in peripheral-blood mononuclear cells (solid circles) collected 7 days (± 2 days) before and at defined time points after infusions is shown for patients who achieved complete remissions, partial responses, or stable disease after treatment. Green arrows indicate CTL infusions, black vertical arrows indicate anti–cytotoxic T-cell lymphocyte antigen-4 infusions, asterisks indicate the start of an alternate treatment, pound signs indicate comfort care, orange arrows indicate concurrent corticosteroid therapy, and blue horizontal arrows indicate ongoing monitoring. (B) The same analysis performed for patients who experienced disease progression after treatment. IL-2, interleukin-2.

10 patients were alive, and two of 10 remained in sustained CRs (Data Supplement).

Polyclonal MART1-Specific IL-21–Primed CTLs Persist In Vivo When Transferred With Concurrent Anti–CTLA-4

A majority of patients had nearly undetectable pre-existing PB mononuclear cell (PBMC) frequencies of endogenous MART1-specific multimer-binding CTLs (median, ≤ 0.05%; range, ≤ 0.05 to 0.21%). Persistence of the infused CTLs was documented for 10 of 10 patients at 6 weeks and for seven of seven evaluable patients at 12 weeks, with median frequencies of 1.6% (range, 0.3% to 2.9%) and 1.1% (range, 0.3% to 2.2%), respectively (Fig 2). The transferred CTLs could be detected for as long as the patients donated PBMCs for analysis, regardless of their tumor response at 12 weeks, with the exceptions of patients 9 and 5, who each received the equivalent of prednisone 1 mg/kg. Both experienced a gradual decline in the frequency of transferred cells to undetectable levels at 6 and 17 weeks, respectively.

Transferred CTLs Detected in PB Exhibit or Acquire Phenotypic and Functional Characteristics of Long-Lived Memory T Cells in Patients Achieving CRs, PRs, or SD

Regardless of the levels detected on multimer-positive CTLs at the time of infusion (Data Supplement), CTL tracking in patients who achieved CRs, PRs, or SD documented a significant increase in the frequency of multimer-positive cells expressing CD28 ($P < .05$), CD27 ($P < .05$), CD127 ($P < .005$), CD62L ($P < .05$), and CCR7 ($P < .005$) at 12 weeks (Fig 3A [left column]). By contrast, although the statistical power to detect differences was limited because of the small sample size (n = 3), the expression of CD28 was lost ($P < .05$), the expression of CD27 and CD127 decreased, and CD62L and CCR7 were never acquired on the transferred cells in patients with PD (Fig 3A [right column]). The expression of CD57 and programmed death-1 (PD1), associated with an exhausted or activated or exhausted phenotype, respectively, was increased on transferred cells found in PBMCs of patients with PD, but not in patients who achieved CRs, PRs, or SD (Fig 3B). We were unable to obtain tumor tissue after treatment to assess the expression of these markers on cells that had reached the tumor microenvironment.

The functional profile of transferred CTLs was determined by gating for IFN-γ–positive cells (Fig 3C). Before cyclophosphamide infusions, less than 0.2% of IFN-γ–producing cells could be detected in PBMCs, including in patients with low but detectable levels of MART1-specific cells, attesting to the lack of pre-existing, functional PB CTLs. Consistent with the maintenance of CD28 expression on the generated cells, preinfusion CTLs secreted IL-2 in addition to IFN-γ and tumor necrosis factor alpha (TNF-α) on exposure to cognate antigen.[36,37] In patients with CRs, PRs, or SD, transferred CTLs continued to produce all three cytokines 6 and 12 weeks post-transfer (Fig 3C [left column]) and for as long as transferred CTLs could be detected by multimer staining (Data Supplement). However, no IFN-γ–secreting cells could be detected post-transfer in patients who had PD (Fig 3C [right column]).

