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T-Cell Therapy for Lymphoma Using Nonengineered Multiantigen-Targeted T Cells Is Safe and Produces Durable Clinical Effects

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abstract

PURPOSE Patients with relapsed lymphomas often fail salvage therapies including high-dose chemotherapy and mono-antigen–specific T-cell therapies, highlighting the need for nontoxic, novel treatments. To that end, we clinically tested an autologous T-cell product that targets multiple tumor-associated antigens (TAAs) expressed by lymphomas with the intent of treating disease and preventing immune escape.

PATIENTS AND METHODS We expanded polyclonal T cells reactive to five TAAs: PRAME, SSX2, MAGEA4, SURVIVIN, and NY-ESO-1. Products were administered to 32 patients with Hodgkin lymphomas (n = 14) or non-Hodgkin lymphomas (n = 18) in a two-part phase I clinical trial, where the objective of the first phase was to establish the safety of targeting all five TAAs (fixed dose, 0.5×10^7 cells/m^2) simultaneously and the second stage was to establish the maximum tolerated dose. Patients had received a median of three prior lines of therapy and either were at high risk for relapse (adjuvant arm, n = 17) or had chemorefractory disease (n = 15) at enrollment.

RESULTS Infusions were safe with no dose-limiting toxicities observed in either the antigen- or dose-escalation phases. Although the maximum tolerated dose was not reached, the maximum tested dose at which efficacy was observed (two infusions, 2×10^7 cells/m^2) was determined as the recommended phase II dose. Of the patients with chemorefractory lymphomas, two (of seven) with Hodgkin lymphomas and four (of eight) with non-Hodgkin lymphomas achieved durable complete remissions (> 3 years).

CONCLUSION T cells targeting five TAAs and administered at doses of up to two infusions of 2×10^7 cells/m^2 are well-tolerated by patients with lymphoma both as adjuvant and to treat chemorefractory lymphoma. Preliminary indicators of antilymphoma activity were seen in the chemorefractory cohort across both antigen- and dose-escalation phases.

INTRODUCTION

Both immune checkpoint blockade and the adoptive transfer of tumor-specific T cells have shown that T-cell immunotherapy is effective in controlling and eradicating both Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL).[1-4] For example, we and others have transferred T cells engineered to recognize CD19-positive lymphomas via transgenic chimeric antigen receptors (CARs) after conditioning chemotherapy (lymphodepletion) and observed durable complete remission (CR) rates of 30%-50%, which have led to the approval of two CAR T-cell products with many more in pivotal trials.[5-9] Nonetheless, > 50% of CD19 CAR T-cell recipients fail to enter CR or ultimately relapse.[10,11]

We hypothesized that the adoptive transfer of CD4+ (helper) and CD8+ (cytotoxic) T cells with native T-cell receptor (TCR) specificity for multiple tumor-associated antigens (mTAAs) would be safe and promote antilymphoma activity by minimizing the risk for antigen-negative relapses.[12,13] In this way, we also had the opportunity to extend T-cell therapy to the majority of lymphoma subtypes (NHL and CD19-negative lymphomas such as HL) that express one or more of these TAAs. Finally, we postulated that tumor lysis mediated by the transferred cells would facilitate the recruitment and activation of endogenous immune cells against additional tumor-expressed antigens (ie, antigen spreading), further extending the breadth and durability of antitumor responses.

Here, we describe the safety and clinical effects of autologous, mTAA-specific T cells directed against PRAME, SSX2, MAGEA4, NY-ESO-1, and SURVIVIN (mTAA-T cells), administered to 32 patients with HL or NHL. We demonstrate that all five TAAs are safe to target at a maximum tested dose level of two doses of 2×10^7 cells/m^2. We also demonstrate objective clinical responses observed at both antigen- and dose-escalation phases. Clinical benefit correlated with the

ASSOCIATED CONTENT

Data Supplement

Protocol

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on December 14, 2020 and published at ascopubs.org/journal/jco on January 28, 2021: DOI https://doi.org/10.1200/JCO.20.02224

CONTEXT

Key Objective

Adoptive T-cell immunotherapy has been effective in treating Epstein-Barr virus–positive —and CD19-positive lymphomas. However, a large proportion of tumors either do not express those or downregulate expression as an escape mechanism. This phase I and II clinical trial examined the safety of administering a nonengineered T-cell product with simultaneous specificity for multiple lymphoma-expressed antigens (PRAME, SSX2, NY-ESO-1, MAGE-A4, and SURVIVIN).

Knowledge Generated

Targeting all five antigens simultaneously was very well-tolerated by patients even at the maximum dose of two infusions of 2×10^7cells/m^2. Six of 15 with active chemorefractory lymphomas entered durable complete remission and responses positively correlated with induction of antigen spreading.

Relevance

This study establishes the safety of a T-cell product specific for a novel cohort of self-antigens that is nonoverlapping with available T-cell therapies. If efficacy is confirmed in pivotal trials, multiple tumor-associated antigen–T cells could be added to the arsenal of immunotherapies for the treatment of chemorefractory lymphomas.

magnitude of antigen spreading induced by week 6 after infusion.

