Efficacy and safety of lifileucel, a one-time autologous tumor-infiltrating lymphocyte (TIL) cell therapy, in patients with advanced melanoma after progression on immune checkpoint inhibitors and targeted therapies: pooled analysis of consecutive cohorts of the C-144-01 study

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ABSTRACT

Background Patients with advanced melanoma have limited treatment options after progression on immune checkpoint inhibitors (ICI). Lifileucel, a one-time autologous tumor-infiltrating lymphocyte (TIL) cell therapy, demonstrated an investigator-assessed objective response rate (ORR) of 36% in 66 patients who progressed after ICI and targeted therapy. Herein, we report independent review committee (IRC)-assessed outcomes of 153 patients treated with lifileucel in a large multicenter Phase 2 cell therapy trial in melanoma.

Methods Eligible patients had advanced melanoma that progressed after ICI and targeted therapy, where appropriate. Melanoma lesions were resected (resected tumor diameter ≥1.5 cm) and shipped to a central good manufacturing practice facility for 22-day lifileucel manufacturing. Patients received a non-myeloablative lymphodepletion regimen, a single lifileucel infusion, and up to six doses of high-dose interleukin-2. The primary endpoint was IRC-assessed ORR (Response Evaluation Criteria in Solid Tumors V.1.1).

Results The Full Analysis Set consisted of 153 patients treated with lifileucel, including longer-term follow-up on the 66 patients previously reported. Patients had received a median of 3.0 lines of prior therapy (81.7% received both anti-programmed cell death protein 1 and anti-cytotoxic lymphocyte-associated protein 4) and had high disease burden at baseline (median target lesion sum of diameters (SOD): 97.8 mm; lactate dehydrogenase (LDH) > upper limit of normal: 54.2%). ORR was 31.4% (95% CI: 24.1% to 39.4%), with 8 complete responses and 40 partial responses. Median duration of response was not reached at a median study follow-up of 27.6 months, with 41% of the responses maintained for ≥18 months. Median overall survival and progression-free survival were 13.9 and 4.1 months, respectively. Multivariable analyses adjusted for Eastern Cooperative Oncology Group performance status demonstrated that elevated LDH and target lesion SOD

WHAT IS ALREADY KNOWN ON THIS TOPIC

Adaptive cell therapy with tumor-infiltrating lymphocytes (TIL) is a promising antitumor therapy being investigated for patients with advanced solid tumors. Lifileucel, an investigational TIL cell therapy, has previously shown an investigator-assessed objective response rate (ORR) of 36% and median duration of response (DOR) not reached at a median follow-up of 33.1 months in 66 patients with advanced melanoma progressing after immune checkpoint inhibitors (ICI) and, if BRAF V600 mutation-positive, BRAF/MEK inhibitors.

WHAT THIS STUDY ADDS

This analysis reports clinically meaningful and durable activity of lifileucel, a novel one-time autologous TIL cell therapy, in the largest prospective, multicenter Phase 2 study in a population of patients with advanced melanoma in the post-ICI setting. Data from 153 lifileucel-treated patients, combining the previously reported 66 patients from Cohort 2 with the 87 previously unreported patients from Cohort 4, demonstrate a 31.4% ORR and median DOR not reached at a median study follow-up of 27.6 months.
The efficacy and safety of lifileucel, a new immunotherapy treatment option, combined with its short 22-day manufacturing duration, address an important unmet need in this patient population with traditionally difficult-to-treat disease who lack effective or approved treatment options in the post-ICI setting.

Background
An estimated 325,000 patients were diagnosed with melanoma worldwide in 2020, with approximately 57,000 deaths. Immune checkpoint inhibitors (ICI) and targeted therapies have revolutionized the treatment of advanced melanoma in the last decade; however, a substantial proportion of patients do not respond or eventually relapse, and treatment options are limited after progression. Many patients who receive first-line single or combination ICI progress by 12–18 months. Primary resistance to ICI is seen in 40%–65% of the patients and acquired resistance in 30%–40%. Moreover, occurrence of immune-related adverse events (irAEs) and subsequent discontinuation of ICI therapy is a clinical challenge.

Conclusions
Investigational lifileucel demonstrated clinically meaningful activity in heavily pretreated patients with advanced melanoma and high tumor burden. Durable responses and a favorable safety profile support the potential benefit of one-time lifileucel TIL cell therapy in patients with limited treatment options in ICI-refractory disease.

Open access
The efficacy and safety of lifileucel, a new immunotherapy treatment option, combined with its short 22-day manufacturing duration, address an important unmet need in this patient population with traditionally difficult-to-treat disease who lack effective or approved treatment options in the post-ICI setting.

How this study might affect research, practice or policy
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Together, these data demonstrate lifileucel efficacy and safety in the largest prospectively enrolled Phase 2 study in a population of patients with advanced melanoma in the post-ICI setting.

METHODS

Patients and study design

The C-144-01 trial (NCT02360579) is a prospective, Phase 2, multicohort, multicenter study in patients with advanced melanoma. Cohort 1 of the trial included patients receiving non-cryopreserved TIL generated using a manufacturing process different from that for lifileucel; patients in Cohort 2 and registrational Cohort 4 received cryopreserved lifileucel. Cohort 3 included patients from Cohorts 1, 2, and 4 who were retreated with lifileucel (online supplemental figure 1). This analysis focuses on sequentially enrolled Cohort 2 (April 2017 to January 2019) and Cohort 4 (February 2019 to December 2019).

