Lifileucel, an Autologous Tumor-infiltrating Lymphocyte Monotherapy, in Patients with Advanced Non-small Cell Lung Cancer Resistant to Immune Checkpoint Inhibitors

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Abstract

In this phase 2 multicenter study, we evaluated the efficacy and safety of lifileucel (LN-145), an autologous tumor-infiltrating lymphocyte cell therapy, in patients with metastatic non-small cell lung cancer (mNSCLC) who had received prior immunotherapy and progressed on their most recent therapy. The median number of prior systemic therapies was 2 (range, 1–6). Lifileucel was successfully manufactured using tumor tissue from different anatomic sites, predominantly lung. The objective response rate was 21.4% (6/28). Responses occurred in tumors with profiles typically resistant to immunotherapy, such as PD-L1–negative, low tumor mutational burden, and *STK11* mutation. Two responses were ongoing at the time of data cutoff, including one complete metabolic response in a PD-L1−negative tumor. Adverse events were generally as expected and manageable. Two patients died of treatment-emergent adverse events: cardiac failure and multiple organ failure. Lifileucel is a potential treatment option for patients with mNSCLC refractory to prior therapy.

Keywords: Lifileucel, tumor-infiltrating lymphocytes, adoptive cell therapy, advanced NSCLC

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Statement of Significance: Autologous tumor-infiltrating lymphocyte therapy lifileucel was administered to 28 patients with heavily pretreated metastatic non-small cell lung cancer (mNSCLC). Responses were observed in patients with driver mutations, and various tumor mutational burdens and PD-L1 expression, potentially addressing an unmet medical need in patients with mNSCLC refractory to prior therapy.

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Introduction

Adoptive cellular therapy using autologous tumor-infiltrating lymphocytes (TIL) – polyclonal immune cells that can recognize and target diverse individualized tumor-specific antigens – has been explored extensively in metastatic melanoma. The first adoptive transfer of TIL in patients with metastatic melanoma was described by Rosenberg et al. at the National Cancer Institute (NCI) in 1988 and involved the infusion of autologous $CD4^+$ and $CD8^+$ T lymphocytes that were expanded ex vivo in the presence of interleukin-2 (IL-2) (1). Since then, multiple clinical trials have demonstrated the potential of TIL cell therapy to generate robust and durable clinical responses in patients with metastatic melanoma (2-7). These encouraging data in melanoma were the impetus for exploring TIL cell therapy in metastatic non-small cell lung cancer (mNSCLC). TIL cell therapy begins with tumor tissue procurement to provide starting material for ex vivo T-cell isolation and expansion to generate the final TIL infusion product. The TIL cell therapy regimen consists of a preparative nonmyeloablative lymphodepletion (NMA-LD) regimen, followed by a one-time TIL infusion and short course of high-dose IL-2. The complexity and cost of manufacturing and delivery processes has previously made multicenter clinical trials difficult to implement, and access to a broader patient population challenging.

The first autologous TIL cell therapy, lifileucel, was recently approved by the U.S. Food and Drug Administration for the treatment of adult patients with unresectable or metastatic melanoma previously treated with a PD-1 blocking antibody, and if BRAF V600 mutation positive, a BRAF inhibitor with or without a MEK inhibitor (7). The approval of lifileucel was based on its efficacy in patients with metastatic melanoma treated in a global multicenter study (8). The approval of lifileucel for melanoma paves the way for exploration of autologous TIL cell therapy in broader patient populations. Beyond melanoma, it is not well established whether metastatic lesions treated with immune checkpoint inhibitor (ICI) with or without cytotoxic chemotherapy can reliably serve as manufacturing sources for

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TIL cell therapy for clinical use or whether patients with other tumor types could tolerate and respond to the TIL cell therapy regimen.