PATIENTS AND METHODS

Patients

Patients with HL or NHL were eligible for infusion on a Baylor College of Medicine and Houston Methodist Hospital Institutional Review Board –approved protocol (H-27471, ClinicalTrials.gov identifier: NCT01333046) if they had received two or more lines of prior therapy (or had received only one line of prior therapy but further chemotherapy was contraindicated) and still had active disease (arm A) or were in remission with a history of prior chemotherapy failure (arm B) (HL, Table 1; NHL, Table 2). Per the US Food and Drug Administration request, this first-in-human clinical trial of mTAA-T cells was (i) restricted to individuals ≥ 18 years and (ii) conducted in two parts—(a) an antigen-escalation phase where the first two patients were administered with a fixed cell dose (0.5×10^7/m^2) and first received a T-cell product targeting PRAME followed 1 month later by a product targeting PRAME + SSX2 and so on until the final cohort received a product targeting PRAME + SSX2 + MAGE-A4 + NY-ESO-1 followed by PRAME + SSX2 + MAGE-A4 + NY-ESO-1 + SURVIVIN (all five TAAs). Once the safety of infusing a product simultaneously targeting five TAAs was established, we then proceeded to (b) the dose-escalation phase where patients received two infusions at 0.5×10^7 cells/m^2 (dose level, DL1), 1×10^7 cells/m^2 (DL2), or 2×10^7 cells/m^2 (DL3), administered 2 weeks apart. The follow-up cutoff date was May 15, 2020. Complete details on the protocol are available in the Data Supplement, online only.

Generation of mTAA-T Cells

Monocyte-derived dendritic cells (DCs) loaded with pepmixes (ie, 15-mer peptides overlapping by 11 amino acids) spanning the TAAs SURVIVIN, SSX2, MAGE-A4, PRAME, and NY-ESO-1 (JPT Peptide Technologies, Berlin, Germany) were cocultured with autologous peripheral blood mononuclear cells in the presence of a Th1-polarizing cytokine cocktail (interleukin [IL] 7 [10 ng/mL], IL12 [10 ng/mL], IL15 [5 ng/mL], and IL6 [10 ng/mL]). From day 10, responder T cells were restimulated weekly with irradiated, pepmix-pulsed DCs in the presence of IL2 (50-100 U/mL) or IL15 (5 ng/mL).[14]

Characterization Studies

A description of the phenotypic and functional studies performed can be found in Data Supplement.

Statistical Analysis

Antigen and dose escalation was performed per protocol using the modified continual reassessment method (mCRM; modification defined in detail in Data Supplement, clinical protocol section 9.1) to determine (i) the maximum number of antigens (up to 5) the T cells could safely target and (ii) the MTD of mTAA-T cells. The MTD is defined as the highest DL at which the probability of a dose-limiting toxicity (DLT) was at most 15%. If no DLTs were observed after the first two protocol-specified doses, patients could receive six additional doses left to the discretion of the treating physician. Up to six additional patients per arm could be accrued to DL3 (or the MTD). For this study, any grade 3 or higher treatment-related adverse event (AE) was considered a DLT. Arms were designated as follows: arm A, active lymphoma and arm B, those who were in remission or adjuvant arm.

Descriptive statistics were used to summarize T-cell product characteristics using mean and SEM. T-cell expansion by week 6 was log-transformed to achieve normality, and comparisons of T-cell expansion, baseline cytokine levels, etc between groups (responders *v* nonresponders, etc) were made using *t*-test for continuous variables. Of note, the small sample size and multiplicity of

observations mean significant findings in correlative assays here are merely hypothesis generating.

RESULTS

Patient Characteristics

Forty-two patients with a diagnosis of lymphoma were eligible to participate, and blood was procured for manufacture. We had three manufacturing failures—two patients from whom we failed to isolate sufficient DCs and one patient whose T cells failed to expand. Six of the remaining 39 patients were not infused because two chose not to participate after procurement (one achieved a CR with bridging therapy and one withdrew consent), two enrolled on other trials, one was diagnosed with MDS after blood procurement, and one went to hospice. Another patient received only one of the two protocol-specified doses and then withdrew consent. Thus, a total of 32 patients, 14 with

TABLE 1. HL—Patient Characteristics

ID	Age/Sex	Stage	Prior Therapies	Time to Relapse After Frontline	Dose Level
Clinical outcomes—adjuvant					
3[a]	39/M	IA	ABVD → relapse → ICE → ASCT	No CR achieved	Ag escalation
7[a]	21/M	IIIA	ABVD → relapse → brentuximab → relapse → GND → Nav + Gem → ASCT	3 mo	Ag escalation
8[a]	34/M	IIIA	ABVD → relapse → ICE → ASCT + XRT → brentuximab	11 mo	Ag escalation
25	41/F	IIIA	ABVD + XRT → relapse → ICE → ASCT → relapse → XRT → brentuximab → DHAP	13 mo	1
30	35/M	IIB, bulky	ABVD → relapse → brentuximab + bendamustine → ASCT → XRT	No CR achieved	3
32	41/M	IIA, bulky	ABVD → PD → ICE → ASCT	2 mo	3
33	25/M	IIA, bulky	ABVD → PD → brentuximab → PD → ICE → XRT → nivolumab → ASCT	No CR achieved	3
Clinical outcomes—active disease					
1[a]	31/F	IVB	ABVD → relapse → ICE → PD → Cis + Gem→ XRT → ASCT → EBV T cells → relapse → brentuximab → PD →Yttrium90 → relapse → CD30 CAR T cells → PD	3 mo	Ag escalation
4[a]	38/M	IIA, bulky	ABVD → XRT → relapse → IGEV → ESHAP → ASCT → XRT→ relapse → GVD → XRT → PD	No CR achieved	Ag escalation
5[a]	44/F	IIA	ABVD → relapse→ ICE → ASCT → PD → brentuximab → SD	5 mo	Ag escalation
9	46/M	IVB, bulky	ABVD → relapse → ICE → ASCT + XRT → relapse → brentuximab → SD	PD on ABVD	1
11	31/F	IIIB	ABVD → relapse → XRT → relapse → ICE → Nav + Gem → ASCT → relapse → HDACi → relapse → brentuximab → PD → bendamustine → relapse → PD1i → SD	No CR achieved	1
19	49/M	IVB	ABVD → relapse → ICE → ASCT → XRT→ relapse → brentuximab → PD → nivolumab → PD → bendamustine → relapse	2 mo	2
15	18/F	IIB	ABVE-variation →XRT→ relapse → I + vinorelbine + bortezomib → brentuximab → PD → PD1i → SD	10 mo	3