Patients were ≥18 years of age with unresectable or metastatic melanoma (stage IIIC or stage IV) per the American Joint Committee on Cancer V.7.46 Patients had documented radiologic disease progression and must have progressed following ≥1 prior systemic therapy including a PD-1-blocking antibody, and if BRAF V600 mutation-positive, a BRAF or BRAF/MEK inhibitor (patients who were either primary or secondary refractory to prior anti-PD-1/programmed death ligand-1 (PD-L1) therapy were eligible); Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1; an estimated life expectancy of ≥3 months; adequate hematologic parameters and organ function; and ≥1 resectable lesion (or aggregate of lesions) providing resected tumor tissue ≥1.5 cm in diameter to generate lifileucel and ≥1 remaining measurable target lesion, as defined by Response Evaluation Criteria in Solid Tumors (RECIST) V.1.1. Patients with organ allograft or prior cell transfer therapy, uveal/ocular melanoma, hypersensitivity to lifileucel or other study drugs, symptomatic and/or untreated brain metastases, chronic systemic steroid therapy, active systemic infections, administration of live or attenuated vaccine within 28 days of non-myeloablative lymphodepletion (NMA-LD), and chronic heart or lung abnormality characterized by left ventricular ejection fraction <45% and forced expiratory volume in 1 s of ≤60% of the predicted value were excluded. Eligibility criteria were the same for Cohorts 2 and 4.33

The study was approved by the Institutional Review Board (IRB) at each site and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines of the International Conference on Harmonization. The Strengthening the Reporting of Observational Studies in Epidemiology cohort reporting guidelines were used to ensure the quality of data reported in this study.47

Lifileucel manufacturing and infusion

Eligible patients underwent resection of a tumor(s) ≥1.5 cm to ≤4.0 cm in aggregate diameter, which was subsequently prospected (ie, trimmed and fragmented) and shipped to a centralized good manufacturing practice (GMP) facility to initiate lifileucel manufacturing. The 22-day GMP manufacturing process that results in a cryopreserved TIL infusion product has been previously described.33 The treatment regimen, consisting of NMA-LD (cyclophosphamide and fludarabine) followed by a single lifileucel infusion and an abbreviated course of high-dose interleukin-2 (IL-2), was administered as described previously.33 No bridging therapy was permitted between tumor resection and lifileucel infusion. The lifileucel manufacturing process and treatment regimen were the same for Cohorts 2 and 4.

Study endpoints and assessments

The original primary endpoint for Cohort 2 was investigator-assessed ORR.33 Cohort 4 was initiated as a single-arm registrational cohort with a prospectively defined primary endpoint of ORR by an independent review committee (IRC); accordingly, the primary endpoint of Cohort 2 was amended to IRC-assessed ORR. Pooled analysis for Cohorts 2 and 4 was not the pre-planned primary analysis; however, it was determined that this analysis would be of value given the identical eligibility, lifileucel manufacturing process, treatment regimen, and central response assessment for the two cohorts. Concordance between investigator-assessed and IRC-assessed ORR was analyzed. The secondary endpoints included DOR, OS, PFS, and safety as assessed by incidence rates, severity, seriousness, relationship to study treatment, and characteristics of treatment-emergent adverse events (TEAEs, defined as any AE with onset after lifileucel infusion through day 30 post-infusion).

Following the end-of-treatment visit, subsequent efficacy assessments occurred every 6 weeks (±3 days) until month 6 (week 24), and then every 3 months (12 weeks) for up to 5 years or until disease progression or start of new anticaner therapy. Survival assessment was conducted every 3 months via phone to obtain survival status and subsequent anticaner therapy information for up to 5 years or death, whichever occurred earlier. AE and serious AE data were graded as per the Common Terminology Criteria for Adverse Events V.4.03.

‘Primary refractory’ to anti-PD-1/PD-L1 therapy was defined as best response of progressive disease (PD) to prior anti-PD-1/PD-L1; the first anti-PD-1/PD-L1 with documented response was considered if multiple anti-PD-1/PD-L1 therapies were received. In a post hoc analysis, we also applied the Society for Immunotherapy of Cancer (SITC) Immunotherapy Resistance Taskforce criteria for ‘primary resistance.’48 Data were analyzed separately for each cohort as specified in the protocol and as a pooled analysis of the two cohorts to allow for subgroup analyses given identical eligibility, lifileucel manufacturing process, treatment regimen, and central response assessment.
Statistical analysis

Based on estimation of ORR using the maximum half width of the two-sided 95% CI of <13.2% when ORR is expected to be 20%–50%, the planned sample size of Cohort 2 was 66 patients. The hypothesis testing for the primary endpoint of ORR in Cohort 4 as assessed by the IRC was prospectively defined as the null hypothesis of ORR ≤10% and alternative hypothesis of ORR >10%. The planned sample size for Cohort 4 was 75 patients, which would provide >90% power to demonstrate statistical significance at a two-sided overall significance level of 0.05 using the exact test and assuming that the true response rate for TIL cell therapy in this population was 25%. The IRC-assessed ORR was analyzed as a binomial proportion with two-sided confidence limits based on the Clopper-Pearson exact method at an overall alpha of 0.05. Time-to-event efficacy endpoints were estimated using Kaplan-Meier product limit method. Assessment of safety data was descriptive. Univariable logistic regression models were used to estimate odds ratios (ORs) with 95% CIs to assess the potential relationship between patient subgroups and ORR. Variables identified from the univariable analysis were examined using the best subset approach to identify independent predictors of ORR of lifileucel using a multivariable logistic regression model. All statistical analyses were conducted using the Statistical Analysis System (SAS) V.9.4.33.

RESULTS

Patients and treatment

Of 189 enrolled patients in the two cohorts, 156 received lifileucel TIL cell therapy infusion and formed the Safety Analysis Set (online supplemental figure 2); 25 patients did not receive lifileucel for patient-related reasons (PD (n=9, 4.8%), death (n=5, 2.6%), AE (n=3, 1.6%), new anticancer treatment (n=2, 1.1%), withdrawal of consent (n=1, 0.5%), withdrawal by patient (n=1, 0.5%), and other reason (n=4, 2.1%)), whereas lifileucel was not available for infusion for 8 patients (4.2%). The Full Analysis Set consisted of 153 patients (Cohort 2, N=66; Cohort 4, N=87) who were treated with 1×10⁹–150×10⁹ TIL cells that met the manufacturing product specification.