These questions have been particularly crucial in patients with mNSCLC. While the combination of chemotherapy plus ICI has revolutionized treatment outcomes, there remains notable unmet medical need in patients with PD-L1–negative tumors and those with primary or acquired resistance to ICI. In preclinical studies, TIL have been isolated and amplified ex vivo from primary NSCLC lesions and demonstrated antitumor activity (9). Additionally, preliminary activity was observed in a phase 1 study of TIL cell therapy in combination with anti-programmed death 1 (anti–PD-1) pathway inhibition in patients with mNSCLC (10). In this context, we report results from a phase 2 multicenter study investigating lifileucel in patients with mNSCLC who were previously treated with ICI therapy.

Results

Patient Disposition and Disease Characteristics

Between January 17, 2019, and January 11, 2021, 39 patients with mNSCLC were enrolled and underwent tumor tissue resection (**Supplementary Figure 1**). Of these 39 patients with tumor tissue resected (Tumor Harvest Set), 28 received a single infusion of lifileucel that met manufacturer's specification and comprised the Full Analysis Set (FAS). Five patients did not receive lifileucel for patient-related reasons. Six patients did not have lifileucel manufactured for reasons of low starting tumor tissue material, insufficient amount of TIL to proceed with manufacturing, contamination of TIL, or presence of necrotic tumor tissue (**Supplementary Figure 1**).

In the FAS (*N* = 28), the median age was 61 years (range, 40–74), and all patients had an Eastern Cooperative Oncology performance status (ECOG-PS) of 0 or 1; 86% of patients had a smoking history. The median number of prior systemic therapies was 2 (range, 1–6). All patients received prior treatment

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with anti–PD-1/PD-L1 antibodies, and 27 (96.4%) patients had received ≥1 line of cytotoxic chemotherapy **(Table 1)**. One-quarter (25% [7/28]) of patients in the FAS had disease that was primary refractory to last anti–PD-1/PD-L1 therapy (best response of progressive disease to last anti–PD-1/PD-L1 therapy). Patients had multiple tumors (median number of target/non-target lesions, 4.5 [range, 2–11]) and median target lesion sum of diameters (SOD) of 79 mm [range, 22–179]). Ten patients (35.7%) had prior brain metastases, and six (21.4%) had liver metastases at baseline (13 [46.4%] had prior brain and/or liver metastases). PD-L1 expression was low (PD-L1 tumor proportion score [TPS] 1%–49%) or negative (PD-L1 TPS <1) in more than half of the tumors (60.7%) **(Table 1)**.

Feasibility and Safety of Tumor Tissue Procurement Surgery

Tumor tissue procurement surgery was generally well tolerated **(Supplementary Table 1**), with a median duration of hospitalization after resection of 2 days (range, 1–6). No patient had TIL cell therapy cancelled due to a surgery-related adverse event (AE). In the Tumor Harvest Set (*N* = 39), 7 grade 3/4 resection-related AEs occurred in five (12.8%) patients (ie, constipation, hypertension, hypotension, hypoxia, non-cardiac chest pain, pneumothorax, subcutaneous emphysema). The most common site of tumor tissue resection for TIL manufacturing was lung (60.7%); multiple other metastatic sites were successfully used for manufacturing of lifileucel, including lymph node, liver, pleura, adrenal gland, and spleen (**Table 1**).

Treatment Administration and Safety

The median time from tumor tissue resection to lifileucel infusion was 35.5 days (range, 28–112). The median number of cyclophosphamide and fludarabine doses was 2 (range, 2–2) and 5 (range, 1-5), respectively. The median number of TIL cells infused was 20.9×10^9 (range, 1.4×10^9–53.2×10^9). The median number of IL-2 doses administered was 5.5 (range, 0–6).

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The safety profile was consistent with the advanced disease and known profiles of NMA-LD and IL-2 (**Table 2, Supplementary Figure 2)** (6,8). All patients experienced grade 3/4 hematologic laboratory abnormalities with first onset date during the period from the start of NMA-LD to 30 days after lifileucel infusion; these events resolved to baseline in 93% of patients with low neutrophils and low leukocytes, 82% of patients with low lymphocytes, 85% of patients with low platelets, and 79% of patients with low hemoglobin.