Abbreviations: ABVD, Adriamycin, bleomycin, vinblastine, and dacarbazine; Ag escalation, antigen-escalation phase; ASCT, high-dose chemotherapy and autologous stem-cell transplantation; CD30 CAR T cells, CD30 chimeric antigen receptor–transduced T cells on a clinical trial; Cis, cisplatin; CR, complete remission; D/ESHAP, dexamethasone or etoposide, methyl prednisolone, high-dose cytarabine, and cisplatin; EBV T cells, Epstein-Barr virus–specific T cells on a clinical trial; F, female; HDACi, histone deacetylase inhibitor; ICE, ifosfamide, carboplatin, and etoposide; IGEV, ifosfamide, gemcitabine, vinorelbine, and prednisone; M, male; Nav, navelbine; Gem, gemcitabine; PD, progressive disease; PD1i, PD-1 checkpoint inhibitor on a clinical trial; SD, stable disease; XRT, radiation therapy.

[a]Patients treated on the antigen-escalation phase.

TABLE 2. NHL—Patient Characteristics

ID	Age/Sex	Disease	Genetics	Stage	Prior Therapies	Time to Relapse After Frontline	Dose Level
Adjuvant							
6[a]	78/F	DLBCL	UNK	IVB	R → RCHOP	Not applicable	Ag escalation
12	78/F	DLBCL	UNK	IVB	R → RCHOP → mTAA-T cells → ***relapse*** → R-bendamustine	13 mo	2
24	54/M	DLBCL	UNK	IVA	RCHOP → R-EPOCH → ***relapse*** → dose-adjusted R-EPOCH + IT chemo → ***relapse*** → R-DHAP → ASCT	9 mo	1
23	61/M	DLBCL	Double hit (c-myc, bcl2)	IVA	R-EPOCH → ASCT → XRT	unknown	1
27	62/M	T cell	ALK1 neg, LCA+	IVA	CHOP + XRT → ASCT	Not applicable	2
26	53/M	Mantle	UNK	IVA	R-HyperCVAD → ***relapse*** → R-ibrutinib → ASCT + XRT	7 y	2
28	67/M	Mantle	UNK	IVA	R-bendamustine + Ara-C → ASCT	Not applicable	3
29	65/F	DLBCL	Triple hit (bcl2, c-myc, bcl6)	IIA	R-EPOCH → ASCT	No CR achieved	3
17	73/F	DLBCL	Double expressor (bcl2+, bcl6+)	IIB	R-CHOP → XRT → ***PD*** → ESHAP → RIE	No CR achieved	3
18	32/F	T-cell ALCL	ALK+	IIA	CHOP → relapse → brentuximab → ***PD*** → crizotinib → CD30 CAR T cells→ ***PD*** → crizotinib → **CR**	53 mo	3
Active disease							
2[a]	55/F	HL/NHL	UNK	IVB	RCHOP + XRT → ICE → ASCT	6 mo	Ag escalation
10	46/F	DLBCL	bcl6+	IVA	RCHOP → ***relapse*** → GDC → ASCT → ***PD***	26 mo	1
13	69/M	NHL	Not done	IVB	EPOCH → romidepsin → ASCT → ***relapse***	No CR achieved	2
14	54/M	DLBCL	Double hit (bcl2, bcl6)	IVA	RCHOP → R-ICE→ ***PD*** → R-ICE → ASCT → ***relapse***	7 mo	2
16	48/M	DLBCL	Double expressor (bcl2+, bcl6+)	IIIA	EPOCH-R → R-ICE → ASCT → XRT	No CR achieved	3
20	54/M	DLBCL	Double hit (c-myc, bcl2)	IIB	EPOCH-R → R-ICE → XRT → IT MTX + XRT → ***SD*** → ASCT → ***PD***	No CR achieved	3
21	64/M	DLBCL	Double hit (bcl-2, unknown and 17p del)	IIIA	R-CHOP + XRT→ ***relapse*** → bendamustine + R → R → ***relapse*** → RICE→ ASCT → ***relapse***	60 mo	3
22	68/M	DLBCL	Normal	IVB	RCHOP → ***relapse*** → GDP → ASCT → ***relapse***	11 mo	3