We then investigated whether the infused CTLs maintained the potential to divide, as reflected by the expression of Ki-67 in vivo.[38] Before infusions, multimer-negative $CD8^+$ T cells contained a mean of 1.1% Ki-67–positive cells, and polyclonal multimer-positive CTL products contained a mean of 81.2% Ki-67–positive cells 14 days after in vitro stimulation (Fig 3D [left columns]). Between 0 and 11 weeks after transfer, Ki-67 expression in transferred multimer-positive CTLs progressively decreased, and expression in host multimer-negative $CD8^+$ T cells transiently increased (Fig 3D [middle columns]). By 12 weeks after CTL infusion, after the completion of exogenous subcutaneous IL-2 and anti–CTLA-4, a small fraction of the transferred CTLs were Ki-67 positive (mean, 3.9%; Fig 3D [right columns]). Expression of this proliferation marker in the multimer-positive cells was significantly higher compared with that in host multimer-negative CD8 T cells, which had returned to pretreatment levels (mean, 1.14%; $P < .001$). When directly compared, cell products administered to patients with PD did not seem less functional than products infused into patients with CRs, PRs, or SD (Data Supplement). Although no correlation with overall survival could be detected, the quantity of TNF-α secreted by CTL products in response to cognate antigen was associated with a decreased likelihood of progression-free survival, and CD57 expression on infused cells after 3 weeks in vivo was associated with an increased likelihood of progression-free survival (Data Supplement).

Evidence of Epitope Spreading in Patients Who Achieved CRs, PRs, or SD

We sought to identify and assess reactivity pre- and post-therapy to melanoma antigen epitopes that could bind to all expressed MHC antigens and could be recognized by host $CD4^+$ and $CD8^+$ T cells. ELISpot analyses were used to assess reactivity of patient-derived T cells toward overlapping peptides spanning the melanoma-associated proteins MART1, NY-ESO1, gp100, tyrosinase, and MAGE A3 (Appendix Fig A1, online only). All patients who achieved CRs, PRs, or SD demonstrated a significantly increased reactivity to the melanoma-associated proteins at one or more time point after CTL infusion (Appendix Fig A1A). In contrast, patients who experienced PD (Appendix Fig A1B) either developed no new reactivity (patient 3) or did not have a significant increase in reactivity (patients 5 and 8). Detectable reactivity to melanoma-associated antigens was also not observed in three other patients with metastatic melanoma who declined therapy (Data Supplement) or in five patients who received CTL clones alone (Data Supplement).

DISCUSSION

We report, to our knowledge, the first prospective use in an ambulatory setting of antigen-specific PB-derived CTLs in combination with CTLA-4 blockade and demonstrate induction of durable antitumor responses in patients with metastatic melanoma.[39] Although the number of patients who participated in this initial study was limited, the results are encouraging compared with those of each individual modality.[4,17,21] Seven of 10 patients achieved a CR, PR, or SD as best response by irRC. Two patients