Two patients died of treatment-emergent adverse events. A 60-year-old woman died of cardiac failure 6 days after last NMA-LD dose, 3 days after lifileucel infusion, and 3 days after last IL-2 dose. The investigator reported this event as not related to any study therapy. Her history of cigarette smoking, apnea syndrome, and recent pulmonary embolism contributed to respiratory insufficiency due to underlying advanced study disease. Fluid overload may have precipitated her heart failure and possibly contributed to respiratory insufficiency. A 61-year-old woman died of multiple organ failure 2 days after last NMA-LD dose, 1 day after lifileucel infusion, and no IL-2 was administered. The investigator reported this event as possibly related to lifileucel, with sepsis reported as an alternative causality. The patient experienced hypotensive episodes and acute respiratory failure and pneumonia (consistent with history of chronic obstructive pulmonary disease) requiring intubation and ventilation before lifileucel infusion. Subsequently, multi-organ failure and hypotension were reported in the setting of pneumonia due to aspiration and possible sepsis.

Efficacy

At the data-cutoff date of February 22, 2022, the median duration of study follow-up was 16 months (range, 0.1+ to 27.6). The objective response rate (ORR) was 21.4% (6 responses) in the FAS (*N* = 28). Investigator-assessed best overall response (BOR) included one complete metabolic response based on a negative fluorodeoxyglucose (FDG)-positron emission tomography (PET) scan initially observed at Day

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196 (~6.4 months) and confirmed by multiple repeat FDG-PET scans, and 5 partial responses (PR; **Table 3)** confirmed by subsequent computed tomography (CT) scans per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 criteria. Tumor tissue resection sites in the responders included lung (*n* = 4), spleen (*n* = 1), and lymph node (*n* = 1). Reduction in tumor burden (as measured by SOD of target lesions) was reported for 19 (79.2%) patients. **(Figure 1A)**.

The median time from lifileucel infusion to BOR was 2.2 months (range, 1.4–6.5). Four of the six (66.7%) confirmed responders had attained a response by their first efficacy assessment at 6 weeks (1.5 months) after lifileucel infusion **(Figures 1B and 1C)**. **Figure 2** shows representative CT scans taken before TIL treatment and 6 weeks after TIL treatment in a patient who achieved a PR. The duration of response ranged from 1.1+ to 26.2+ months. Responses deepened over time, with continued SOD reduction after initial assessment in all but one responder; in addition, one patient who achieved a PR at their first assessment (SOD reduction of 44% at week 6) subsequently achieved complete metabolic response (based on a negative FDG-PET scan). Another patient who initially had stable disease (SD) achieved PR at 6 months. At the time of the data cutoff, responses were ongoing in both patients (complete metabolic response, 26.2+ months; PR, 8.7+ months) without subsequent local or systemic therapies. Notably, all responders had received at least 2 prior lines of therapy. Of the 4 patients for whom response was not ongoing at the time of the data cut, 3 patients experienced radiographic disease progression per RECIST 1.1 and 1 patient died due to bowel perforation. The cause of radiographic progression in 1 patient was unequivocal progression of non-target disease, 1 patient had both target and non-target lesion progression, and 1 was due to development of a new lesion.

PD-L1 Expression, Clinical, and Molecular Features

The baseline median neutrophil-to-lymphocyte ratio was 2.74 (range 1.16, 9.43) in the responder group and 4.31 (1.05, 16.65) in the non-responder group. Baseline LDH was elevated in 17% of patients (1 of 6) in the responder group as compared with 50% of those (11 of 22) in the non-responder group.

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Two responders previously had PR as best response to prior anti–PD-1/PD-L1 blockade, and four responders had progressive disease or SD as best response to prior anti–PD-1/PD-L1 blockade. Upon treatment with lifileucel, one patient with a PD-L1–negative tumor attained a complete metabolic response as assessed by PET/CT scan. Another patient achieving a PR also had a PD-L1–negative tumor. The remaining four patients with PR had PD-L1–positive tumors with TPS between 5% and 90% **(Figure 1C)**.