Abbreviations: Ag escalation, antigen-escalation phase; Ara-C, cytarabine; ASCT, high-dose chemotherapy and autologous stem-cell transplantation; CD30 CAR T cells, CD30 chimeric antigen receptor–transduced T cells on a clinical trial; CHOP, cyclophosphamide, Adriamycin, vinctristine, and prednisone; CR, complete remission; D/ESHAP, dexamethasone or etoposide, methyl prednisolone, high-dose cytarabine, and cisplatin; DA, dose-adjusted; EPOCH, etoposide along with same agents as in CHOP; F, female; GDC/P, gemcitabine, dexamethasone, and carboplatin or cisplatin; HL, Hodgkin lymphomas; ICE, ifosfamide, carboplatin, and etoposide; IT chemo, intrathecal chemotherapy; M, male; NHL, non-Hodgkin lymphomas; PD, progressive disease; R, rituximab; R-HyperCVAD, rituximab plus high dose cyclophosphamide, vincristine, doxorubicin and dexamethasone; SD, stable disease; UNK, unknown; XRT, radiation therapy.

[a]Patients treated on the antigen-escalation phase.

HL (Table 1) and 18 with aggressive NHL (DLBCL [n = 12], mantle-cell lymphoma [n = 2], T-cell lymphoma [n = 3], and composite lymphoma [HL and DLBCL, n = 1]) (Table 2), were treated per protocol. Eight patients were infused on the antigen-escalation phase of the study, whereas the remaining 24 were infused on the dose-escalation phase of the study. Three patients (Pt 14, 15, and 16) received an additional dose (total three doses instead of the protocol-specified two doses) of mTAA-T cells.

Seven patients with HL received mTAA-T cells as adjuvant therapy (median four prior lines of therapy; range 3-5), whereas the remaining seven received mTAA-T cells to treat relapsed or refractory (R/R) disease following a median of five lines (range 4-8) of prior therapies. In the NHL cohort, 10 patients were infused as adjuvant therapy (median three prior lines of therapy; range 1-5), whereas the remaining eight were treated for R/R disease (median three prior lines of therapy; range 3-4) (Tables 1 and 2 and Data Supplement, only online, which also details TAA expression on available pretreatment biopsies).

Phenotype and Specificity of mTAA-T Cells

T cells underwent 2-4 rounds of in vitro stimulation with pepmix-loaded DCs for an average of 33 (± 3) days in culture. We achieved a mean 8.3 ± 1.0–fold increase (Fig 1A), and final cell numbers achieved are shown in Data

Supplement. Products were almost exclusively CD3+ T cells (mean ± SEM: 95.6% ± 1.0%), with a mixture of CD4+ (46.6% ± 3.9%) and CD8+ (40.7% ± 3.6%) subsets possessing both central (CD45RO+/CD62L+ 22.6% ± 2.9%) and effector memory markers (CD45RO+/CD62L−: 31.6% ± 3.7%) and an activated phenotype subset evidenced by upregulation of CD69+ (33.6% ± 2.0%) (Figs 1B and 1C). Data Supplement and Figure 1D demonstrate the specificity of the expanded mTAA-T cells in the antigen-escalation phase and for all lines generated, respectively, as determined by interferon-γ enzyme-linked immune absorbent spot. Of the 32 (of 39) lines that were generated using all five antigens as a stimulus and were subsequently characterized, PRAME induced the strongest activity (87 ± 23 spot-forming cells [SFC]/2 × 10^5 cells), followed in descending order by SSX2 (36 ± 16 SFC), MAGE-A4 (25 ± 11 SFC), NY-ESO-1 (25 ± 12 SFC), and SURVIVIN (17 ± 9 SFC). Finally, we observed < 10% lysis (a release criterion for infusion) of nonpulsed autologous phytohaemagglutinin blasts at an effector to target ratio of 20:1 (mean 2.0 ± 0.0%, n = 47) to rule out potential for autoreactivity (Fig 1E).

Safety

Antigen escalation. All eight patients received two infusions of 0.5 × 10^7 cells/m^2 administered 1 month apart. Without any DLTs seen, we achieved the primary objective of demonstrating safety of targeting up to 5 TAAs (Table 3).

Dose escalation. A total of 24 patients received the minimum protocol-specified two cell doses, 2 weeks apart (six patients at DL1 [three per arm, 0.5 × 10^7 cells/m^2], six at DL2 [three per arm, 1 × 10^7 cells/m^2], and 12 at DL3 [five on arm A and seven on arm B, 2 × 10^7 cells/m^2]). In total, there were six treatment-related events, headache (n = 3) and nausea and/or dysgeusia (n = 3), which are known side effects of the cryopreservative dimethyl sulfoxide. All treatment-related events were grade < 3 (Data Supplement) and thus not dose-limiting. Notably, there were no autoreactivity syndromes, cytokine release syndrome, or neurotoxicities. We observed eight grade ≥ 3 hematologic toxicities: six neutropenias and two lymphopenias. In all cases, patients had preexisting neutropenia or lymphopenia at grade 2 with trends of worsening to grade ≥ 3 even before T-cell infusions. After infusion, there was transient worsening with subsequent resolution to baseline without intervention in all but one case (Pt 32). Pt 32 was a patient with HL who received rituximab-based transplant conditioning (rituximab use in this case was the patient's first exposure and was per an investigational protocol at our site), was diagnosed with delayed rituximab neutropenia, and received granulocyte colony-stimulating factor. Indeed, all patients with neutropenia had a baseline diagnosis of rituximab-related or chemotherapy-related neutropenia. All other grade ≥ 3 AEs by study phase and dose level are shown in Table 3 (patient-by-patient AEs are shown in Data Supplement). Thus, we achieved the dual primary objectives of demonstrating safety of targeting five TAAs simultaneously and at a maximum tested dose of 2 × 10^7 cells/m^2 given twice, 2 weeks apart. Although MTD was not reached, since efficacy was demonstrated at DL3 and below, DL3 was chosen as the recommended phase II dose.