Baseline patient demographic and clinical characteristics are summarized in table 1. Most patients had cutaneous melanoma (n=83 (54.2%)); a minority had mucosal (n=12 (7.8%)) or acral (n=10 (6.5%)), and the remainder had either unknown primary or insufficient information (n=47 (30.7%)). At baseline, the median target lesion sum of diameters (SOD) was 97.8 mm, proportion of patients with >3 target and non-target lesions was 75.8%, and proportion of patients with lesions in ≥3 anatomic sites was 71.2%. Baseline liver and/or brain metastasis was reported for 47.1% of the patients. Elevated baseline lactate dehydrogenase (LDH) levels were observed in 54.2% of the patients, with LDH levels 1–2×upper limit of normal (ULN) in 35.3% and >2×ULN in 19.0% of the patients. Patients had received a median of 3.0 prior lines of therapy (range, 1–9). All patients had received prior anti-PD-1/PD-L1 therapy; 81.7% of the patients had also received anti-CTLA-4 therapy, and 53.6% had received combination anti-PD-1/anti-CTLA-4 therapy; 25.0% of the patients had received BRAF/MEK inhibitors. More than half of the patients (n=83 (54.2%)) were considered primary refractory to prior anti-PD-1/PD-L1 therapy.

The median cumulative duration of anti-PD-1/PD-L1 therapy before lifileucel was 7 months. ICI retreatment was common; 73.9% (n=113) of patients were retreated with ICI and received a median of 2.0 lines (range, 1–7) of ICI-containing therapy (online supplemental figure 3). The anatomic site of tumor resection for TIL manufacturing was lymph node/skin/subcutaneous (46.4%), visceral organ (27.5%), and other (26.1%; including muscle, soft tissue, bone, limb/extremity, and others). Of the 189 patients who had tumor resected, lifileucel manufacturing was terminated for two early patient withdrawals; of the remaining 187 patients, lifileucel was manufactured in 179 (95.7%; n=8, lifileucel not available) and within specification in 177 (94.7%). The median time from resection to lifileucel infusion was 33.0 days. The median number of TIL cells infused was 21.1×10⁹ (range, 1.2×10⁹–99.5×10⁹). The median number of IL-2 doses administered was 6.0 (range, 0–6).

Efficacy

ORR and DOR

As of September 15, 2021, ORR as assessed by IRC was 31.4% (95% CI: 24.1% to 39.4%), with 8 complete responses (CRs) and 40 partial responses (PRs) (ORR for Cohort 2: 34.8% (95% CI: 23.5% to 47.6%); Cohort 4: 28.7% (95% CI: 19.5% to 39.4%); table 2). The concordance rate of IRC-assessed and investigator-assessed ORR for the pooled cohorts was 90.8% (Cohen’s Kappa coefficient 0.8 (95% CI: 0.7 to 0.9; p<0.0001). Of the 140 patients evaluable for changes of target lesion SOD, 78.6% (110/140) had SOD reduction (figure 1A). Median time from lifileucel infusion to best response was 1.5 months (range, 1.3–29.6 months; figure 1B). Thirty-nine (81.3%) responders had achieved an IRC-assessed response at the time of first response assessment. Six patients (12.5%) who were initially assessed as a PR achieved confirmed CR, and 10 patients (20.8%) improved from stable disease (SD) to PR. Response to lifileucel was observed across all subgroups analyzed (figure 1C), regardless of age, prior anti-CTLA-4 use, BRAF/mutation status, PD-L1 status, and mucosal/non-mucosal subsets. In multivariable analyses adjusted for COG PS, elevated LDH and target lesion SOD >median were independently correlated with ORR (p=0.008); patients with normal LDH and SOD median had greater likelihood of response than those with either (OR: 2.08) or both (OR: 4.42) of these risk factors.