Baseline tumor samples from 20 patients were available for genomic analysis. Overall, key oncogenic driver mutations were seen in 13 patients (responders and non-responders) – *KRAS* mutations (including one *KRAS* ^{G12C}) were seen in the tumors from 11 patients, and *EGFR* alterations (including *EGFR* gene amplification, as well as exon 19 deletion and T790M mutation) were identified in the tumors from 3 patients; one patient's tumor (3B-16) harbored both *KRAS* and *EGFR* mutations (**Figure 3A**). Among the responders, one patient's tumor (3B-17, PR) harbored the *KRAS* ^{G12C} point mutation and *MET* amplification, and one patient's tumor (3B-26, PR) harbored the *KRAS* ^{G12D} mutation. Tumors of patients 3B-02 and 3B-25 were not assessed for mutations, and tumors of patients 3B-22 and 3B-28 showed no detectable actionable driver mutations (**Figures 1C and 3A**). The tumor from patient 3B-22 did have mutations in *STK11* and *KEAP1*, which are typically associated with poor outcomes to anti-PD-1/PD-L1 therapy (11,12).

The median tumor mutational burden (TMB) exome equivalent was 7.71 (1.4–69.48) mutations/Mb; conversion to exome equivalent was calibrated as reported in Vega et al (13) (**Figure 3B**). There was no significant difference in TMB between responders and non-responders to lifileucel (P = 0.79, **Figure 3C**).

Circulating tumor DNA (ctDNA) was assessed using pre- (Day -7) and post-TIL infusion (Day 42) blood from three of the six responders. Patient 3B-17, one of the long-term responders, had low but detectable levels of *KRAS* ^{G12C} (0.41 VAF, 1.4 mutant molecules/mL plasma) in pre-infusion blood, with

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clearance of this mutation in the ctDNA post-infusion. Patient 3B-22, who had ongoing response at the time of ctDNA sample collection on Day 42 and progressive disease later at Day 126, had high levels of ctDNA in pre-infusion blood (2,771 mutant molecules/mL plasma) and reduced levels post-infusion (672 mutant molecules/mL plasma, Day 42) (**Supplementary Figure 3A-3E**). No mutations were detected in plasma from patient 3B-26 at either timepoint.

Phenotype of TIL Infusion Product and T-Cell Clonal Dynamics

TIL infusion product was available for phenotypic analysis from 27 of 28 patients. The memory T-cell subset composition (i.e., central memory T cells [$CCR7^+CD45RA^-$, TCM] and effector memory T cells [$CCR7^-CD45^-$, TEM] **Supplementary Figure 4A**) did not correlate with response to lifileucel (**Supplementary Table 2**). Additionally, proportions of CD4 or CD8 T cells and expression of differentiation, activation/exhaustion, and immune-checkpoint markers by $CD4^+$ and $CD8^+$ T cells (**Supplementary Figures 4B and 4C**) were not associated with response to lifileucel (all P >0.05 between responders [n = 6] and non-responders [n = 21] using Kruskal-Wallis test; **Supplementary Table 2**).

T-cell receptor (TCR) repertoire dynamics and persistence were assessed using unique TCR β chain complementarity-determining region 3 (uCDR3) sequences (i.e., clonotypes) from baseline tumor, TIL infusion product, and pre- (Day -7) and post-TIL infusion (Day 42) blood samples. Day 42 was chosen for TCR repertoire analysis because it corresponds to the first response assessment and has the largest number of samples. The TCR repertoire of all sample types was highly polyclonal, and clonality of the post-infusion blood more closely resembled that of the TIL infusion product than the tumor or pre-infusion blood (**Figure 4A**). A mean of 3090 unique CDR3 clones was present in baseline tumor samples, and a mean of 4076 in TIL infusion products, with a mean of 417 shared clones between tumors and TIL infusion products (~5.5% of total clonotypes; **Figure 4B**). These shared clones persisted in post-infusion

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blood through Month 6 in both responders and non-responders (**Figure 4C**); small sample sizes preclude statistical analyses of association with response. Common CDR3s in the beta chain shared by up to 9 patients have been found in TIL drug products.