Clinical Outcomes

HL. At the 8-week disease assessment, all seven patients in the adjuvant cohort remained in continued complete remission (CCR), which was sustained in all but one at a median follow-up of 3.8 years (range: 2-5.2 years) (Fig 2A, top panel). Of the seven patients infused on the active disease cohort, two achieved complete and durable remissions without additional therapies and both remain in long term (> 3 years) remission (Fig 2A, bottom panel).

NHL. All 10 patients in the adjuvant cohort remained in CCR at their 8-week assessment, and only two subsequently relapsed at a median follow-up of 2.3 years (range: 1-4 years) (Fig 2B, top panel).

Of the eight patients treated for active disease, four patients entered complete and durable CR (> 3 years) without any other therapies (Fig 2B, bottom panel). Notably, four of the six responding patients achieved a CR after the 8-week disease assessment time point.

Kinetics of Response

The six patients in the active cohort (both HL and NHL combined) who achieved a CR with T cells alone represented a variety of lymphoma subtypes, DLBCL, T-cell lymphoma, and HL, and responses were seen in antigen- and dose-escalation phases with no apparent correlation with dose levels. We observed a similar pattern of response across the clinical responders where clinical response coincided with increased numbers of T cells directed against targeted and/or nontargeted antigens in peripheral blood followed by tumor regression, which was often (n = 4) after the week-8 staging scans (Data Supplement). Of note, this in vivo T-cell expansion (against targeted antigens) was derived from the infused product on the basis of TCR deep-sequencing studies performed on available material in two responders (Data Supplement).

In Vivo T-Cell Function

Given the nature of our T-cell therapy, we investigated whether there was an immune signature associated with superior clinical outcomes. Thus, we analyzed the behavior of our T cells in vivo and grouped our patients as follows: (i) those infused as adjuvant therapy vs treated for active disease, (ii) those with HL versus NHL, and (iii) those who responded to therapy (defined as a sustained CCR or achievement of a CR absent other therapies) versus nonresponders. We interrogated the peripheral blood of infused patients at multiple time points after infusion to examine the expansion of the infused mTAA-T cells. In addition, we looked for endogenous immune activation as evidenced by in vivo antigen spreading, ie, the emergence of T-cell