Median DOR was NR (95% CI: 8.3 months to NR) at a median study follow-up of 27.6 months (Cohort 2: NR (95% CI: NR to NR); Cohort 4: 10.4 months (95% CI: 4.1 to NR); figure 2A); 41.7% of the patients had responses
## Table 1  
Patient demographics and baseline characteristics of Cohorts 2, 4, and pooled Cohorts 2 and 4 (Full Analysis Set)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cohort 2 (n=66)</th>
<th>Cohort 4 (n=87)</th>
<th>Cohort 2+4 (N=153)</th>
</tr>
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<tbody>
<tr>
<td>Median age, years (range)</td>
<td>55.0 (20–79)</td>
<td>58.0 (25–74)</td>
<td>56.0 (20–79)</td>
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<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>39 (59.1)</td>
<td>44 (50.6)</td>
<td>83 (54.2)</td>
</tr>
<tr>
<td>Female</td>
<td>27 (40.9)</td>
<td>43 (49.4)</td>
<td>70 (45.8)</td>
</tr>
<tr>
<td>Screening ECOG performance status, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>42 (63.6)</td>
<td>62 (71.3)</td>
<td>104 (68.0)</td>
</tr>
<tr>
<td>1</td>
<td>24 (36.4)</td>
<td>25 (28.7)</td>
<td>49 (32.0)</td>
</tr>
<tr>
<td>Melanoma subtype,* n (%)</td>
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<td></td>
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<tr>
<td>Cutaneous</td>
<td>39 (59.1)</td>
<td>44 (50.6)</td>
<td>83 (54.2)</td>
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<tr>
<td>Mucosal</td>
<td>4 (6.1)</td>
<td>8 (9.2)</td>
<td>12 (7.8)</td>
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<tr>
<td>Acral</td>
<td>4 (6.1)</td>
<td>6 (6.9)</td>
<td>10 (6.5)</td>
</tr>
<tr>
<td>BRAF V600-mutated, n (%)</td>
<td>17 (25.8)</td>
<td>24 (27.6)</td>
<td>41 (26.8)</td>
</tr>
<tr>
<td>PD-L1 status,† n (%)</td>
<td></td>
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<tr>
<td>TPS ≥1%</td>
<td>37 (56.1)</td>
<td>39 (44.8)</td>
<td>76 (49.7)</td>
</tr>
<tr>
<td>TPS &lt;1%</td>
<td>12 (18.2)</td>
<td>20 (23.0)</td>
<td>32 (20.9)</td>
</tr>
<tr>
<td>Melanoma stage at study entry, n (%)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>IIIC</td>
<td>9 (13.6)</td>
<td>1 (1.1)</td>
<td>10 (6.5)</td>
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<tr>
<td>IV</td>
<td>57 (86.4)</td>
<td>86 (98.9)</td>
<td>143 (93.5)</td>
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<tr>
<td>Liver and/or brain lesions by IRC, n (%)</td>
<td>28 (42.4)</td>
<td>44 (50.6)</td>
<td>72 (47.1)</td>
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<tr>
<td>Median target lesion SOD (range), mm</td>
<td>95.8 (13.5–271.3)</td>
<td>99.5 (15.7–552.9)</td>
<td>97.8 (13.5–552.9)</td>
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<tr>
<td>Baseline lesions in ≥3 anatomic sites, n (%)</td>
<td>44 (66.7)</td>
<td>65 (74.7)</td>
<td>109 (71.2)</td>
</tr>
<tr>
<td>Baseline target and non-target lesions,‡ n (%)</td>
<td>22 (33.3)</td>
<td>14 (16.1)</td>
<td>36 (23.5)</td>
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<tr>
<td>&gt;3</td>
<td>43 (65.2)</td>
<td>73 (83.9)</td>
<td>116 (75.8)</td>
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<td>LDH, n (%)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>≤ULN</td>
<td>39 (59.1)</td>
<td>31 (35.6)</td>
<td>70 (45.8)</td>
</tr>
<tr>
<td>1−2×ULN</td>
<td>19 (28.8)</td>
<td>35 (40.2)</td>
<td>54 (35.3)</td>
</tr>
<tr>
<td>&gt;2×ULN</td>
<td>8 (12.1)</td>
<td>21 (24.1)</td>
<td>29 (19.0)</td>
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<tr>
<td>Prior systemic therapies, n (%)</td>
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<tr>
<td>Median number of therapies (range)</td>
<td>3.0 (1–9)</td>
<td>3.0 (1–8)</td>
<td>3.0 (1–9)</td>
</tr>
<tr>
<td>Anti-PD-1/PD-L1</td>
<td>66 (100)</td>
<td>87 (100)</td>
<td>153 (100)</td>
</tr>
<tr>
<td>Anti-CTLA-4</td>
<td>53 (80.3)</td>
<td>72 (82.8)</td>
<td>125 (81.7)</td>
</tr>
<tr>
<td>Anti-PD-1 plus anti-CTLA-4 combination</td>
<td>34 (51.5)</td>
<td>48 (55.2)</td>
<td>82 (53.6)</td>
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<tr>
<td>BRAF±MEK inhibitor</td>
<td>15 (22.7)</td>
<td>24 (27.6)</td>
<td>39 (25.5)</td>
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<td>IL-2</td>
<td>7 (10.6)</td>
<td>6 (6.9)</td>
<td>13 (8.5)</td>
</tr>
<tr>
<td>Primary refractory to anti-PD-1/PD-L1,§ n (%)</td>
<td>42 (63.6)</td>
<td>41 (47.1)</td>
<td>83 (54.2)</td>
</tr>
<tr>
<td>Median cumulative duration of anti-PD-1/PD-L1 therapy before lifileucel (range), months</td>
<td>5.1 (1.4–51.1)</td>
<td>10.0 (0.7–75.8)</td>
<td>7.0 (0.7–75.8)</td>
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<tr>
<td>Anatomic site of resection</td>
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<tr>
<td>Lymph node/skin/subcutaneous</td>
<td>28 (42.4)</td>
<td>43 (49.4)</td>
<td>71 (46.4)</td>
</tr>
<tr>
<td>Visceral organ</td>
<td>12 (18.2)</td>
<td>30 (34.5)</td>
<td>42 (27.5)</td>
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<tr>
<td>Other¶</td>
<td>26 (39.4)</td>
<td>14 (16.1)</td>
<td>40 (26.1)</td>
</tr>
</tbody>
</table>

*Forty-seven patients (30.7%) had melanoma of other subtypes (including unknown primary subtype or insufficient information).
†Forty-five patients (29.4%) in the pooled cohorts had missing PD-L1 status.
§One patient in Cohort 2 had missing data on the number of baseline target and non-target lesions.
‡Primary refractory to anti-PD-1/PD-L1 was defined as patients who had best response of progressive disease to prior anti-PD-1/PD-L1; the first anti-PD-1/PD-L1 with documented response was considered if multiple anti-PD-1/PD-L1 therapies were received.
¶Other sites of resection included muscle, soft tissue, bone, limb/extremity, and others.
CTLA-4, cytotoxic T-lymphocyte-associated protein 4; ECOG, Eastern Cooperative Oncology Group; IL-2, interleukin 2; IRC, independent review committee; LDH, lactate dehydrogenase; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1; SOD, sum of diameters; TPS, tumor proportion score; ULN, upper limit of normal.
maintained for ≥18 months, and 39.6% of the responses were ongoing at the time of data cut.

Survival
The median OS was 13.9 months (95% CI: 10.6 to 17.8) (figure 2B), and 12-month OS rate was 54.0% (95% CI: 45.6% to 61.6%). In an analysis of survival by response at 1.5 months after lifileucel infusion (first planned response evaluation), the median OS in responders was NR (95% CI: 22.5 months to NR) (online supplemental figure 4). The median PFS was 4.1 months (95% CI: 2.8 to 4.4) (figure 2C); the 12-month PFS rate was 28.3% (95% CI: 20.8% to 36.3%).

