Clinical Cancer Research

First-in-Class, First-in-Human Study Evaluating LV305, a Dendritic-Cell Tropic Lentiviral Vector, in Sarcoma and Other Solid Tumors Expressing NY-ESO-1

Neeta Somaiah[1], Matthew S. Block[2], Joseph W. Kim[3], Geoffrey I. Shapiro[4], Khanh T. Do[4], Patrick Hwu[5], Joseph P. Eder[3], Robin L. Jones[6,7], Hailing Lu[8], Jan H. ter Meulen[8], Chet Bohac[8], Michael Chen[8], Frank J. Hsu[8], Sacha Gnjatic[9], and Seth M. Pollack[6,7]

Abstract

Purpose: LV305 is a modified, third-generation, nonreplicating, integration-deficient lentivirus-based vector designed to selectively transduce dendritic cells *in vivo*. LV305 induces expression of the New York Esophageal Squamous Cell Carcinoma-1 (NY-ESO-1) cancer testis antigen in dendritic cells, promoting immune responses against NY-ESO-1–expressing tumors. This phase I study evaluated the safety, immunogenicity, and preliminary efficacy of LV305 in patients with sarcoma or other solid tumors.

Patients and Methods: Adults with previously treated, advanced, NY-ESO-1–positive solid tumors and limited tumor burden were eligible. LV305 was administered every 3 weeks by intradermal injection in four dose cohorts (Cohort 1: 10^8 vector genomes (vg) x 3 doses; Cohorts 1A, 2, and 3: 10^8 vg, 10^9 vg, 10^{10} vg x 4 doses).

Results: Thirty-nine patients were enrolled: 3 patients each in Cohorts 1, 1A, and 2, and 30 patients in Cohort 3. No dose-limiting toxicities were observed. Tumor types included sarcoma ($n = 24$), ovarian ($n = 8$), melanoma ($n = 6$), and lung cancer ($n = 1$). All treatment-related adverse events were grade 1 or 2. Common treatment-related adverse events were fatigue (49%), injection site reactions (46%), and myalgia (21%). The disease control rate was 56.4% in all patients and 62.5% in sarcoma patients. One patient with synovial sarcoma achieved a partial response lasting >36 months. Anti–NY-ESO-1-specific $CD4^+$ and/or $CD8^+$ T cells were induced in 57% of evaluable sarcoma patients. Induction of an anti–NY-ESO-1 immune response was associated with improved 1-year survival in an exploratory analysis.

Conclusions: This first-in-class, first-in-human study of LV305 demonstrated a favorable safety profile, induction of antigen-specific responses, and potential clinical activity in patients with advanced cancer.

Introduction

Successful solid tumor immunotherapy requires activation of a broad population of T cells that recognize tumor cells. T cells are activated by antigen-presenting cells, including dendritic cells (DC). DCs are uniquely equipped to process and present antigen to T cells in the context of MHC along with costimulatory molecules, and are effective for stimulating naïve T cells. The engineering of therapeutic technologies that selectively activate DCs and induce them to process and present tumor-specific antigens to $CD8^+$ T cells could be an important step toward the development of more effective solid tumor immunotherapies (1).

Modified, third-generation, lentivirus-based vectors (LV) are a novel approach for *in vivo* DC-targeted vaccination. Unlike many other viral vectors, LV can transduce nondividing DCs, thus inducing the *de novo* transcription of the encoded antigen. Cytoplasmically expressed antigens can be directly presented to the immune system via MHC-I molecules for induction of $CD8^+$ T cells; this direct presentation is hypothesized to be up to 10^5 times more efficient than the loading of exogenous antigens onto MHC class I via cross-presentation (2). Furthermore, the RNA of LV is able to trigger toll-like receptor (TLR3, TLR7/TLR8) signaling which stimulates the maturation of DCs, increases their expression of costimulatory molecules (e.g.,

[1]Department of Sarcoma Medical Oncology, MD Anderson Cancer Center, Houston, Texas. [2]Department of Oncology, Mayo Clinic, Rochester, Minnesota. [3]Department of Medicine (Medical Oncology), Yale School of Medicine, New Haven, Connecticut. [4]Early Drug Development Center, Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts. [5]Department of Melanoma Medical Oncology, MD Anderson Cancer Center, Houston, Texas. [6]Division of Oncology, University of Washington, Seattle, Washington. [7]Fred Hutchinson Cancer Research Center, Seattle, Washington. [8]Immune Design Corp., Seattle, Washington and South San Francisco, California. [9]Immune Monitoring Center, Icahn School of Medicine at Mount Sinai, New York, New York.

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

Current address for R.L. Jones: Royal Marsden and Institute of Cancer Research, London.

Corresponding Author: Neeta Somaiah, The University of Texas MD Anderson Cancer Center, 1400 Holcombe Blvd., Unit 450, FC12.3038, Houston, TX 77030. Phone: 713-563-5056; Fax: 713-794-1934; E-mail: nsomaiah@mdanderson.org

Clin Cancer Res 2019;25:5808–17

doi: 10.1158/1078-0432.CCR-19-1025

©2019 American Association for Cancer Research.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/25/19/5808/2052901/5808.pdf by guest on 25 April 2024

Translational Relevance

LV305 is a novel lentiviral-based cancer vaccine that induces expression of the New York Esophageal Squamous Cell Carcinoma-1 (NY-ESO-1) cancer testis antigen in dendritic cells. LV305 was derived from the ZVex platform, which is a third-generation, replication-incompetent, and integration-deficient lentiviral vector engineered to deliver antigen-encoding gene(s) selectively to dendritic cells *in vivo*. To our knowledge, this is the first lentiviral vector directly injected into patients as a cancer vaccine. In this first-in-human study, LV305 was well-tolerated in patients with advanced solid tumors, with only grade 1 or 2 treatment-related adverse events (AE) reported and no treatment-related serious AEs. LV305 effectively induced $CD4^+$ and $CD8^+$ T-cell responses against NY-ESO-1–expressing tumor cells, as measured by biological and molecular assays, and these immune responses were associated with potential clinical benefit. These results demonstrate the feasibility and potential therapeutic impact of direct injection of a dendritic-cell tropic lentiviral vector into patients as a cancer vaccine.

CD40, CD80, CD86), upregulates MHC Class II expression, and improves the interactions of DCs with T cells (3, 4). Thus, LV may be more effective at inducing T-cell responses than immunotherapeutic approaches that rely on exogenous antigens and cross-presentation.

The New York Esophageal Squamous Cell Carcinoma-1 [NY-ESO-1 (CTAG1B)] expressing LV305 is derived from the ZVex platform, which was engineered to deliver antigen-encoding gene(s) selectively to DCs *in vivo*. The envelope protein of ZVex is derived from the Sindbis virus envelope glycoprotein which interacts with the DC-specific, C-type lectin receptor DC-SIGN (CD209). ZVex is a third-generation LV that is replication-incompetent and integration-deficient due to extended deletion of the vector backbone and integrase inactivation (5). These redundant molecular safety features make persistence and integration of the vector very unlikely and enable safe parenteral injection compared with other third-generation LV currently used for *ex vivo* gene transfer in cell-based therapies.

LV305 induces the expression of full-length NY-ESO-1 protein (5–7), which is a highly immunogenic cancer testis antigen expressed in up to 40% of non–small cell lung, ovarian, and melanoma cancers, and in greater than 70% of certain sarcoma subtypes [e.g., synovial sarcoma (SS) and myxoid/round cell liposarcoma (MRCL); refs. 8–11]. NY-ESO-1–directed vaccination has obtained some promising clinical results in early phase I/II studies, and adoptive cell therapy has resulted in partial responses (PR) in 60% of NY-ESO-1–expressing sarcomas in small phase I trials (12). In preclinical studies, a single immunization with LV305 transduced DCs *in vivo*, generated multifunctional CD8 T-cell responses, and controlled the growth of NY-ESO-1–expressing murine tumors (7).