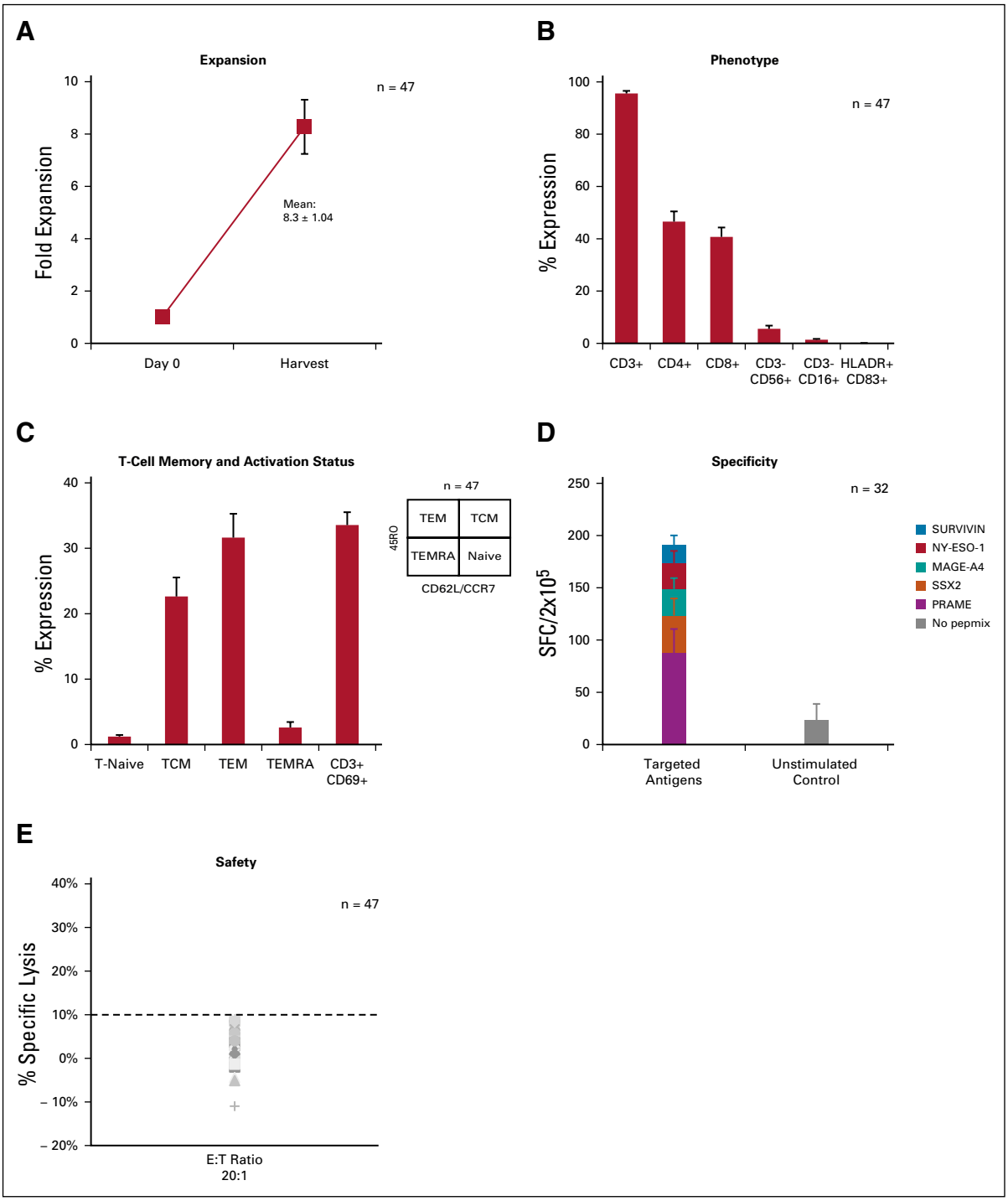

FIG 1. Characterization of autologous multiple tumor-associated antigen–T cells. Fold expansion (A), phenotype (B), and memory and activation profile (C) of mTAA–T-cell lines. (D) TAA-directed specificity as measured by enzyme-linked immune absorbent spot for 32 products generated using all five antigens as a stimulus. Data are shown as spot-forming cells (SFCs) ± SEM, and each color represents an individual antigenic specificity. (E) Lack of in vitro multiple tumor-associated antigen–T-cell cytolytic activity against autologous (nonmalignant) targets at an effector to target ratio of 20:1.

responses directed against tumor-expressed antigens not targeted by the infused product.

We saw no significant differences in the peak fold expansion of T cells by week 6 after infusion when we analyzed patients on the basis of the presence or absence of active disease (Fig 3A) or disease type (HL *v* NHL; Fig 3B). When we examined the immune response in patients who responded to our mTAA-T cells versus nonresponders, we observed expansion of T cells directed against our five target TAAs in both the patient groups (Fig 3C, left panel).

TABLE 3. All Grade ≥ 3 Adverse Events by Dose Level

Incident	No. of Events	Description
Antigen escalation		
Influenza A infection	1	Managed supportively, with resolution
Hyperkalemia	1	S-T suspected
DL1		
Neutropenia	1	Drug-related (suspect newly started S-T on top of preexisting rituximab-related neutropenia, resolved when S-T was discontinued)
Lymphopenia	1	Baseline lymphopenia, only transiently worse (grade 2-3) and resolved to baseline without intervention. Similar trends noted before T-cell infusion.
DL2		
Urinary tract obstruction	1	Prostatic hypertrophy suspected
Diarrhea	1	Antibiotic-associated
Hypertension	1	Worsening from baseline essential hypertension
DL3		
Neutropenia	5	All had received > 4 cycles of rituximab in combination with chemotherapy agents within preceding 3 months and all had a trend of grade 2-3 neutropenia prior to infusion. In all but one case, the neutropenia was transient and self-limited. One patient was given G-CSF with resolution.
Lymphopenia	1	Baseline lymphopenia, only transiently worse (grade 2-3) and resolved to baseline without intervention. Similar trends noted before T-cell infusion.
Cheilitis	1	Vitamin deficiency suspected versus herpes cheilitis
Clostridium difficile infection	1	Complicated with hypotension needing ICU stay. Resolved with antibiotics alone.

Abbreviations: G-CSF, granulocyte colony-stimulating factor; ICU, intensive care unit; S-T, sulfamethoxazole-trimethoprim.

Importantly though, we observed antigen spreading, as evidenced by the detection of T cells directed against nontargeted tumor-expressed antigens, which was superior in responders versus nonresponders (Fig 3C, right panel; $P = .022$). The specificity profile and trends of circulating TAA-specific T cells at multiple time points after infusion are demonstrated in Data Supplement (active *v* adjuvant, HL *v* NHL, and responders *v* nonresponders).

DISCUSSION

In this study, we evaluated the safety and clinical effects of transferring autologous mTAA-T cells to patients with lymphoma at high risk of relapse (n = 17) or to treat active R/R disease (n = 15). We show that T-cell products specific for up to five TAAs and infused at doses of up to 2×10^7 cells/m^2 twice, 2 weeks apart, can be safely administered to patients with lymphoma regardless of disease status (in remission or active) or lymphoma subtype. When administered to treat R/R active lymphoma, we observed objective clinical responses [in 6/15 patients (40%), ongoing for > 3 years] that appeared to be independent of dose. Furthermore, among responders, we demonstrate a direct correlation between the in vivo clinical effects of mTAA-T cells and the induction of antigen spreading.