Evidence of peripheral TCR repertoire remodeling was observed, with a higher proportion of the TCR repertoire derived from TIL infusion product clones in post-infusion than in pre-infusion blood in nearly all patients, regardless of response (**Figure 4D**).

Discussion

For the first time in a multicenter phase 2 clinical trial, we demonstrate the feasibility and efficacy of one-time centrally manufactured autologous TIL cell therapy in patients with mNSCLC who had received prior anti-PD-1/PD-L1 therapy and whose disease progressed on their most recent therapy. Lifileucel was successfully generated in a centralized TIL manufacturing process, and administered to 28 patients.

The initial protocols for TIL cell therapy were primarily developed for patients with melanoma and renal cell carcinoma (2,14-18). In the current study, TIL infusion product was successfully manufactured from a heavily pretreated population of patients with mNSCLC, and AEs related to surgery were as expected and manageable. Overall, the lifileucel regimen demonstrated a safety profile generally consistent with the underlying advanced disease and known safety profiles of NMA-LD and IL-2, comparable with that observed in previous TIL cell therapy studies (6,19,20). The primary toxicities were cytopenias occurring after preparative NMA-LD, which typically resolved within 2 weeks of treatment. Patients received a similar median number of IL-2 doses (5.5) as patients treated with lifileucel in previous studies in metastatic melanoma (6,8). Thus, this study, amongst other proof of concepts, establishes that patients with mNSCLC, including patients with poor baseline characteristics, can tolerate tumor tissue

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procurement surgery, including that of lung lesions, and can have lifileucel successfully manufactured and administered, with TEAEs that were as expected and manageable.

However, disease-specific factors in mNSCLC deserve further discussion. A significant number of patients had a history of liver and/or brain metastases (46.4%), which likely reflects a more aggressive disease phenotype in these patients. Additionally, NSCLC is known to directly impact pulmonary function and patients with smoking-related NSCLC have a high prevalence of cardiac and pulmonary comorbidities which may pose challenges before or after administration of the TIL regimen. Of the patients who underwent tumor tissue resection for lifileucel manufacturing, four experienced complications related to the underlying disease that made them ineligible to receive lifileucel. Additionally, six patients did not have lifileucel manufactured. Although centralization of TIL manufacturing is a substantial advancement, manufacturing and administration of lifileucel at earlier timepoints in a patient's disease course when the disease is less aggressive could enable more patients with lung cancer to successfully complete the TIL cell therapy regimen. The ongoing clinical trial IOV-LUN-202 (NCT04614103) is enrolling a population of NSCLC patients with fewer prior lines of therapy and includes an exploratory option for tumor tissue procurement and lifileucel manufacturing prior to disease progression to minimize the time between disease progression and initiation of TIL cell therapy. Furthermore, a separate cohort of the current clinical trial IOV-COM-202 (NCT03645928) enrolled patients with mNSCLC who were naïve to ICIs (21).

The ORR with lifileucel per RECIST v1.1 was 21.4% (6/28), and responders included patients with PD-L1–negative, TMB-low, and *STK11*-mutant tumors, who are often considered resistant to immunotherapy in mNSCLC. Durable clinical benefit with ongoing responses at time of data cutoff were observed in two of the six responding patients, including a patient with a PD-L1–negative tumor. Notably, five of the six responders showed deepening of responses over time, with continued SOD reduction after initial

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assessment, indicating the potential of one-time lifileucel TIL cell therapy to generate durable and deepening responses in a subset of patients with ICI-treated mNSCLC, supporting further investigation.