This first-in-human phase I study examined the clinical safety, immunogenicity, and preliminary efficacy of LV305 in patients with advanced sarcoma or other solid tumors expressing NY-ESO-1.

Patients and Methods

Study design and oversight

A phase I, open-label trial of LV305 was conducted to evaluate the safety and tolerability of LV305 administered intradermally (ID) in patients with locally advanced, relapsed, or metastatic cancers expressing NY-ESO-1.

Secondary objectives included evaluation of immunogenicity. Exploratory objectives included evaluation of responses every 8 weeks by immune-related response criteria (irRC) modified to use RECIST (v1.1), duration of response, progression-free survival (PFS), time to progression, and biomarkers. Three escalating doses of LV305 in four cohorts were assessed using a 3+3 sequential dose-escalation design, with expansion at the maximum safe dose.

The trial was conducted in accordance with the ethical principles in the Declaration of Helsinki, International Conference on Harmonization guidelines for Good Clinical Practice, and the code of Federal Regulations. The protocol was reviewed by the National Institutes of Health Recombinant DNA Advisory Committee and followed their approved guidelines and was approved by local Institutional Review Boards and Biosafety Committees. All patients provided written, informed consent. This trial is registered on Clinicaltrials.gov (NCT02122861).

Patients

Eligible patients were ≥18 years of age with locally advanced, relapsed, and/or metastatic cancer with low tumor burden as defined in Supplementary Table S1. During screening, tumor expression of NY-ESO-1 was determined by IHC (performed by Mosaic Laboratories) on tumor samples collected prior to screening using mouse monoclonal antibody E978 (Sigma). Eligible NY-ESO-1 expression was defined as ≥5% and later amended to include any tumor cell–specific positivity (as determined by a central lab pathologist) to allow enrollment of lower-expressing tumor types. Patients were not required to have HLA typing to determine eligibility.

During dose escalation, eligible NY-ESO-1–positive tumor types included sarcomas (any subtype), melanoma, non–small cell lung cancer (NSCLC), ovarian cancer (including fallopian tube carcinoma), and breast cancer. During expansion at the maximum safe dose, tumor types included sarcomas (SS and MRCL subtypes), melanoma, NSCLC, ovarian cancer (including fallopian tube carcinoma), and urothelial carcinoma of the urinary tract.

Eligible patients must have had an inadequate response, relapse, and/or unacceptable toxicity with one or more prior systemic, surgical, or radiation cancer therapies, except patients with NSCLC and breast cancer who must have two or more prior therapies. Inadequate response was defined as having persistent disease or ≥50% risk of recurrence per investigator assessment despite standard or curative intent treatment. An Eastern Cooperative Oncology Group performance status of 0 or 1, life expectancy of ≥6 months, and electrocardiogram without evidence of clinically significant arrhythmia or ischemia were required.

Exclusion criteria included prior treatment with other NY-ESO-1–targeting agents, uveal melanoma, unstable brain metastases, significant immunosuppression from corticosteroids or other immunosuppressive medications, significant autoimmune disease, myocardial infarction within 6 months or New York Heart

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/25/19/5808/2052901/5808.pdf by guest on 25 April 2024

Association Grade III or IV heart failure, inadequate organ function (marrow, hepatic, renal), and clinically significant infection.

Study treatment

LV305 (1 mL per dose) was administered ID once every 3 weeks using a MicronJet needle (Nanopass Technologies Ltd.). Each 1 mL dose was divided into 8 separate injections of 125 μL each; two 125 μL injections were administered to each upper arm and two 125 μL injections were administered to each thigh. Patients were enrolled in one of four cohorts: Cohort 1 received 1×10^8 viral genomes (vg) x 3 doses, Cohort 1A received 1×10^8 vg x 4 doses, Cohort 2 received 1×10^9 vg x 4 doses, and Cohort 3 received 1×10^{10} vg x 4 doses. Concurrent treatment with corticosteroids or immunosuppressive medications (e.g., methotrexate, cyclosporine, azathioprine) was prohibited.

Clinical assessments

Adverse events (AEs) were assessed regularly during treatment, and AE severity was graded per NCI Common Terminology Criteria for Adverse Events v.4.03. During dose escalation, a dose-limiting toxicity (DLT) was defined as any treatment-related grade ≥3 AEs that occurred during the 28 days following the first dose of LV305, with the exception of alopecia, vomiting or diarrhea controlled by optimal anti-emetics or anti-diarrheals, grade 3 fatigue, asymptomatic grade 3 laboratory AEs, or grade 3 systemic reactions (e.g., fever, headache, influenza-like symptoms, myalgia, malaise, nausea) that returned to baseline or grade 1 within 3 days. Data were reviewed by an independent data monitoring committee for DLT during the specified time period for each dose cohort and then for all patients at least quarterly thereafter.

Tumor imaging and clinical assessments were performed by the investigator at screening (baseline) and then every 8 weeks until disease progression. After progression, long-term follow-up was conducted every 12 weeks for any serious AEs (SAE) possibly related to LV305, disease status, and survival. The presence or absence of detectable LV305 genome in peripheral blood mononuclear cells (PBMC) was assessed and monitored for up to 2 years from first dose of LV305.

Tumor response was assessed by the investigator using irRC (13, 14) modified to incorporate RECIST v 1.1 criteria (15). The objective response rate was calculated as the proportion of patients with complete response (CR) or PR, and the disease control rate (DCR) was calculated as the proportion of patients with CR, PR, or stable disease (SD) for a minimum of 2 months from start of therapy. Clopper–Pearson exact 95% confidence interval (CI) was provided.

Immune response assessments and criteria for induced responses

Baseline tumor biopsies were obtained from all patients to screen for presence of NY-ESO-1 expression on tumor specimens by IHC. Posttreatment biopsies for exploratory biomarker analysis were optional in patients with accessible tumors. Peripheral blood samples were collected for immunologic testing at baseline and on days 14, 28, 63, and 84. These samples were processed into plasma and PBMC over Ficoll gradients and stored cryogenically for immunologic testing. Leukapheresis (1.5 to 2 blood volume process) were collected on each patient before and after treatment. PBMCs isolated from leukapheresis samples were cryopreserved for immunologic testing and exploratory biomarkers.

Details of the criteria for preexisting and induced antibody and T-cell responses have been published previously (16). For this study, plasma was analyzed for the presence of anti–NY-ESO-1 antibodies using an ELISA with recombinant NY-ESO-1 protein; a titer >1:100 was considered positive. The induction of anti–NY-ESO-1 antibody response was defined for this study as a ≥4-fold increase in the titer or the presence of a newly positive response after the first dose of LV305. Anti–NY-ESO-1 $CD4^+$ and $CD8^+$ T-cell responses were measured by IFNγ detecting enzyme-linked ImmunoSpot (ELISPOT) using isolated $CD4^+$ and $CD8^+$ T cells from PBMCs expanded *in vitro* with a NY-ESO-1 peptide pool (20-mer peptides, 10-mer overlap) and considered positive if there were >50 spot-forming units/50,000 cells observed for NY-ESO-1 peptides and a ≥2-fold increase in spot-forming units compared with a negative control. The induction of an anti–NY-ESO-1 $CD4^+$ or $CD8^+$ T-cell response was defined as *de novo* positive or ≥2-fold rise in the number of spot-forming units on the ELISPOT assay after the first dose of LV305. In addition, a FACS-based intracellular cytokine staining (ICS) assay for IFNγ or TNFα after stimulation with NY-ESO-1 peptide pool was used, and staining ≥2-fold above the baseline value was considered positive for T-cell responses.