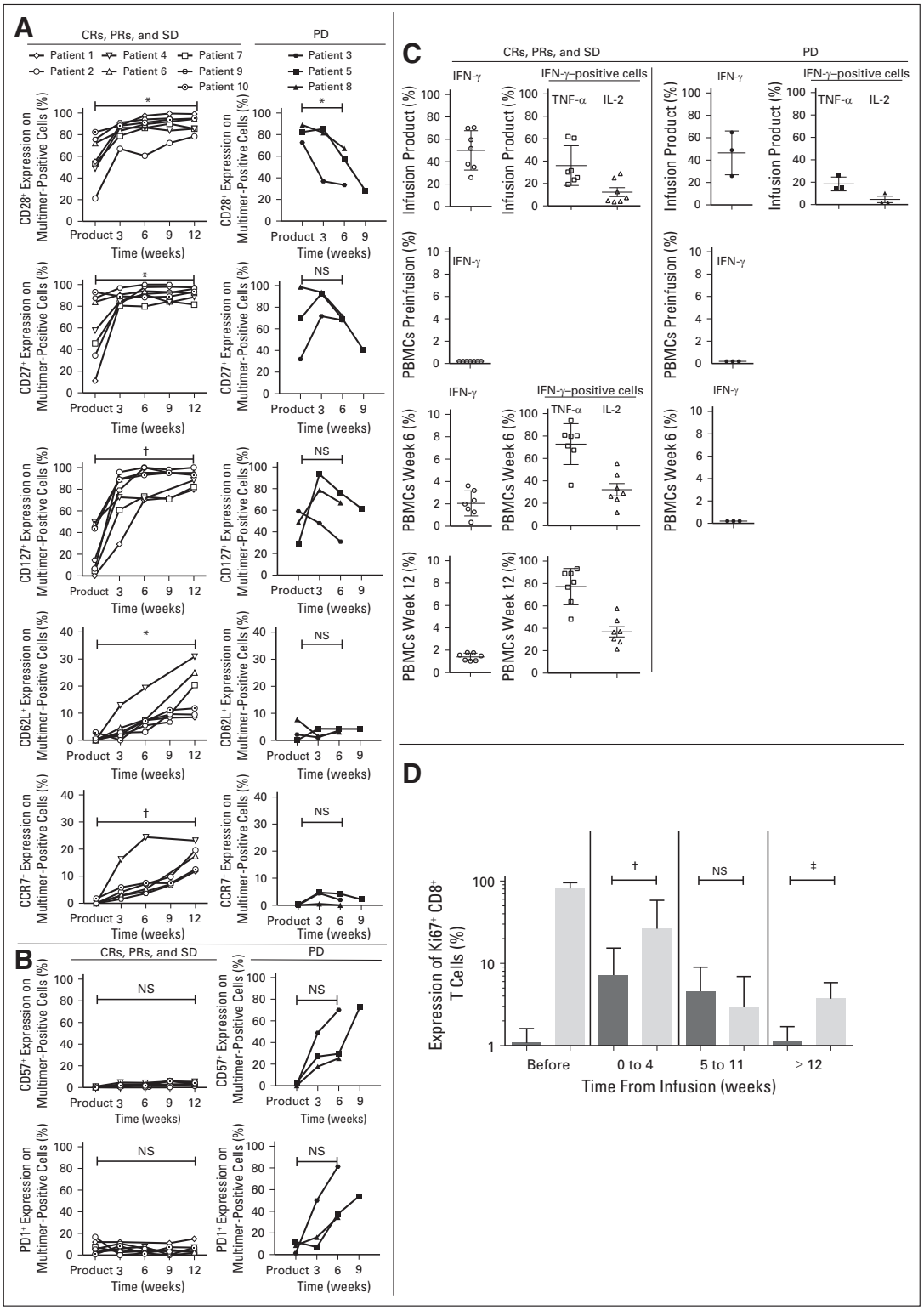

Fig 3. Phenotypic and functional characteristics of transferred melanoma-reactive cytotoxic T cells (CTLs). (A) Expression of CD27, CD28, CD127, CD62L, and CCR7 (*y*-axis) on gated multimer-positive cells for $CD8^+$ CTL products immediately before infusion and after 3, 6, 9, and 23 weeks in vivo for patients who achieved complete remissions (CRs), partial responses (PRs), or stable disease (SD). (B) The same analysis in panel A for expression of CD57 and programmed death-1 (PD1; *y*-axis) performed for patients who experienced progressive disease (PD). (C) Open symbols indicate patients who achieved CRs, PRs, or SD (left column); solid symbols indicate patients who experienced PD (right column). Left plots: percentage of cells within the infusion products producing interferon gamma (IFN-γ) in response to MART1 peptide and in peripheral-blood mononuclear cells (PBMCs) before and 6 and 12 weeks after the CTL infusion; right plots: respective percentages of tumor necrosis factor alpha (TNF-α) and interleukin-2 (IL-2) cells among IFN-γ–positive cells. (D) Mean intranuclear Ki67 expression of endogenous $CD8^+$ multimer-negative cells (black columns) and preinfusion CTL products and multimer-positive $CD8^+$ T cells (gray columns) at indicated time points for all patients combined. Two-tailed paired *t* tests were used for statistical analysis. NS, not significant. (*) $P < .05$. (†) $P < .005$. (‡) $P < .001$.

remained in sustained CRs without additional therapy more than 3 and 4 years later, respectively. These results were achieved with a toxicity profile comparable to that of ipilimumab monotherapy.[31]

Compared with our previous trials, infused CTLs in this study consisted of polyclonal, pMHC multimer-sorted T-cell lines that had undergone shorter ex vivo manipulation (≤ 6 weeks) and fewer cell divisions.[4,6,7,14] The transferred CTLs persisted in vivo (seven of seven evaluable patients at 12 weeks), which is in sharp contrast with our previous results (two of 11 CTL clones beyond 2 weeks).[4] CTLs were exposed to IL-21 during primary stimulation, which has been shown to promote retention of CD27, CD28, and CD127 on naïve T cells.[8,10-13] Although this cannot be precisely determined retrospectively, it is likely that the infused cells in our trial originated mostly from the autologous IL-21–responsive naïve T-cell pool, because few if any MART1-specific multimer-positive cells were detected preinfusion.