Given that the proposed mechanism of action of TIL cell therapy is distinct from that of ICI, predictive biomarkers for immunotherapy with ICI may not be applicable in this context. In the current study, the most durable response occurred in a patient with a PD-L1–negative tumor (TPS <1%), suggesting that lifileucel activity is not limited by PD-L1 expression. Additionally, mutations in *STK11* and *KEAP1*,which play a role in ICI resistance in lung cancer (11,12), were identified in the tumors from responders. In our study, TMB did not seem to correlate with response, as has been seen for ICI (22). Thus, lifileucel may have a uniquely different mechanism of action relative to ICI in NSCLC. A phase 1 study of TIL plus nivolumab in NSCLC similarly observed two complete responses ongoing for >1.5 years in PD-L1–low or –negative lung tumors, including a never-smoker whose tumor was TMB-low and harbored an *EGFR* mutation (9). Therefore, lifileucel could be particularly useful in NSCLC patients who may not experience benefit from PD-1/PD-L1 blockade.

Consistent with prior studies of lifileucel (6), we found no association between composition of the TIL product (i.e., memory, differentiation, activation/exhaustion, and immune-checkpoint markers by $CD4^+$ and $CD8^+$ T-cells) and response to lifileucel. However, infusion of lifileucel clearly led to peripheral TCR repertoire changes, with notable expansion and persistence of clonotypes present in the TIL infusion product. How this remodeling affects lifileucel clonal dynamics in the peripheral blood and its relationship to antitumor T-cell responses is currently under investigation. In the future, comprehensive and longitudinal peripheral and intratumoral monitoring, including on-treatment and post-progression biopsies, may also be critical to improve our understanding of the intrinsic and extrinsic factors associated with response and emergence of resistance to TIL cell therapy in lung cancer.

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Recent analyses in metastatic melanoma also demonstrated that prior exposure and longer duration of exposure to ICI was associated with worse outcomes with TIL cell therapy (23,24). Prior ICI experience was associated with decreased detection of T cells reactive against neoantigens despite similar predicted neoantigen loads, suggesting that ICI exposure prior to tumor resection could be inversely correlated with expansion of tumor-reactive T cells (24). To that end, recent data demonstrated the safety of combining pembrolizumab with lifileucel in ICI-naïve patients with advanced (unresectable or metastatic) melanoma; advanced, recurrent, or metastatic head and neck squamous cell carcinoma; and persistent, recurrent, or metastatic cervical cancer (25). Additionally, preliminary activity was recently observed in a phase 1 study of TIL cell therapy plus anti–PD-1 therapy in patients with mNSCLC who underwent tumor resection prior to exposure to anti–PD-1 (9). Given the different mechanisms of action of TIL cell therapy and ICI, the promising signals in earlier settings, and the favorable risk-benefit profile demonstrated in the current study, evaluation of lifileucel with or without the addition of PD-1 pathway blockade earlier in the NSCLC disease course is currently underway (NCT03645928 and NCT04614103). Recently, in the IOV-COM-202 study with lifileucel plus pembrolizumab, patients with ICI-naïve mNSCLC demonstrated an encouraging ORR of 42.1% for the entire cohort and ORR of 58.3% for patients with *EGFR*-wild type disease. Durable and deepening responses up to 15.4 months and beyond were observed (21), thus supporting the use of this combination earlier in the disease course.

As another approach, genetic modification while maintaining polyclonality of TIL is feasible and may confer a functional advantage to TIL as a potential therapeutic option in patients with advanced solid tumors (26-30). As an example, IOV-4001, a TALEN®-mediated PD-1–inactivated TIL cell therapy product, is under investigation in patients with metastatic melanoma and advanced NSCLC, including those resistant to prior anti–PD-1/PD-L1 (NCT05361174).