T-cell receptor beta variable chain sequencing and statistical analysis

T-cell receptor beta (TCRβ) repertoire in baseline and post-treatment PBMC were evaluated by TCRβ chain sequencing of complementarity-determining region (CDR)3 on PBMC and tumor biopsies. Immunosequencing of the CDR3 regions of human TCRβ chains was performed using the ImmunoSEQ Assay (Adaptive Biotechnologies). Extracted genomic DNA was amplified in a bias-controlled multiplex PCR, followed by high-throughput sequencing. Sequences were collapsed and filtered in order to identify and quantitate the absolute abundance of each unique TCRβ-CDR3 region for further analysis as previously described (17–19).

Statistical analyses of TCRβ sequencing results

Shannon's Entropy, H, is defined as $-\sum_{i=1}^{N} p_i \log_2(p_i)$, where p_i is the proportional abundance of clone i, and N is the total number of unique TCR receptor rearrangements. Clonality is related to Shannon's Entropy and defined by $1 - \frac{H}{\log_2 N}$, where $\frac{H}{\log_2 N}$ is also known as Pielou's evenness (20). Clonality values range from 0 to 1 and describe the shape of the immune repertoire's frequency distribution: clonality values approaching 0 indicate a very even distribution of frequencies, whereas values approaching 1 indicate an increasingly asymmetric distribution in which a few clones are present at high frequencies. Richness of a repertoire was calculated using the Daley–Smith Estimate (R package: preseqR, v3.0.1), which combines a rational function approximation with Good and Toulmin's nonparametric empirical Bayes power series estimator (21, 22). The potential association between TCR clonality and overall survival (OS) was analyzed using the Kaplan–Meier method.

Exploratory analyses

PFS and OS were evaluated using the Kaplan–Meier method and compared using a log-rank test stratified by disease type. The OS rates at 1-year are presented, with P values for difference in 1-year OS calculated using Z statistics of difference in survival

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/25/19/5808/2052901/5808.pdf by guest on 25 April 2024

rates/square root of sum of variance. HR and its 95% CI were calculated using a stratified Cox model stratified by disease type.

Results

Baseline demographics and disease characteristics

Screening for NY-ESO-1 expression was performed on previously collected tumor samples from patients with eligible tumor types; the percentages of NY-ESO-1–positive specimens in screened patients are presented by tumor type in Supplementary Table S2; the highest percentages were seen in patients with MRCL (93%) and SS (68%–76%). A total of 39 patients were enrolled. The majority of enrolled patients had sarcoma (62%; $n = 24$); other tumor types enrolled were ovarian (21%; $n = 8$), melanoma (15%; $n = 6$), and NSCLC (3%; $n = 1$; Table 1). The most common sarcoma subtypes enrolled were SS ($n = 13$) and MRCL ($n = 6$). Eighty-seven percent of all enrolled patients had stage IV disease, and 28% had ≥3 prior systemic anticancer therapies (Table 1).

Table 1. Baseline demographics and disease characteristics

	Sarcoma patients ($n = 24$)	All patients ($n = 39$)
Median (range) age, years	47 (25–72)	56 (25–75)
Male/female gender, *n* (%)	11 (46)/13 (54)	15 (38)/24 (62)
Diagnosis, *n* (%)		
SS	13 (54)	13 (33)
MRCL	6 (25)	6 (15)
Other sarcoma[a]	5 (21)	5 (13)
Ovarian	N/A	8 (21)
Melanoma	N/A	6 (15)
NSCLC	N/A	1 (3)
Stage at diagnosis, *n* (%)		
II	1 (4)	1 (3)
III	2 (8)	4 (10)
IV	21 (88)	34 (87)
NY-ESO-1 expression[b], *n* (%)		
≤5%	0	11 (28)
>5%–25%[c]	4 (17)	15 (38)
>25%–50%	2 (8)	2 (5)
>50%–75%	2 (8)	3 (8)
>75%–100%	16 (67)	19 (49)
Median (range) time since diagnosis, years	3.4 (0.5–13.4)	2.9 (0.5–18.2)
Median (range) time since last treatment, months	4.9 (0.7–66)	4.8 (0.7–66)
Prior therapy, *n* (%)		
Radiation	21 (88)	26 (68)
Systemic anticancer therapy	20 (83)	32 (84)
Number of prior systemic anticancer therapies, *n* (%)		
0 lines[d]	4 (17)	9 (23)
1 line	10 (42)	15 (38)
2 lines	3 (13)	4 (10)
≥3 lines	7 (29)	11 (28)
Tumor status at study entry, *n* (%)		
NED[e]	6 (25)	9 (23)
SD	5 (21)	9 (23)
Evidence of any tumor growth	13 (54)	21 (54)
Mean tumor burden at study entry[f], mm	45.3 (±18.9)	41.9 (±18.3)

Abbreviation: NA, not applicable.

[a]Other sarcoma subtypes included leiomyosarcoma ($n = 2$) and rhabdomyosarcoma ($n = 1$), solitary fibrous tumor ($n = 1$), and Ewing sarcoma ($n = 1$).

[b]Among patients with nonsarcoma tumor types, high expression (≥50%) was detected in 50% of melanoma patients (3/6) and the only NSCLC patient; the remaining 3 melanoma patients had low (<1%) expression. All patients with ovarian cancer had NY-ESO-1 expression in ≤5% of tumor cells, including 5 with expression <1%.

[c]Includes any tumor-specific positivity up to <25%.

[d]All patients had prior therapy with at least one of the following: surgery, radiation, systemic anticancer therapy; patients with no prior systemic anticancer therapies had prior local radiation and/or surgery.

[e]NED was determined by investigator assessment of baseline imaging. If there were no measurable target lesions nor any evidence of nontarget lesions, investigators made assessment of NED.

[f]Sum of target lesion diameters; 19 patients (11 with sarcoma) classified as NED or with only presence of nontarget lesions were excluded.

Table 2. Summary of AEs

n (%)	Cohort 1 ($N = 3$)	Cohort 1A ($N = 3$)	Cohort 2 ($N = 3$)	Cohort 3 ($N = 3$)	Expansion ($N = 27$)	All patients ($N = 39$)
Any DLT	0	0	0	0	0	0
Any AE	3 (100)	3 (100)	3 (100)	3 (100)	27 (100)	39 (100)
Any tmt-rel AE	3 (100)	3 (100)	3 (100)	3 (100)	22 (81)	34 (87)
Grade ≥3 tmt-rel AE	0	0	0	0	0	0
SAE	0	0	0	0	4 (15)	4 (10)[a]
tmt-rel SAE	0	0	0	0	0	0
Treatment-related AE in ≥2 patients, *n* (%)		Grade 1	Grade 2	Grade ≥3	Any Grade	
Fatigue		12 (31)	7 (18)	0	19 (49)	
Injection site reaction[b]		18 (46)	0	0	18 (46)	
Myalgia		6 (15)	2 (5)	0	8 (21)	
Nausea		4 (10)	0	0	4 (10)	
Stomatitis		2 (5)	1 (3)	0	3 (8)	
Headache		3 (8)	0	0	3 (8)	
Bilirubin conjugated increased		3 (8)	0	0	3 (8)	
Malaise		2 (5)	0	0	2 (5)	
Pyrexia		2 (5)	0	0	2 (5)	
Diarrhea		2 (5)	0	0	2 (5)	
Blood creatinine increased		2 (5)	0	0	2 (5)	
Arthralgia		2 (5)	0	0	2 (5)	
Paresthesia		2 (5)	0	0	2 (5)	
Nasal congestion		2 (5)	0	0	2 (5)	

Abbreviation: Tmt-rel, treatment-related.