Post hoc analyses
In the subset of 83 patients who were primary refractory to prior anti-PD-1/PD-L1 therapy by study criteria, lifileucel produced an ORR of 31.3% (95% CI: 21.6% to 42.4%), with 6 CRs (7.2%) and 20 PRs (24.1%). Forty (48.2%) of these patients had best response to lifileucel of SDR non-CR/non-PD, and 13 (15.7%) had PD; four patients were non-evaluable. Median DOR was NR (95% CI: 15.1 months to NR) for this subpopulation. Using SITC Immunotherapy Resistance Taskforce criteria, 109 patients (71.2%) were considered primary resistant to prior anti-PD-1/PD-L1 therapy, with an ORR of 33.0% (95% CI: 24.3% to 42.7%) and median DOR NR (95% CI: 12.5 months to NR).

In patients who received prior anti-PD-1 and anti-CTLA-4 combination, the ORR was 26.8%, and median DOR was NR (95% CI: 4.1 months to NR). In patients who received any prior anti-CTLA-4 treatment, the ORR for lifileucel was 32.8%, and in patients who received anti-CTLA-4 therapy as last treatment prior to lifileucel, ORR was 31.8%.

There was no difference in TIL dose manufactured across anatomic sites of resection (online supplemental figure 5A). Target lesion SOD reductions were seen across the range of total TIL doses (online supplemental figure 5B).

Safety
All patients in the Safety Analysis Set (N=156) experienced ≥1 TEAE (any grade) during the course of the study. Grade 3/4 TEAEs occurring in ≥30% of the patients included thrombocytopenia (76.9%), anemia (50.0%), and febrile neutropenia (41.7%) (table 3). The TEAE profile was consistent with the underlying disease and known safety profiles of NMA-LD and IL-2 regimens and was similar between cohorts. Anaphylactic and infusion-related reactions that were reported by the investigator as related to lifileucel specifically were seen in 2 (1.3%; grade 3/4) and 6 (3.8%; grade 1/2) patients, respectively. Six deaths occurred within 30 days after infusion, four of which were attributed to AEs and two to PD. Of the four grade 5 TEAEs, three were assessed by the investigator as not related to lifileucel but related to NMA-LD and/or IL-2 (ie, pneumonia, arrhythmia, acute respiratory failure) and one as related to all components of the regimen (ie, intra-abdominal hemorrhage). Tumors were resected from diverse sites with minimal surgical morbidity; grade 3/4 tumor-resection AEs related to surgery were seen in 6 (3.2%) patients (online supplemental table 1).

Table 2 Efficacy outcomes by IRC assessment for Cohorts 2, 4, and pooled Cohorts 2 and 4 (Full Analysis Set)

<table>
<thead>
<tr>
<th>Response (RECIST V.1.1)*</th>
<th>Cohort 2 (n=66)</th>
<th>Cohort 4 (n=87)</th>
<th>Pooled Cohorts 2+4 (N=153)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR, n (%)</td>
<td>23 (34.8)</td>
<td>25 (28.7)</td>
<td>48 (31.4)</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(23.5 to 47.6)</td>
<td>(19.5 to 39.4)</td>
<td>(24.1 to 39.4)</td>
</tr>
<tr>
<td>Best overall response, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>5 (7.6)</td>
<td>3 (3.4)</td>
<td>8 (5.2)</td>
</tr>
<tr>
<td>PR</td>
<td>18 (27.3)</td>
<td>22 (25.3)</td>
<td>40 (26.1)</td>
</tr>
<tr>
<td>SD</td>
<td>24 (36.4)</td>
<td>47 (54.0)</td>
<td>71 (46.4)</td>
</tr>
<tr>
<td>Non-CR/non-PD†</td>
<td>1 (1.5)</td>
<td>0</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>PD</td>
<td>15 (22.7)</td>
<td>12 (13.8)</td>
<td>27 (17.6)</td>
</tr>
<tr>
<td>Non-evaluable‡</td>
<td>3 (4.5)</td>
<td>3 (3.4)</td>
<td>6 (3.9)</td>
</tr>
<tr>
<td>Median DOR,§ months (range)</td>
<td>NR (1.4+ to 45.0+)</td>
<td>10.4 (1.4+ to 26.3+)</td>
<td>NR (1.4+ to 45.0+)</td>
</tr>
</tbody>
</table>

*Objective response refers to patients with the best overall response of CR and PR. 95% CI for ORR was calculated using the Clopper-Pearson exact test.
†Patient did not have measurable target lesions by IRC and had best overall response of non-CR/non-PD per IRC assessment.
‡Six patients were non-evaluable for response (five due to early death; one due to new anticancer therapy).
§Based on responders and using Kaplan-Meier product-limit estimates.
¶Note: + refers to censored.
**Based on the reverse Kaplan-Meier method.
CR, complete response; DOR, duration of response; IRC, independent review committee; NR, not reached; ORR, objective response rate; PD, progressive disease; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease.
Figure 1  (Continued)
Most TEAEs were expected and manageable, and the incidence decreased rapidly over the first 2 weeks after lifileucel infusion (figure 3).

**DISCUSSION**

TIL cell therapy has emerged as a promising option to treat advanced ICI-refractory solid tumors due to the polyclonal nature of the infusion product targeting different tumor antigens, which is thought to attenuate the potential for immune escape via loss of target antigen expression by neoplastic cells. However, few prospective studies have investigated the efficacy and safety of TIL cell therapy after disease progression on ICI and targeted therapy, the current standards of care in advanced melanoma. In the current Phase 2 trial that included 153 patients enrolled in two consecutive cohorts in the ICI era, one-time lifileucel treatment using cryopreserved products demonstrated an ORR of 31.4% and median DOR was NR at a median study follow-up of 27.6 months—a marked improvement over the therapies available for patients in the post-ICI setting. Durability of responses and deepening of responses over time are consistent with ongoing antitumor activity of persisting tumor-specific TIL clones, further supporting the use of lifileucel as a novel treatment option in this patient population to address a highly unmet need.