Patients who achieved CRs, PRs, or SD shared a number of biologic correlates. Compared with the infused product, persisting CTLs expressed or acquired phenotypic and functional characteristics associated with long-lived memory T cells (including markers associated with survival [CD28, CD27, and CD127][40,41]; lymph-node homing [CD62L and CCR7]; and production of IFN-γ, TNF-α, and IL-2),[41,42] suggesting the preferential survival or expansion of this subset. These favorable characteristics may also have been facilitated by CTLA-4 blockade such that infused $CD28^+$ CTLs experienced unopposed stimulation or enhanced signaling through binding of the costimulatory ligands CD80 and/or CD86. Although these ligands are present at low levels on more than 80% of melanoma cells,[43] higher expression can be induced by inflammatory cytokines, such as IFN-γ, which would have been provided by the responding transferred CTLs.[44] Consequently, transferred $CD28^+$ cells may have gained proliferative or survival advantages related to B-cell lymphoma extra large ($Bcl\text{-}X_L$) expression and autocrine production of IL-2.[40] In contrast, no IFN-γ–secreting cells could be detected posttransfer in patients with PD.

Epitope spreading was observed in all patients with CRs, PRs, and SD, likely a consequence of transferred CTL–induced tumor lysis and heightened T-cell activation fostered by anti–CTLA-4.[24] Released tumor antigens presented by local antigen-presenting cells may have promoted activation of new responses to nontargeted melanoma-associated proteins.[23,24,45] Whether such T-cell responses, toward wild-type or nonevaluated tumor-specific mutations,[46] induced the decrease in tumor size observed in some patients cannot be ascertained. However, the combination may represent a strategy to specifically increase the number and strength of T cells targeting multiple antigens of the patient's own tumor, which may be particularly relevant when targeting non-oncogenic antigens, such as MART1.[47]

Although the intrapatient pre- and postinfusion immunobiologic results suggest the combination of anti–CTLA-4 plus adoptive T-cell therapy mediates the observed effects, tumor regressions observed in this single-arm phase I study cannot be definitely attributed to either component because of the lack of matched comparison groups. Further study is warranted to determine the specific contributions of anti–CTLA-4, persistence of memory T cells (potentially facilitated by anti–CTLA-4), and epitope spreading. Long-term CRs and PRs were not observed in all patients, suggesting that mechanisms limiting antitumor efficacy are still operative. Indeed, transferred CTLs isolated from patients with PD expressed the exhaustion or activation markers CD57 and PD1,[36,37] suggesting interventions that block the PD1–PD1 ligand axis may further enhance therapeutic activity.[48] The finding that CTLs from the PB of patients with SD, PRs, or CRs did not express PD1 is distinct from reports that PD1 expression in the tumor environment reflects the presence of activated functional CTLs.[49] This could, however, reflect the fact that tumor-reactive CTLs in PB represent a renewable source of memory cells that can migrate and function in the tumor environment. Overall, the continued development of improved cell-culture methods and immune-modulatory antibodies holds great promise for designing immunotherapeutic combinations that have increased efficacy and reduced toxicity.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

T-Cell Therapy Using Interleukin-21–Primed Cytotoxic T-Cell Lymphocytes Combined With Cytotoxic T-Cell Lymphocyte Antigen-4 Blockade Results in Long-Term Cell Persistence and Durable Tumor Regression