[a]SAEs were grade 3 small intestinal obstruction ($n = 2$), grade 3 abdominal pain, and grade 3 hypoxia.

[b]Number of unique patients who experienced one or more of the following AE: injection site pruritus ($n = 10$ patients), injection site reaction ($n = 7$ patients), injection site pain ($n = 5$ patients), and injection site erythema ($n = 4$ patients).

NY-ESO-1 expression was high (>50% of tumor cells by IHC; $n = 18$) or moderate (>25%–50% of tumor cells by IHC; $n = 2$) in 83% of sarcoma patients (20/24; Table 1). Among patients with other tumor types, high expression was detected in 50% of melanoma patients (3/6) and the only NSCLC patient. Ovarian cancer had the lowest NY-ESO-1 expression levels, with expression in ≤5% of tumor cells in all enrolled subjects.

DLT and AEs

Twelve patients received LV305 during dose escalation, 3 patients in each dose cohort. No DLTs were observed, and the MTD was not reached. Enrollment was expanded to enroll 27 additional patients at the highest dose level tested, 1×10^{10} vg x 4 doses.

Thirty-four patients (87%) experienced at least one treatment-related AE. The most common were fatigue (49%), injection site reaction (46%, includes the terms injection site pruritus, injection site pain, injection site erythema, and injection site reaction), and myalgia (21%; Table 2). All treatment-related events were grade 1 or 2 in severity. No grade ≥3 treatment-related AE and no treatment-related SAE were observed. Thirty patients (77%), including 20 of 24 (83%)

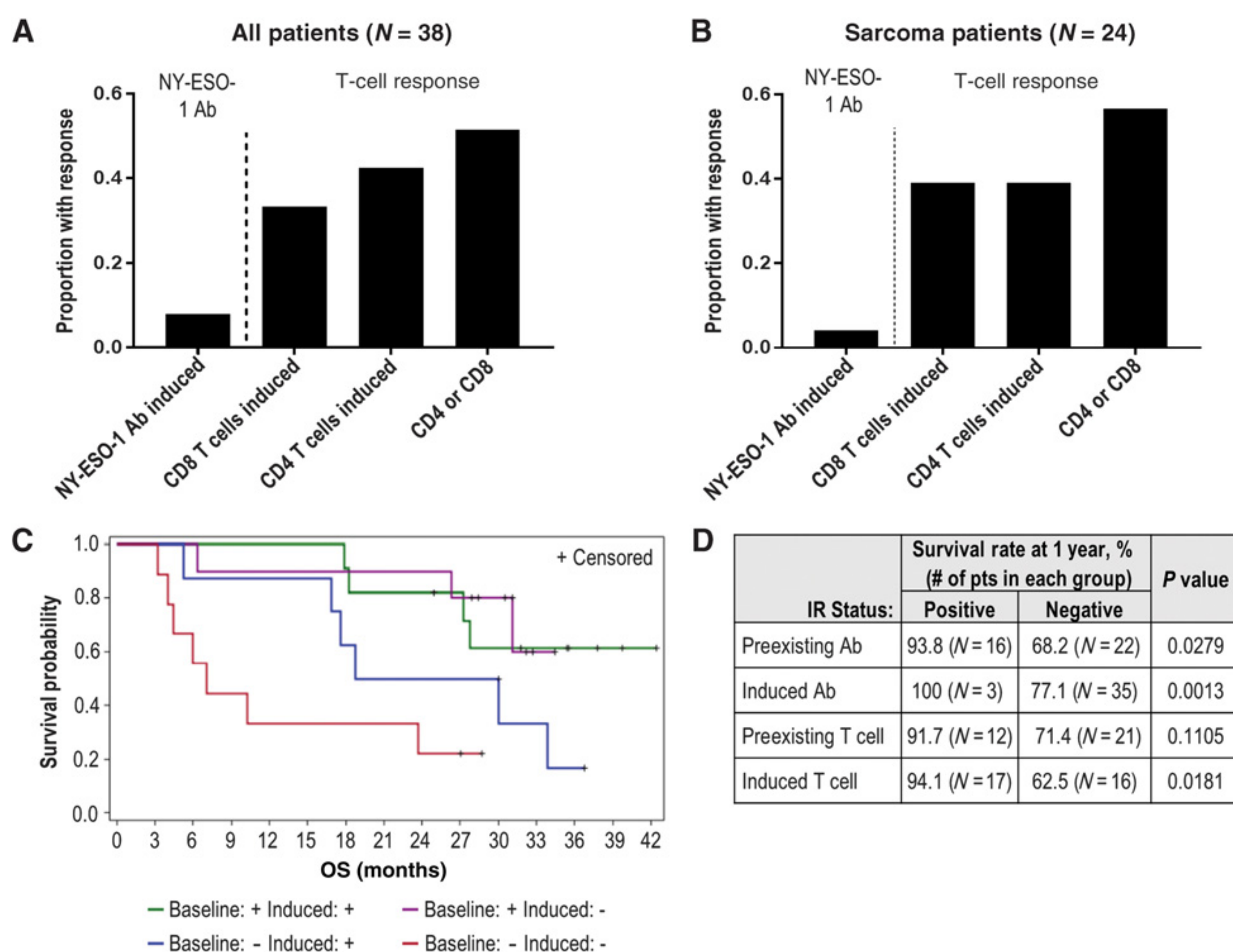
Downloaded from http://aacrjournals.org/clincancerres/article-pdf/25/19/5808/2052901/5808.pdf by guest on 25 April 2024

sarcoma patients, completed all planned therapy, and no patients discontinued due to AE.

Seventy-four percent of patients had at least 1 evaluable post-treatment assessment for the persistence of the LV305 genome; 49% had 2 or more assessments, 38% were tested at approximately 12 months, and 26% were tested ≥22 months after baseline (including 2 patients at >3 years). The presence of LV305 genome was not detected in any patient at any timepoint on study.

Immune responses

The presence of anti–NY-ESO-1 T-cell responses was evaluable in 85% of patients, and the presence of anti–NY-ESO-1 antibodies in plasma was evaluable in 97%. Preexisting anti–NY-ESO-1 T-cell responses were detected at baseline in 36% of patients (39% of sarcoma patients), and anti–NY-ESO-1 antibodies were present at baseline in 42% of patients (39% of sarcoma patients). Figure 1A summarizes LV305-induced IR in all patients, and Fig. 1B summarizes LV305-induced IR in all sarcoma patients. LV305-induced $CD4^+$ or $CD8^+$ T cells were detected by ELISPOT and/or ICS in 52% of patients (57% of sarcoma patients) at ≥1 timepoint on study, and 18% at ≥2 timepoints. The survival rate at 1 year was 94.1% in patients with induction of anti–NY-ESO-1 T cells versus 62.5% in those without T-cell induction ($P = 0.0181$), and 91.7% in patients with preexisting anti–NY-ESO-1 T-cells versus 71.4% in those without ($P = 0.1105$; Fig. 1C and D). LV305-induced anti–NY-ESO-1 antibodies were detected by ELISA in 8% of patients (4% of sarcoma patients). The survival rate at 1 year was 100% in patients with induction of anti–NY-ESO-1 antibody versus 77.1% in those without antibody induction ($P = 0.0013$), and 93.8% in patients with preexisting anti–NY-ESO-1 antibody at



IR Status:	Survival rate at 1 year, % (# of pts in each group) Positive	Negative	P value
Preexisting Ab	93.8 (N = 16)	68.2 (N = 22)	0.0279
Induced Ab	100 (N = 3)	77.1 (N = 35)	0.0013
Preexisting T cell	91.7 (N = 12)	71.4 (N = 21)	0.1105
Induced T cell	94.1 (N = 17)	62.5 (N = 16)	0.0181

Figure 1.