Existing and recently approved therapies have shown limited benefit in the post-ICI setting in advanced melanoma. Studies assessing combination anti-CTLA-4 and anti-PD-1 therapy after anti-PD-1/PD-L1 therapy failure have been performed in ipilimumab-naïve patients or have included patients whose disease has progressed after adjuvant anti-PD-1 treatment only and sometimes used immune-related RECIST criteria-based responses. The patients in these studies were generally not as heavily pretreated as those in the present study, thus precluding efficacy comparisons. The recently approved combination of anti-LAG3 and anti-PD-1 antibodies (relatlimab plus nivolumab) and investigational anti-LAG3 antibody, fianlimab, in combination with cemiplimab have shown only modest response rates when administered as second-line treatment after progression on ICI therapy. Similarly modest response rates were observed in a small retrospective analysis of second-line anti-CTLA-4 therapy after progression on first-line relatlimab and nivolumab, suggesting emergence of cross-resistance to subsequent therapy after disease progression on ICI.

In the context of these recent data and the lack of data from appropriately sized prospective, multicenter, independently reviewed RECIST-based studies in this setting, an ORR of 31.4% with lifileucel, with nearly half of the responses maintained ≥18 months in heavily pretreated patients in the post-ICI and targeted therapy (where indicated) setting, is encouraging. Further, in patients who achieved a response at 1.5 months after lifileucel infusion (time of first efficacy assessment), median OS was NR, suggesting that early response to lifileucel may be predictive of long-term outcomes and may be a good indicator of sensitivity, in contrast to ICI where imaging results at a similar time point can be misleading.

In the present study, some notable differences were observed in the baseline characteristics of patients in the later-enrolled Cohort 4 compared with Cohort 2, which included a higher proportion of patients with >3 lesions, elevated LDH, and liver and/or brain metastasis. In addition, patients in Cohort 4 received nearly twice the cumulative duration of prior anti-PD-1/PD-L1 therapy. These indicators of greater disease burden and more difficult-to-treat disease in Cohort 4, many of which are well-known negative prognostic factors for response and survival in patients with advanced melanoma treated with ICI, may have contributed to the differences in the ORR point estimates and median DOR, although the 95% CI were overlapping.

Although the eligibility criteria (same for Cohorts 2 and 4) allowed for patients to receive lifileucel after progression on first-line ICI, the median number of prior lines of therapy was 3.0. As illustrated in online supplemental figure 3, most patients received multiple regimens of ICI-based therapy prior to receiving lifileucel. In addition, as shown in multivariable analyses, favorable clinical characteristics of lower tumor burden (ie, target lesion SOD < median, normal LDH) are associated with greater likelihood of response. The ORR of 31.4% in heavily pretreated patients in this study is encouraging compared with response rates of 29% in patients treated with anti-CTLA-4 and anti-PD-1 therapy after first-line ICI treatment failure. The use of lifileucel after first progression on ICI may thus represent a unique opportunity to intervene with a one-time therapy that can lead to durable responses.

Patients whose disease progresses after combination ICI therapy and BRAF/MEK inhibitors (when appropriate) have no effective therapeutic options that give
Figure 2  DOR in confirmed responders (PR or better) by IRC assessment per Response Evaluation Criteria in Solid Tumors V.1.1 (A), OS (B), and PFS (C) for pooled Cohorts 2 and 4. DOR, duration of response; IRC, independent review committee, NR, not reached; OS, overall survival; PFS, progression-free survival; PR, partial response.
Clinically meaningful results. Lifileucel was efficacious in this subset of patients (ORR: 26.8%), which constituted 53.6% of the entire cohort, and thus, lifileucel could be considered as their next line of therapy.

In addition, the ORR was 31.3% in the subset of patients who were primary refractory to anti-PD-1/PD-L1 therapy per the study criteria, consistent with earlier observations. Notably for patients with disease defined as primary resistant per the SITC Immunotherapy Resistance Taskforce, the ORR was 33.0%, and median DOR was NR (95% CI: 12.5 months to NR). The efficacy of lifileucel in these patient subsets suggests that lifileucel is a viable option for patients progressing after first-line ICI monotherapy and in those progressing after combination therapy.

Recently, the Netherlands Cancer Institute reported results of a randomized Phase 3 clinical trial of autologous, in vitro expanded, non-cryopreserved TIL compared with ipilimumab in 168 patients with unresectable or metastatic melanoma who had received a maximum of one prior line of systemic therapy (86% had received prior anti-PD-1 therapy (first-line or adjuvant)). This study met its primary endpoint with a 6-month PFS rate of 53% in patients treated with TIL cell therapy compared with 21% in those treated with ipilimumab. An ORR of 49% was seen with TIL cell therapy compared with 21% with ipilimumab.55 Consistent with these earlier-line clinical findings, biological findings, particularly anti-PD-1-induced immunoediting, loss of neoantigens and

---

**Table 3** TEAEs occurring in ≥30% of the patients (Safety Analysis Set (N=156))*

<table>
<thead>
<tr>
<th>Preferred term, n (%)</th>
<th>Any grade</th>
<th>Grade 3/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocytopenia</td>
<td>129 (82.7)</td>
<td>120 (76.9)</td>
</tr>
<tr>
<td>Chills</td>
<td>117 (75.0)</td>
<td>8 (5.1)</td>
</tr>
<tr>
<td>Anemia</td>
<td>97 (62.2)</td>
<td>78 (50.0)</td>
</tr>
<tr>
<td>Fever</td>
<td>81 (51.9)</td>
<td>17 (10.9)</td>
</tr>
<tr>
<td>Neutropenia†</td>
<td>66 (42.3)</td>
<td>45 (28.8)</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>65 (41.7)</td>
<td>65 (41.7)</td>
</tr>
<tr>
<td>Hypophosphatemia</td>
<td>58 (37.2)</td>
<td>41 (26.3)</td>
</tr>
<tr>
<td>Leukopenia†</td>
<td>54 (34.6)</td>
<td>42 (26.9)</td>
</tr>
<tr>
<td>Hypotension</td>
<td>52 (33.3)</td>
<td>17 (10.9)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>51 (32.7)</td>
<td>6 (3.8)</td>
</tr>
<tr>
<td>Lymphopenia†</td>
<td>49 (31.4)</td>
<td>38 (24.4)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>48 (30.8)</td>
<td>2 (1.3)</td>
</tr>
</tbody>
</table>