We have previously demonstrated the benefit of treating Epstein-Barr virus (EBV)–associated malignancies with adoptively transferred EBV-specific T cells.[15-18] However, the majority of lymphomas do not express EBV antigens, limiting the broader applicability of this approach. Furthermore, lymphomas are susceptible to immunological editing as an evasion mechanism, which is a phenomenon we have encountered in our EBV studies.[15-18] Therefore, we hypothesized that effective immunotherapy for lymphoma would require targeting multiple tumor-expressed antigens both to enhance antitumor activity and to prevent immune escape. So, in this safety study, we infused T cells with specificity for five nonviral TAAs upregulated or overexpressed by malignant cells to 32 patients with a variety of lymphoma subtypes shown to express one or more of these antigens.[19-24] To establish the safety of targeting each TAA individually, we first conducted an antigen-escalation phase followed by a traditional dose-escalation phase to establish an MTD. Although an MTD was not reached, we observed an exquisite safety profile up to and including at the maximum tested dose (DL3), thus leading us to choose a dose of 2×10^7 cells/m^2 ($\times$ 2 infusions) as the phase II dose. Ultimately, the absence of infusion-related toxicities likely reflects a number of factors including the pattern of normal tissue expression of our target antigens, the small cell doses administered without prior lymphodepletion so

FIG 2. Clinical outcomes of infused patients. Swimmers plots depicting outcomes after infusion in patients with HL (A) and NHL (B). Those who were in remission at the time of infusion in each group are labeled adjuvant, and those who had active lymphoma at the time of infusion are labeled as active disease. Those who were infused on the antigen-escalation phase of the study are indicated. HL, Hodgkin lymphomas; NHL, non-Hodgkin lymphomas.

that in vivo expansion was driven by physiologic TCR simulation, and the fact that our cells were not engineered to enhance TCR affinity—a practice that has induced unexpected cross-reactivity.[25,26] In addition, we achieved objective responses (all CRs) in those with active lymphoma at the time of infusion with no evident dose-response correlation. Furthermore, consistent with other immunotherapies including adoptively transferred EBV-specific T cells[15-18] and immune checkpoint inhibitors[27-30], antigen spreading, which was seen across all dose levels, was significantly associated with long-term responses.

T-cell immunotherapy with CD19 CAR T cells has produced unprecedented responses, even in patients with aggressive and rapidly growing lymphomas. However, approximately half of all treated patients subsequently relapse.[10,11] Another challenge associated with CAR T cells is the management of immune effector–induced toxicities to the CNS and CRS.[31,32] Thus, mTAA-T cells, if confirmed to be efficacious in planned pivotal trials, could complement the existing standard of care for CD19-positive lymphomas without additive toxicities. Moreover, they could be applied to the treatment of lymphomas not expressing CD19 and as demonstrated by this trial could be safely used to consolidate the beneficial effects of prior cytotoxic chemotherapies or as a treatment for R/R disease. In the current study, the mean time for mTAA manufacture was 33 days, which in combination with the study inclusion or exclusion criteria may have led to the selection of patients with lower disease burden and/or slower growing tumors (as compared with those enrolled to CAR-based trials)—limitations that could be overcome with the advent of prospectively generated and thus immediately available therapies such as allogeneic CAR T cells or bispecific T-cell engagers. However, it should be noted that our responders had refractory disease after 2 or more prior curative-intent therapies. Therefore, taken together, our findings provide preliminary indications of clinical responses that warrant efficacy-based confirmatory trials in R/R settings.

It is important to note that this phase I and II trial included small numbers of patients with heterogeneous diseases infused with different mTAA–T-cell products (antigen- *v* dose-escalation phases), which would dilute efficacy estimates. In addition, estimates of clinical benefit of mTAA-T cells in the adjuvant cohort are impossible to discern from this single-arm trial. Therefore, now that safety has been established, we expect to fully define the clinical effects of mTAA-T cells as CR rate in a larger phase II trial of R/R patients as well as a randomized comparative trial in the adjunctive setting.

In summary, our findings demonstrate that autologous mTAA-T cells can be reproducibly manufactured from heavily pretreated patients with lymphoma. The infused cells were well-tolerated at the highest antigen and dose levels tested, leading to a recommended phase II dose of two infusions of 2×10^7 cells/m^2. We also observed single-agent clinical effects in R/R patients with lymphoma