LV305-induced immune responses and correlation with OS. **A** and **B,** The proportion of patients with induction of anti–NY-ESO-1 immune responses are summarized in **A** for all patients with samples available for analysis ($n = 38$) and **B** for sarcoma patients ($n = 24$). **C,** One-year OS rates and P values by preexisting antibody, induced antibody, preexisting T-cell, or induced T-cell responses. Both induced antibody and T-cell responses are significantly associated with survival rate at 1 year. **D,** Kaplan–Meier curves summarizing OS by the presence or absence of baseline immunity and treatment-induced immune responses (based on antibody or T-cell positivity). Green, baseline positive, induction positive (+,+); purple, baseline positive, induction negative (+,−); blue, baseline negative, induction positive (−, +); red, baseline negative, induction negative (−, −). A significant difference in OS was observed between the +,+ and −,− groups ($P = 0.0137$) and between the +,− and −,− groups (P value = 0.0215). OS was not significantly different between the +,+ and +,− groups (P value = 0.520) or between the −, + and −, − groups (P value = 0.0215).

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/25/19/5808/2052901/5808.pdf by guest on 25 April 2024

baseline versus 68.2% in those without ($P = 0.0279$). Neither preexisting immunity (Ab or T cells) nor induced IR was associated with NY-ESO-1 expression in tumor in a logistic regression model that included immunity as the response variable and NY-ESO-1 expression as the independent variable (data not shown).

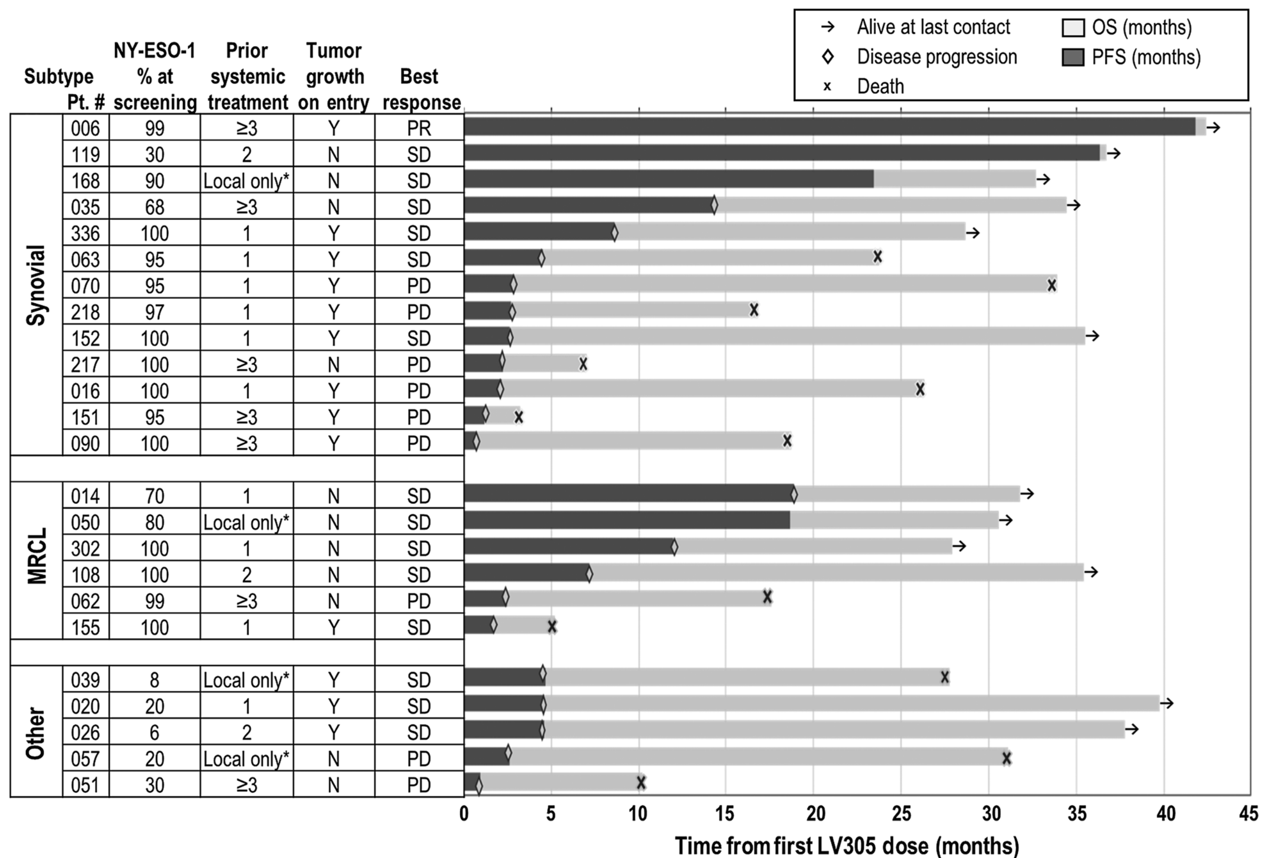
Exploratory tumor response assessments

A durable PR was observed in 1 sarcoma patient, and an unconfirmed PR was observed in 1 lung cancer patient. In the patient with lung cancer, previously undetected lesions were identified in the central nervous system (an immune sanctuary site) within 1 month of the initial observation of PR and were associated with symptomatic deterioration and discontinuation from the study. An additional 20 patients (51.3%) had SD, based on modified irRC, for a DCR of 56.4%.

Among patients with sarcoma, 1 patient (4.2%; patient no. 006) achieved PR and 14 patients (58.3%) had SD, for a DCR of 62.5% (Fig. 2). The median duration of disease control in these 15 patients was 5.9 months (95% CI, 1.8–16.8 months). Among patients with SS, the DCR was 53.8%, with a median duration of 9.8 months (95% CI, 0 months–not assessable). In sarcoma patients who had evidence of any tumor growth tumor at study entry, as determined by site investigators, 61.5% (8 of 13) achieved disease control following LV305, compared with 63.6% of patients (7 of 11) who had SD or no evidence of disease (NED) with high risk of recurrence per investigator assessment.

The sarcoma patient who achieved a PR (patient no. 006) had evidence of tumor growth at study entry after having received extensive prior therapies, including multiple tumor-related surgeries, radiation, single-agent ifosfamide, ifosfamide/adriamycin, trabectedin, and pazopanib. This patient initially had SD upon completion of all dosing of LV305 with a slow tumor regression over time resulting in a PR approximately 1.5 years after completion of LV305 dosing (16). The tumor continued to regress to a near-CR state, measuring less than 1 cm at 42 months after the first dose of LV305 (Fig. 3A). This patient did not receive any tumor-related treatment after LV305 administration. This patient had both preexisting anti–NY-ESO-1 antibodies and T cells; however, the evidence of tumor growth at time of enrollment despite extensive prior therapies indicates the preexisting anti–NY-ESO-1 immune response was inadequate to control the tumor. Following LV305 dosing, the patient developed an induced and durable anti–NY-ESO-1 IR. We have previously shown that NY-ESO-1–specific $CD4^+$ and $CD8^+$ T cells were induced in this patient (16). TCRβ repertoire

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/25/19/5808/2052901/5808.pdf by guest on 25 April 2024



Subtype	Pt. #	NY-ESO-1 % at screening	Prior systemic treatment	Tumor growth on entry	Best response
Synovial	006	99	≥3	Y	PR
	119	30	2	N	SD
	168	90	Local only*	N	SD
	035	68	≥3	N	SD
	336	100	1	Y	SD
	063	95	1	Y	SD
	070	95	1	Y	PD
	218	97	1	Y	PD
	152	100	1	Y	SD
	217	100	≥3	N	PD
	016	100	1	Y	PD
	151	95	≥3	Y	PD
	090	100	≥3	Y	PD
MRCL	014	70	1	N	SD
	050	80	Local only*	N	SD
	302	100	1	N	SD
	108	100	2	N	SD
	062	99	≥3	N	PD
	155	100	1	Y	SD
Other	039	8	Local only*	Y	SD
	020	20	1	Y	SD
	026	6	2	Y	SD
	057	20	Local only*	N	PD
	051	30	≥3	N	PD

Note: Dark gray bar shows time through last tumor assessment. The uppermost bar represents the 1 sarcoma patient who achieved a partial response.
* Local treatment includes surgery or radiotherapy.