*Other relevant events: Grade 3/4 TEAEs commonly observed with cellular therapies or IL-2 included immune effector cell-associated neurotoxicity syndrome and cytokine release syndrome (investigator-assessed, no confirmatory serum cytokine levels measured) in one patient, and capillary leak syndrome (due to IL-2) in seven patients. Grade 3/4 uveitis was reported in three patients.

†All patients had grade 4 laboratory abnormality per the Common Terminology Criteria for Adverse Events V.4.03 for leukopenia, neutropenia, and lymphopenia during the treatment-emergent period. Only clinically significant laboratory abnormalities as per investigators were reported as adverse events. IL-2, interleukin-2; TEAE, treatment-emergent adverse event.

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**Figure 3** Incidence of AEs over time (Safety Analysis Set).* All occurrences of AEs were counted if a patient experienced a new onset of the same AE at different time points. If multiple records were reported on the electronic case report form because of toxicity grade decrease of the same AE that had not been resolved, then the event was counted once with the highest grade reported. *Fourteen events were reported after month 12 (grade 1, n=6; grade 2, n=6; grade 3, n=1, grade 5, n=1). AE, adverse event; D, day; M, month.
neoadvertig presentation, and associated relative paucity of neoadventig-reactive cells within final TIL products of patients with prior anti-PD-1 exposure, add weight to consideration of intervention with lifileucel early in the treatment course.

In the recent DREAMseq trial, ICI therapy (nivolumab/ipilimumab) preceding BRAF/MEK inhibitors (dabrafenib/trametinib) yielded a substantially higher 2-year OS rate (72%), longer median DOR (NR), and higher rate of ongoing responses (88%) compared with patients who received BRAF/MEK inhibitors preceding ICI therapy (2-year OS: 52%, median DOR: 12.7 months, ongoing responses: 51%). Adoption of ICI use before BRAF/MEK inhibitors based on this study has been a paradigm shift in the management of advanced melanoma. A long-term follow-up analysis of patients treated by the National Cancer Institute Surgery Branch going back to the pre-ICI and BRAF tyrosine kinase inhibitor era showed an inverse relationship between response to TIL and prior anti-PD-1 therapy or BRAF/MEK inhibitors. A higher rate of durable responses and melanoma-specific survival was demonstrated, especially in the ICI-naive population receiving TIL, driven by firm biological rationale as recently shown by Levi et al.6 In PD-1 inhibitor-naive patients, treatment with lifileucel in combination with pembrolizumab produced an ORR of 60% (CR rate, 30%), supporting the potential for improved response rates, including CR rates, with earlier TIL cell therapy. Nevertheless, lifileucel produced durable responses and a favorable safety profile across subgroups of heavily pretreated patients with high tumor burden, regardless of age, BRAF mutation status, PD-L1 status, baseline ECOG PS status, and presence of liver and/or brain lesions at baseline, which supports a potential benefit for a broad population of patients with melanoma.

In line with prior reports, TEAEs were consistent with the known safety profiles of NMA-LD and IL-2, with a majority of TEAEs occurring within the first 2 weeks post-lifileucel infusion and no new safety signals reported in the combined analysis. The transient and manageable nature of AEs support the potential benefit of one-time treatment with lifileucel.

Lifileucel was successfully manufactured for 94.7% of the eligible resected tumors, reflecting the ability to manufacture lifileucel using tumors from diverse anatomic sites and supporting the feasibility and scalability of the 22-day GMP process. The short-duration manufacturing process (compared with a historical production time of 5–7 weeks) may benefit patients who have exhausted all approved treatment options. Rapid transition of TIL cell therapy from clinical trials into clinical practice will allow broader access, and the success of the treatment regimen will require close coordination across multidisciplinary teams, including surgeons, medical oncologists, and cell therapy physicians. Surgical best practices for tumor resection for TIL manufacturing are well established to address possible practice variations. Further innovation is underway to develop TIL regimens that use IL-2 analogs to enhance antitumor responses by continuous support of growth and activity of the infused TIL product, but also mitigate the toxicities associated with currently available recombinant IL-2. Moreover, efforts to reduce the doses of available cytotoxic agents or develop novel lymphodepletion regimens may allow for broader use.

In summary, one-time treatment with lifileucel TIL cell therapy demonstrated clinically meaningful activity in heavily pretreated patients with advanced melanoma with a high tumor burden and advanced disease in a larger population and with a longer follow-up duration, consistent with data from the previously published Cohort 2. Responses were durable and AEs were transient and manageable in a population with traditionally difficult-to-treat disease that currently has no approved treatments. These findings thus support the potential of lifileucel to fulfill a large unmet medical need for novel therapeutic options distinct from ICI in patients with advanced melanoma.