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In summary, TIL cell therapy represents a feasible, individualized, and polyclonal potential treatment option for patients with mNSCLC. This is the first study to demonstrate the efficacy and safety of centrally manufactured autologous TIL cell therapy as a single modality in patients with mNSCLC after treatment with anti–PD-1/PD-L1 therapy. These results are encouraging and warrant further investigation of lifileucel in patients with mNSCLC.

Methods

Study Design

IOV-COM-202 (NCT03645928) is a prospective, open-label, multicohort, non-randomized, multicenter phase 2 study evaluating the efficacy and safety of lifileucel in combination with ICI and as a monotherapy in multiple solid tumors. The study consists of 7 cohorts spanning advanced (unresectable or metastatic) melanoma (Cohorts 1A, 1B, 1C); advanced, recurrent, or metastatic head and neck squamous cell carcinomas (HNSCC; Cohort 2A), and mNSCLC (Cohorts 3A, 3B, 3C). Data from cohort 3B, which evaluated lifileucel monotherapy in previously treated (1–3 prior systemic therapies) patients with mNSCLC, are reported here. The treatment schema is shown in **Supplementary Figure** 5). **Supplementary Table 3** provides information about the representativeness of the study population in relation to the population at large.

Written informed consent was obtained from all patients. The study was conducted in full compliance with the principles of the Declaration of Helsinki (as amended in Tokyo, Venice, Hong Kong, and South Africa), ICH guidelines, and with the laws and regulations of the country in which the research was conducted. Institutional review boards provided initial approval and continuing review of the study.

Inclusion Criteria

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Patients had a diagnosis of stage III or IV mNSCLC, with confirmed radiographic progression on or after most recent treatment. Progression on ≥1 prior systemic therapy with ICI, including PD-1 or PD-L1 blocking antibody was required, except for patients with actionable oncogenic mutations as part of 1–3 lines of prior systemic therapy. Patients with tumors harboring known oncogene drivers (e.g., *EGFR, ALK, ROS*) that are sensitive to targeted therapies must have progressed after ≥1 line of recommended targeted therapy. Patients must have had ≥1 resectable lesion (or aggregate lesions) of a minimum 1.5 cm in diameter post-resection for TIL production and ≥1 remaining lesion for response assessment. Eligible patients were ≥18 years of age, with an ECOG-PS of 0 or 1 and adequate organ function and required a sufficient washout period from previous anticancer regimen(s).

Exclusion Criteria

Key exclusion criteria included untreated or symptomatic brain metastases, receipt of an organ allograft or prior cell transfer therapy consisting of a lymphodepleting regimen, current steroid therapy, active illness, primary immunodeficiency, and pregnancy or breastfeeding.

Lifileucel manufacturing and infusion

Eligible patients underwent resection of a tumor(s) measuring a minimum of 1.5 cm in diameter postresection in aggregate diameter, which was prosected (ie, trimmed and fragmented) and shipped to a centralized good manufacturing practice (GMP) facility. The manufacture of lifileucel by a 22-day GMP process involves the ex vivo expansion of the TIL cells in the presence of IL-2, OKT3, and irradiated allogeneic PBMC feeder cells, followed by harvesting, formulation, cryopreservation, and shipment to the clinical site for infusion.

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Treatment Regimen

Patients received an NMA-LD regimen consisting of cyclophosphamide (60 mg/kg) daily for two days followed by fludarabine (25 mg/m^2) daily for five days. The cryopreserved lifileucel autologous TIL product was thawed and administered as a single infusion approximately 24 hours after the last dose of fludarabine. Lifileucel infusion was followed by up to 6 doses of intravenous IL-2 (600,000 IU/kg) approximately every 8 to 12 hours, with the first dose administered between 3 and 24 hours after completion of the TIL infusion.