Figure 2.
Select baseline characteristics and clinical outcome in patients with sarcoma ($N = 24$) treated with LV305.

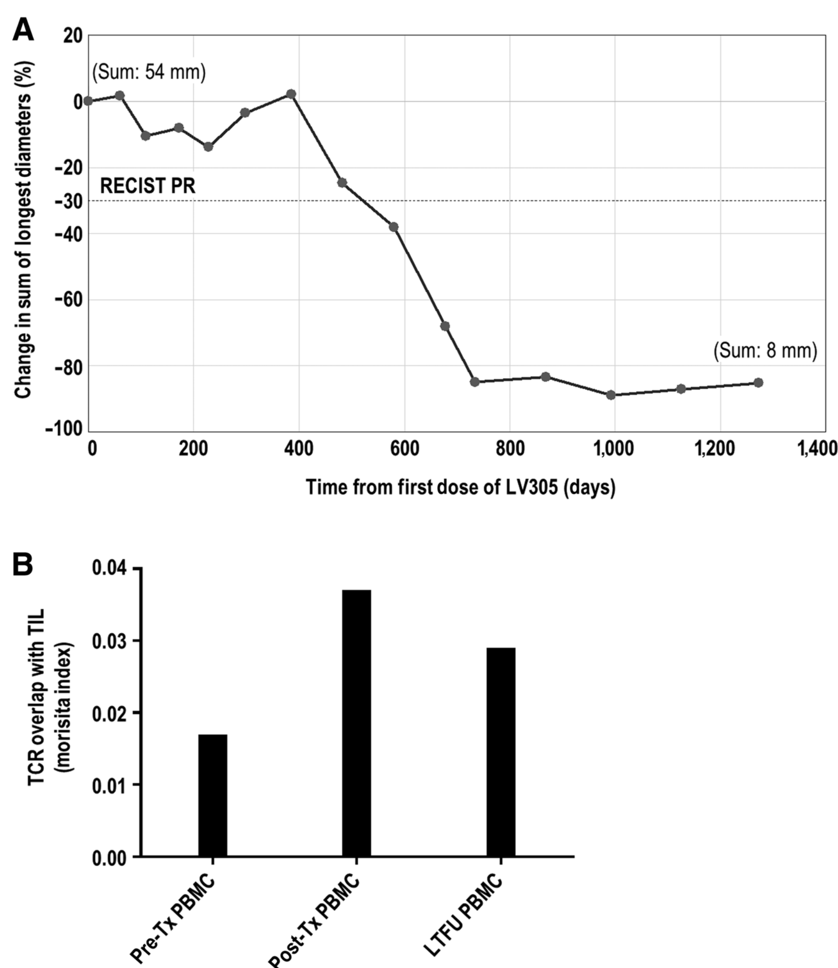


Figure 3.
Tumor regression over time and persistent immune response in a sarcoma patient treated with LV305. **A,** The percent change in tumor size (sum of longest diameters) over time in the patient with a PR. A PR was first measured approximately 1.5 years after completion of LV305 treatment, after which the tumor continued to regress to a near-CR state, measuring less than 1 cm after 42 months of follow-up (on day 1273). **B,** The results of TCRβ variable chain repertoire analysis using tumor biopsy and PBMCs collected at baseline (pre-Tx), day 84 (post-Tx), and at 2-year follow-up (LTFU) from Patient 1-1. Increased TIL sequences were detected in PBMC post-LV305 treatment, and these sequences continued to be present 2 years after treatment.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/25/19/5808/2052901/5808.pdf by guest on 25 April 2024

analysis using PBMC samples collected from this patient at baseline, day 84, and at 2-year follow-up showed increased tumor-infiltrating lymphocyte (TIL) sequences in PBMC post-LV305 dosing, and the tumor NY-ESO-1–specific sequences continued to be present 2 years after completion of LV305 dosing (Fig. 3B).

Exploratory PFS and OS analysis

The median duration of follow-up was 27.9 months (range, 3.2–42.4 months). Median PFS was 4.6 months (95% CI, 2.7–11.7 months) in all patients and 4.6 months (95% CI, 2.5–8.6 months) in patients with sarcoma (Supplementary Fig. S1A). Median PFS was 2.8 months, 9.6 months, and 4.6 months in patients with SS, MRCL, and other sarcoma subtypes, respectively (Supplementary Fig. S1B).

Median OS was 31.1 months [95% CI, 18.8 months–not available (NA)] in all patients. In patients with sarcoma, the median OS was 33.9 months (95% CI, 18.8 months–NA) with OS rates of 83.3% and 66.7% at 12 and 24 months, respectively (Supplementary Fig. S1C). OS was similar across sarcoma subtypes (Supplementary Fig. S1D).

Sarcoma patients at high risk of recurrence per investigator assessment with NED at time of enrollment ($n = 6$) had longer PFS than patients with SD ($n = 5$) or evidence of tumor growth ($n = 13$) at enrollment; median PFS was 16.6 months (95% CI, 0.9 months–NA) in patients with NED; 2.6 months (95% CI, 2.2 months–NA) in patients with SD; and 2.8 months (95% CI, 1.7–4.6 months) in those with any evidence of tumor growth. Median OS was not reached in sarcoma patients with NED at time of enrollment, and was 31.11 months (95% CI, 7.03 months–NA) in patients with SD and 27.79 months (95% CI, 16.85–NA) in those with any evidence of tumor growth.

A *post hoc* exploratory analysis revealed significantly longer OS in patients with increased T-cell clonality (higher than median post-/preclonality ratio) in posttreatment PBMC among all patients ($P = 0.0116$; Fig. 4A) and sarcoma patients ($P = 0.0184$; Fig. 4B), indicating LV305-induced clonal expansion of T-cell anti–NY-ESO-1 responses may be associated with OS.

Discussion

LV305 is the first vaccine designed to selectively induce expression of an antigen-encoding gene in DCs *in vivo*. This first-in-human study of this first-in-class agent demonstrates the potential of a LV vaccine to induce antigen expression in DCs and to promote IR and tumor responses in patients.