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Ethics approval | This study involves human participants and was approved by Providence Health & Services Institutional Review Board (IRB) (PDX15-117); Atlantic Health System IRB (763583); Advarra IRB (PRE00076076); Yale University IRB #2, 3, 4B, 5, 6—Human Investigation Committee IB Oncology, II, III, IV, 1A (1506016043); Western IRB (20161098); University of Louisville IRB (16:0817) (initial approval reference ID: 746942); Mount Sinai Medical Center IRB (16-49-H-059 (Federalwide Assurance [FWA] 00017178)); NYU School of Medicine IRB (1-000084); Sutter Health IRB—(SHIRB) (2016.124 (IRB Net # 931782); Orlando Health IRB #1 (1092019 (reference: 17.069.060); Human Research Protection Program, University of Minnesota (STUDY00001236); UC San Diego Human Research Protections Program (171801); Thomas Jefferson University Institutional Review Board (17C.598); Fred Hutchinson Cancer Research Center IRB (9925); Roswell Park Cancer Institute IRB (STUDY0000465/P541177); Medical College of Wisconsin’s Froedtert Hospital IRB—Human Research Protection Program (PRO00031199); Office of the Human Research Protection Program (OHRPP) (IRB#19-000536); University of Miami Human Subject Research Office (HSRO)—IRB (IRB #17099026); CEM HM Hospitals (Tracking w EudraCT#- 2017-000760-15); Egészsegügyi Tudományos Tanács Klinikai Farmakológiai Etikai Bizottsága (GYEI/47943-5/2017); Comité de Protection des Personnes (CPP) Sud-Ouest et Outre Mer IRB (Tracking w EudraCT#- 2017-000760-15); London—West London & GTAC Research Ethics Committee (EudraCT#- 2017-000760-15; MHRA: 48580; IRAS: 229812; REC: 17/LO/1471); Ethikkommission der Medizinischen Fakultät der Technischen Universität München (Tracking w EudraCT#- 2017-000760-15); and Commission Cantonale d’Éthique de la Recherche sur l’Être Humain (CER-VD) (2017-02031). Participants gave informed consent to participate in the study before taking part.

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18. National Comprehensive Cancer Network, Inc. Referenced with permission from the NCCN clinical practice guidelines in oncology (NCCN Guidelines®) for melanoma, cutaneous V.2.2022, 2022To view the most recent and complete version of the guideline, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way.
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SUPPLEMENTARY MATERIALS

Supplementary Figure 1. C-144-01 Study Design

Four cohorts were enrolled in the C-144-01 trial as shown in the study design. The total planned sample size was approximately 171 patients. The present report focused on the efficacy and safety of lifileucel in patients enrolled in Cohorts 2 and 4.

Abbreviations: ICI, immune checkpoint inhibitors; TIL, tumor-infiltrating lymphocytes.
Supplementary Figure 2. CONSORT Diagram for Pooled Cohorts 2 and 4

- 189 patients enrolled (Tumor Harvest Set)
  - 33 (17.5%) did not receive lifileucel
    - Progressive disease; n=9 (4.8%)
    - Lifileucel not available; n=8 (4.2%)
    - Death; n=5 (2.6%)
    - Adverse event; n=3 (1.6%)
    - New anti-cancer treatment; n=2 (1.1%)
    - Consent withdrawal; n=1 (0.5%)
    - Withdrawal; n=1 (0.5%)
    - Other reason; n=4 (2.1%)

- 156 received lifileucel (Safety Analysis Set)
- 153 received lifileucel and analyzed for efficacy (Full Analysis Set)
  - Received lifileucel <1 billion cells; n=1 (0.5%)
  - Lifileucel not meeting product specifications; n=2 (1.1%)
Supplementary Figure 3. Sankey Plot of Treatment Patterns

The Sankey plot shows the treatment journey of the patients with various regimen types used. The R package networkD3 was used to generate the Sankey plot.

1L–10L represent lines of therapy.

Abbreviations: ICI, immune checkpoint inhibitors; L, line of therapy.
Supplementary Figure 4. Overall Survival, by Response Status at 1.5 Months After Lifileucel Infusion

Abbreviations: OS, overall survival; NR, not reached

Survivors at 1.5 months (~6 weeks, first response assessment after lifileucel infusion) were stratified by response status, and overall survival from the 1.5-month landmark was computed using Kaplan Meier methods.
Supplementary Figure 5. Site of Tumor Resection and Infused Cell Dose

Abbreviations: BOR, best overall response; CR, complete response; IRC, Independent Review Committee; PD, progressive disease; PR, partial response; SD, stable disease; SOD, sum of diameters.
### Supplementary Table 1. Tumor-resection AEs* Related to Surgery Occurring in >1 Patient (Any Grade) in the Tumor Harvested Set (N=189)

<table>
<thead>
<tr>
<th>Preferred Term, n (%)</th>
<th>Tumor Harvested Set (N=189)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any Grade</td>
<td>Grade 3/4</td>
<td></td>
</tr>
<tr>
<td>Number of patients reporting &gt;1 tumor-resection AE related to surgery</td>
<td>60 (31.7)</td>
<td>6 (3.2)</td>
<td></td>
</tr>
<tr>
<td>Procedural pain</td>
<td>22 (11.6)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>5 (2.6)</td>
<td>1 (0.5)</td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>4 (2.1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>3 (1.6)</td>
<td>1 (0.5)</td>
<td></td>
</tr>
<tr>
<td>Cellulitis</td>
<td>3 (1.6)</td>
<td>2 (1.1)</td>
<td></td>
</tr>
<tr>
<td>Flank pain</td>
<td>3 (1.6)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Incision site erythema</td>
<td>3 (1.6)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Seroma</td>
<td>3 (1.6)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>2 (1.1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Erythema</td>
<td>2 (1.1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Incision site pain</td>
<td>2 (1.1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Localized edema</td>
<td>2 (1.1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Lymphocele</td>
<td>2 (1.1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Postoperative wound infection</td>
<td>2 (1.1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Wound dehiscence</td>
<td>2 (1.1)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AE, adverse event; NMA-LD, nonmyeloablative lymphodepletion.
*Tumor-resection AEs refer to AEs that started after tumor resection and before the start of NMA-LD.

For patients who did not receive NMA-LD, AEs up to 30 days from tumor resection were included.