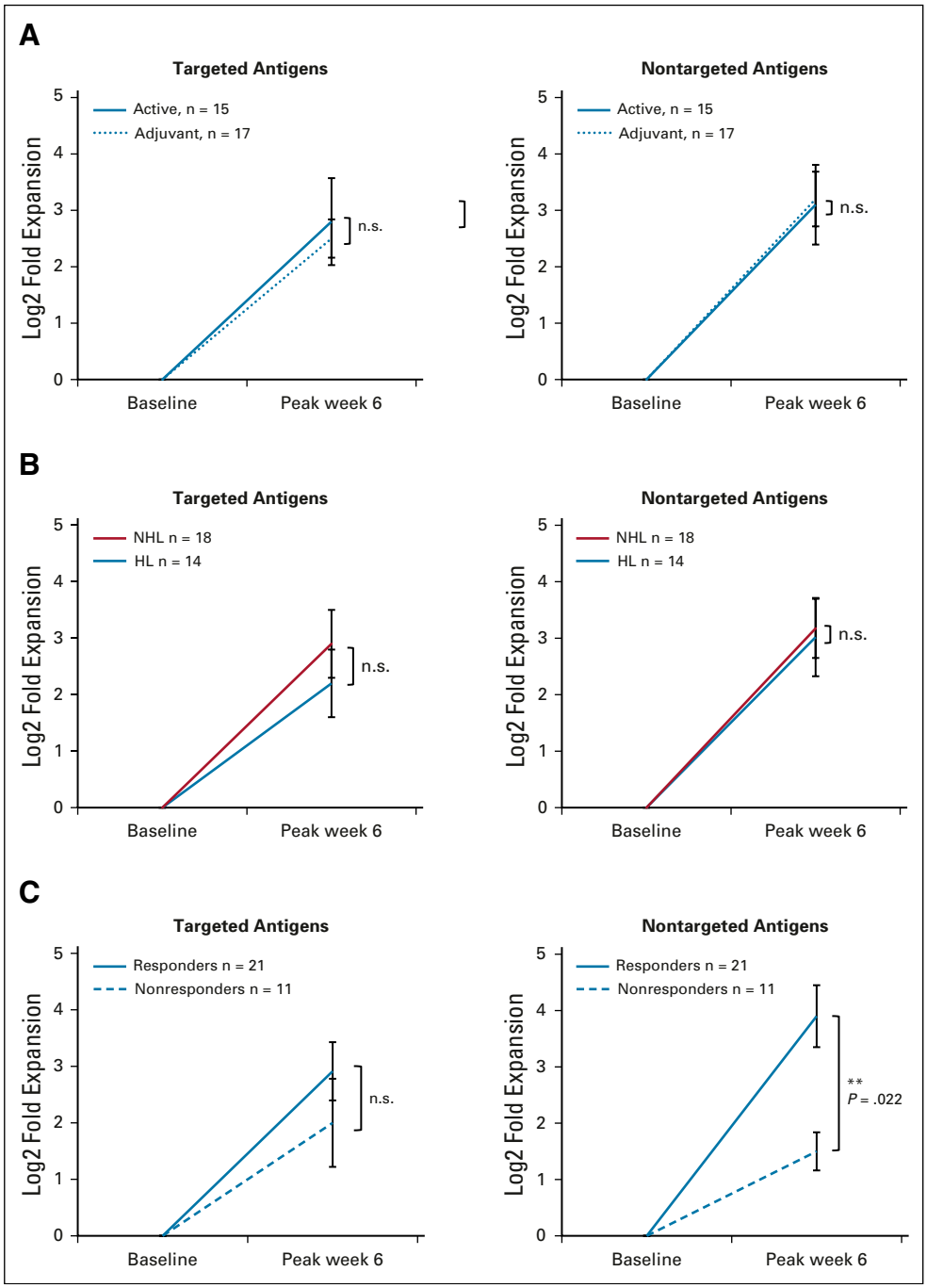

FIG 3. In vivo behavior of multiple tumor-associated antigen–T cells. Expansion of T cells specific for targeted tumor-associated antigens and other nontargeted tumor-associated antigens in patients with active disease versus those infused as adjuvant (A); patients with HL vs those with NHL (B); and responders (defined as continued complete remission and complete remission) versus nonresponders (C). Results are reported as log-transformed fold expansion values (mean ± SEM) by week 6 after infusion. Statistical significance was assessed by *t*-test for continuous variables. **Denotes statistical significance. HL, Hodgkin lymphomas; n.s., nonsignificant; NHL, non-Hodgkin lymphomas.

coupled with the induction of antigen spreading. Ultimately, both safety and clinical responses were seen in patients with HL and a spectrum of NHLs using a T-cell product targeting antigens that are distinct and complimentary to CAR-based and immune checkpoint inhibitor approaches.

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PRIOR PRESENTATION

Presented in part at the 57th Annual Meeting of the American Society of Hematology, Orlando, FL, December 5-8, 2015, and in part at the Annual Meeting of the American Society of Blood and Marrow Transplantation, Honolulu, HI, February 18-22, 2016.

SUPPORT

Supported by NIH SPORE in lymphoma 5P50CA126752 (PI: Malcolm Brenner and Helen Heslop, Project leaders Ann Leen, George Carrum), Leukemia & Lymphoma society SCOR award (PI: Helen Heslop, project leaders: Ann Leen and Premal Lulla), ASH Scholar Award (PI: Premal Lulla). Leukemia Texas Research grant (PI: Premal Lulla), ASBMT New Investigator Award (PI: Premal Lulla), CPRIT Texas Access to Cellular Therapies (TACCT) (PI: Adrian Gee), CPRIT Early Career Clinical Investigator Award (PI: Premal Lulla).

CLINICAL TRIAL INFORMATION

NCT01333046 (TACTAL)

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at DOI https://doi.org/10.1200/JCO.20.02224.

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ACKNOWLEDGMENT

We thank patients and their families who participated in this trial, the clinicians, staff, and integral personnel in the clinic and the laboratory. We would specifically like to acknowledge Deborah Lyon and Natalia Lapteva for quality assurance and quality control and Walter Mejia for assisting with formatting figures.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

T-Cell Therapy for Lymphoma Using Nonengineered Multiantigen-Targeted T Cells Is Safe and Produces Durable Clinical Effects

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No potential conflicts of interest were reported.