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Appendix

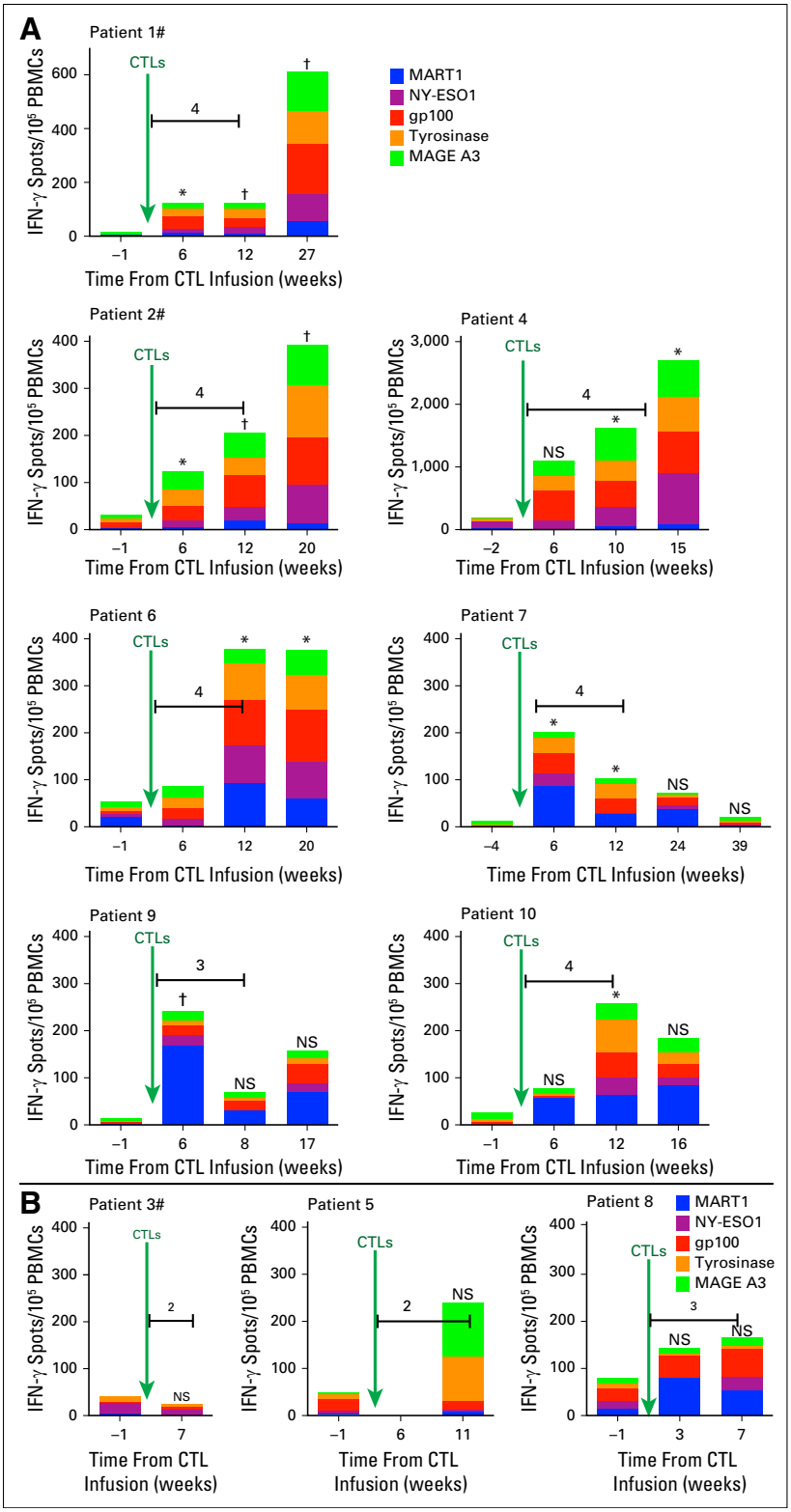

Fig A1. Reactivity to nontargeted epitopes. Interferon gamma (IFN-γ) spots per 10^5 peripheral-blood mononuclear cells (PBMCs; *y*-axis) at indicated time points (*x*-axis) before and after the infusion of polyclonal cytotoxic T cells (CTLs) generated in the presence of interleukin-21 for patients who (A) achieved complete remissions, partial responses, or stable disease or (B) experienced disease progression after receiving treatment. Pound signs indicate patients who had received prior ipilimumab, green arrows indicate CTL infusions, and horizontal lines indicate ipilimumab administration with the total number of doses received indicated immediately above. The scales of the *y*-axis for graphs for patients 1 (maximum, 700 spots/10^5 PBMCs) and 4 (maximum, 3,000 spots/10^5 PBMCs) are different from all others (maximum, 400 spots/10^5 PBMCs). Two-tailed paired *t* tests were used for statistical analysis. NS, not significant. (*) $P < .05$. (†) $P < .005$.