LV305 was administered ID in the outpatient setting and was well-tolerated with only grade 1 or 2 treatment-related AEs reported and no treatment-related SAEs. LV305 effectively

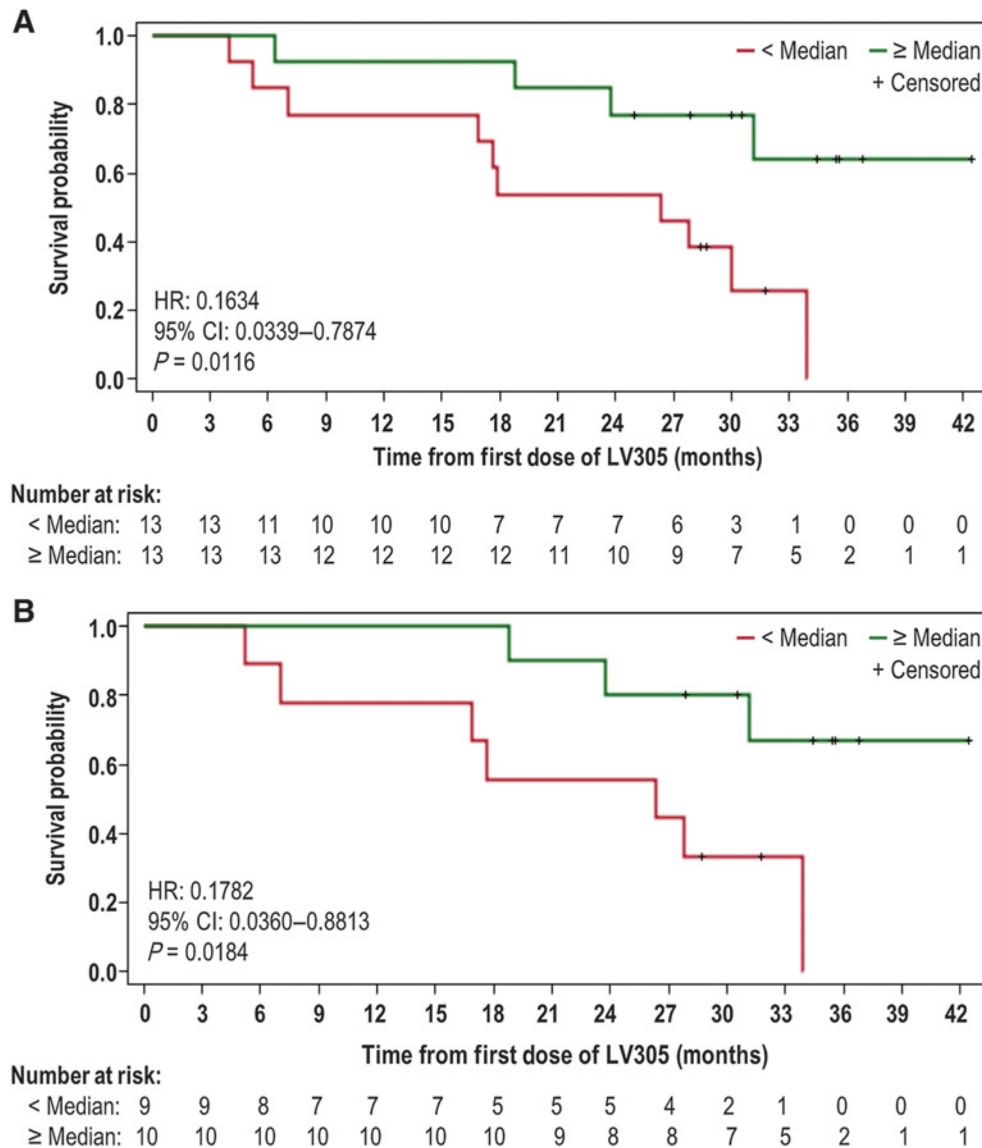


Figure 4.

OS by TCR clonality ratio in PBMCs. Kaplan–Meier analysis of OS by TCR clonality ratio (posttreatment/pretreatment) is presented for all patients with samples available for analysis ($n = 26$; **A**) and patients with sarcoma with samples available for analysis ($n = 19$; **B**) for whom a complete dataset was available. Patients with a TCR clonality ratio greater than or equal to the median are represented with green lines, and patients with a TCR clonality ratio less than the median are represented with red lines. The median clonality ratio was 1.025 (range, 0.43–4.37) among all patients and 1.050 (range, 0.43–4.37) among sarcoma patients.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/25/19/5808/2052901/5808.pdf by guest on 25 April 2024

induced an anti–NY-ESO-1 IR that was broad, tumor-specific, and durable. IR consisted of both anti–NY-ESO-1 antibody and $CD4^+$/$CD8^+$ T-cell responses, as measured by cell-based and molecular assays, with measurable responses ongoing as long as 2+ years after treatment. LV305 was associated with increases of preexisting immune responses to NY-ESO-1 as well as *de novo* IR, suggesting LV305 may act not only to prime, but also to boost IR. LV305 administration resulted in a greater induction of T-cell responses than antibody responses, which is not unexpected as NY-ESO-1 is expressed in relatively low amounts in DC and presumably not secreted.

The data indicate that LV305-induced anti–NY-ESO-1 IR can lead to tumor regression over time, as evident in one patient with synovial sarcoma who achieved a near-CR. In this patient, a relatively high level of preexisting T cells was boosted, with the durability of this T-cell response measured for over 2 years. Although no objective responses were observed in the other patients, 62.5% of all sarcoma patients and 61.5% of sarcoma patients with growing tumor at study entry maintained or achieved SD. These data suggest that the main mechanism of action of LV305 in this patient population may be slowing of tumor growth. A similar observation has been made with the *ex vivo*–generated DC-based prostate cancer vaccine sipuleucel-T, which also expresses a single tumor antigen (23).

In addition, in exploratory analyses, the induction of an anti–NY-ESO-1 response following LV305 (either a *de novo* response or

an increase in a preexisting response) was associated with improved OS. Increased clonality (clonal expansion of antitumor T cells as measured by TCR beta CDR3 sequencing) also appeared to be associated with improved outcomes. This molecular evidence for a T-cell–mediated effect of LV305 on the clinical course of the disease warrants further study.

The induction of anti–NY-ESO-1 $CD8^+$ T-cell IR can take several months to occur, and bulky tumors can be immunosuppressive, which is the reason enrollment to this study was limited to patients with low or moderate tumor burden. The favorable clinical outcomes of this group indicate that single-agent LV305 may have an optimal effect when given as a maintenance therapy. Alternatively, for patients with a larger, bulky tumor burden, LV305 may prove to play a role in combination immunotherapy strategies. Further investigation of LV305 as a solid tumor immunotherapy is merited, possibly in combination with a boosting vaccine and/or other agents, such as anti–PD-L1 checkpoint inhibitors. Further development of LV305 is planned in combination with G305, as the combination therapy "CMB305." G305 is a cancer vaccine composed of a full-length recombinant NY-ESO-1 protein and the synthetic TLR4 agonist glucopyranosyl lipid A in stable emulsion (GLA-SE; ref. 24). LV305 and G305 have different but complementary mechanisms of action, and in preclinical models, priming DCs with LV305 and then boosting with G305 significantly increased NY-ESO-1–directed antitumor activity. Studies of CMB305 alone or in combination the anti–PD-L1 checkpoint inhibitor atezolizumab are ongoing (NCT02609984, NCT02387125). Additional opportunities for further development of LV305 continue to be explored.

Although limited by the exploratory nature and the small number of patients, the favorable safety profile, promising tumor outcomes, and durable IR observed in this first-in-class, first-in-human study demonstrate the feasibility and potential therapeutic impact of direct injection of a DC tropic LV vector into patients as a cancer vaccine.

Disclosure of Potential Conflicts of Interest

N. Somaiah was a consultant/advisory board member for Immune Design at the time of this study. M.S. Block reports receiving commercial research grants from Immune Design, Marker Therapeutics, and Merck. J.W. Kim reports receiving commercial research grants from Immune Design, and is a consultant/advisory board member for Dendreon, AstraZeneca, and, Clovis. G.I. Shapiro reports receiving commercial research grants from Lilly, Merck EMD-Serono, Sierra Oncology, and Merck, and is a consultant/advisory board member for Pfizer, Lilly, G1 Therapeutics, Astex, Almac, Roche, Bicycle Therapeutics, Merck-EMD Serono, Fusion Pharmaceuticals, Sierra Oncology, Bayer, Ipsen, Cybrexa Therapeutics, Angiex, Daiichi Sankyo, and Seattle Genetics. K.T. Do is a consultant/advisory board member for Seattle Genetics. P. Hwu holds ownership interest (including patents) in Dragonfly and Immatics, and is a consultant/advisory board member for Dragonfly, GlaxoSmithKline, Immatics, and Sanofi. R.L. Jones is a consultant/advisory board member for Adaptimmune, Blueprint, Clinigen, Eisai, Epizyme, Daichii, Deciphera, Immune Design, Lilly, Merck, Pharmamar, and Tracon. J.H. ter Meulen and F.J. Hsu hold ownership interest (including patents) in Immune Design. S. Gnjatic reports receiving commercial research grants from Immune Design, Pfizer, Genentech, and Bristol-Myers Squibb, and is a consultant/advisory board member for Merck and OncoMed. S.M. Pollack reports receiving other commercial research support from Immune Design. H. Lu, J.H. ter Meulen, C. Bohac, M. Chen, and F.J. Hsu were employees of Immune Design during the study. J.H. ter Meulen and F.J. Hsu hold ownership interest (including patents) in Immune Design. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: N. Somaiah, P. Hwu, R.L. Jones, F.J. Hsu, S.M. Pollack
Development of methodology: N. Somaiah, R.L. Jones, H. Lu, F.J. Hsu, S. Gnjatic, S.M. Pollack
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): N. Somaiah, M.S. Block, J.W. Kim, G.I. Shapiro, K.T. Do, J.P. Eder, R.L. Jones, H. Lu, S. Gnjatic, S.M. Pollack
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.W. Kim, G.I. Shapiro, K.T. Do, P. Hwu, J.P. Eder, R.L. Jones, H. Lu, J.H. ter Meulen, C. Bohac, M. Chen, F.J. Hsu, S. Gnjatic, S.M. Pollack
Writing, review, and/or revision of the manuscript: N. Somaiah, J.W. Kim, G.I. Shapiro, K.T. Do, P. Hwu, J.P. Eder, R.L. Jones, H. Lu, J.H. ter Meulen, C. Bohac, F.J. Hsu, S. Gnjatic, S.M. Pollack
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): N. Somaiah, R.L. Jones, C. Bohac, F.J. Hsu, S. Gnjatic
Study supervision: N. Somaiah, R.L. Jones, C. Bohac, F.J. Hsu

Acknowledgments

Authors wish to thank Seunghee Kim-Schulze, Kevin Tuballes, and Mahlet Yishak for technical assistance, and Janis Leonoudakis, PhD, for medical writing assistance. S. Gnjatic acknowledges Mount Sinai's Cancer Center Support Grant P30CA196521. This study was funded by Immune Design.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received March 29, 2019; revised May 28, 2019; accepted June 19, 2019; published first June 21, 2019.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/25/19/5808/2052901/5808.pdf by guest on 25 April 2024

References

1. Constantino J, Gomes C, Falcão A, Neves BM, Cruz MT. Dendritic cell-based immunotherapy: a basic review and recent advances. Immuno Res 2017;65:798–810.
2. Zinkernagel RM. On the role of dendritic cells versus other cells in inducing protective CD8+ T cell responses. Front Immunol 2014;5:30.
3. Liu MA. Immunologic basis of vaccine vectors. Immunity 2010;33:504–15.
4. Breckpot K, Escors D, Arce F, Lopes L, Karwacz K, Van Lint S, et al. HIV-1 lentiviral vector immunogenicity is mediated by Toll-like receptor 3 (TLR3) and TLR7. J Virol 2010;84:5627–36.
5. Tareen SU, Kelley-Clarke B, Nicolai CJ, Cassiano LA, Nelson LT, Slough MM, et al. Design of a novel integration-deficient lentivector technology that incorporates genetic and posttranslational elements to target human dendritic cells. Mol Ther 2014;22:575–87.
6. Odegard JM, Kelley-Clarke B, Tareen SU, Campbell DJ, Flynn PA, Nicolai CJ, et al. Virological and preclinical characterization of a dendritic cell targeting, integration-deficient lentiviral vector for cancer immunotherapy. J Immunother 2015;38:41–53.
7. Albershardt TC, Campbell DJ, Parsons AJ, Slough MM, ter Meulen J, Berglund P. LV305, a dendritic cell-targeting integration-deficient ZVexTM-based lentiviral vector encoding NY-ESO-1, induces potent anti-tumor immune response. Mol Ther Oncolytics 2016;3:16010.
8. Jungbluth AA, Antonescu CR, Busam KJ, Iversen K, Kolb D, Coplan K, et al. Monophasic and biphasic synovial sarcomas abundantly express cancer/testis antigen NY-ESO-1 but not MAGE-A1 or CT7. Int J Cancer 2001;94:252–6.
9. Pollack SM, Jungbluth AA, Hoch BL, Farrar EA, Bleakley M, Schneider DJ, et al. NY-ESO-1 is a ubiquitous immunotherapeutic target antigen for patients with myxoid/round cell liposarcoma. Cancer 2012;118:4564–70.
10. CT Database. Published data of protein expression in neoplasias for CTAG1B (NY-ESO-1) [Internet]. Available from: http://www.cta.lncc.br/modelo.php?idgene=101&idmeta=15
11. Park TS, Groh EM, Patel K, Kerkar SP, Lee CCR, Rosenberg SA. Expression of MAGE-A and NY-ESO-1 in primary and metastatic cancers. J Immunother 2016;39:1–7.

12. Thomas R, Al-Khadairi G, Roelands J, Hendrickx W, Dermime S, Bedognetti D, et al. NY-ESO-1 based immunotherapy of cancer : current perspectives. Front Immunol 2018;9:947.
13. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbé C, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: Immune-related response criteria. Clin Cancer Res 2009;15:7412–20.
14. Nishino M, Giobbie-Hurder A, Gargano M, Suda M, Ramaiya NH, Hodi FS. Developing a common language for tumor response to immunotherapy: Immune-related response criteria using unidimensional measurements. Clin Cancer Res 2013;19:3936–43.
15. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228–47.
16. Pollack SM, Lu H, Gnjatic S, Somaiah N, O'Malley RB, Jones RL, et al. First-in-human treatment with a dendritic cell-targeting lentiviral vector-expressing NY-ESO-1, LV305, induces deep, durable response in refractory metastatic synovial sarcoma patient. J Immunother 2017;40:302–6.
17. Robins HS, Campregher P V, Srivastava SK, Wacher A, Turtle CJ, Kahsai O, et al. Comprehensive assessment of T-cell receptor beta-chain diversity in alphabeta T cells. Blood 2009;114:4099–107.
18. Carlson CS, Emerson RO, Sherwood AM, Desmarais C, Chung MW, Parsons JM, et al. Using synthetic templates to design an unbiased multiplex PCR assay. Nat Commun 2013;4:2680.
19. Robins H, Desmarais C, Matthis J, Livingston R, Andriesen J, Reijonen H, et al. Ultra-sensitive detection of rare T cell clones. J Immunol Methods 2012;375:14–9.
20. Kirsch I, Vignali M, Robins H. T-cell receptor profiling in cancer. Mol Oncol 2015;9:2063–70.
21. Good IJ, Toulmin GH. The number of new species, and the increase in population coverage, when a sample is increased. Biometrika 1956;43: 45–63.
22. Daley T, Smith AD. Predicting the molecular complexity of sequencing libraries. Nat Methods 2013;10:325–7.
23. Sims RB. Development of sipuleucel-T : autologous cellular immunotherapy for the treatment of metastatic castrate resistant prostate cancer. Vaccine 2012;30:4394–7.
24. Mahipal A, Ejadi S, Gnjatic S, Kim-Schulze S, Lu H, Ter Meulen JH, et al. First-in-human phase 1 dose-escalating trial of G305 in patients with advanced solid tumors expressing NY-ESO-1. Cancer Immunol Immunother 2019;68:1211–22.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/25/19/5808/2052901/5808.pdf by guest on 25 April 2024