Study Endpoints

The primary endpoints of the study were ORR as assessed by investigator per RECIST v1.1 (**Supplementary Figure 1**) and incidence of grade ≥3 TEAEs (defined as AEs that occurred from the time of TIL infusion, up to 30 days after TIL infusion or start of a new anticancer therapy). The secondary endpoints were complete response (CR) rate, duration of response (DOR), progression-free survival (PFS), and overall survival (OS). ORR was defined as the proportion of patients who achieved either a confirmed PR or CR as BOR, as assessed by the investigator per RECIST v1.1. Additionally, according to RECIST v1.1, FDG-PET was used to upgrade a response to a CR in cases where it was difficult to distinguish residual disease from normal tissue (31). DOR was measured from the first time the response criteria (PR/CR) were met until the date of progressive disease documentation or death. Patients not experiencing progressive disease or who did not die prior to data cut or the final database lock had their event times censored on the last date that an adequate tumor assessment was made before the start of a new anticancer therapy. Exploratory endpoints included assessment of in vivo persistence of T cells comprising the TIL product and predictive and pharmacodynamic biomarkers of clinical response to TIL therapy.

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Assessment Schedule

Tumor response assessments by investigator using CT with contrast of the chest and abdomen were performed at week six (Day 42), then every six weeks until month six (Week 24), and every three months thereafter until disease progression or start of a new anticancer therapy, or participation in the study for five years (Month 60) from day 0, whichever occurred first. Consistent with RECIST v1.1, all reported responses (CR or PR) were confirmed by a subsequent CT scan.

TEAEs and SAEs of any attribution were assessed from the time of enrollment until 30 days after the last dose of study treatment (lifileucel infusion); during long-term follow-up, only SAEs related to lifileucel were collected. TEAEs were assessed per the Common Terminology Criteria for Adverse Events (CTCAE) v4.03. AE summaries were based on patient incidence counts and their related percentages, with separate listings for severity and investigator-assessed relationship with study treatment.

Tumor Tissue Collection for Gene Mutations and Protein Expression Levels

If adequate tissue was available during tumor tissue resection for TIL manufacturing, tissue material for studying gene mutations (e.g., *EGFR, ALK, ROS*), and protein levels (e.g., PD-L1 testing) was obtained at the same time and from the same anatomic location(s) as the material harvested for TIL generation. Tumor samples were processed to obtain formalin-fixed paraffin-embedded (FFPE) samples.

Assessment of Tumor PD-L1 status

When available, results of PD-L1 TPS assessment were provided to the sponsor in the screening enrollment packet. In addition to the historical TPS score, if sufficient tumor was available at the time of resection for TIL manufacturing, FFPE tumor blocks were prepared and analyzed for PD-L1 levels (PD-L1 22C3 pharmDx Pan Tumor assay, Neogenomics, Fort Myers, FL).

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Next Generation Sequencing (NGS) of Tumor Tissues and ctDNA for Detection of Mutations and TMB

Sample processing from FFPE tissue, library preparation, hybrid capture, and NGS were performed at Personal Genome Diagnostics, Inc. (PGDx; Baltimore, MD). NGS to assess for mutations and TMB was performed on DNA isolated from FFPE tumor samples using the PGDx elio^{TM} tissue complete RUO assay. NGS of ctDNA was performed using the PGDx elio^{TM} plasma complete assay. Whole blood was collected into ctDNA BCT (Streck, Inc., La Vista, NE), a blood collection tube that stabilizes cell-free DNA. Plasma was isolated according to manufacturer's recommendations and stored at -80°C until DNA extraction. DNA extraction, library preparation, and sequencing were performed at the PGDx laboratory.

TCR Repertoire Analysis

In vivo persistence of T cells comprising lifileucel was assessed by monitoring the presence of TIL product-specific TCR β chain CDR3 sequences in the patient's blood over time, as previously described (32,33). TIL product CDR3 sequences were also assessed in patients' tumors. Briefly, the TCR repertoire of the lifileucel lots and corresponding tumor (FFPE), and pre- and post-infusion peripheral blood mononuclear cell (PBMC) samples from patients who underwent tumor resection for the purpose of lifileucel manufacturing were established by RNA-seq: Total RNA was extracted, using Qiagen's RNeasy Mini Kit protocol. TCR β CDR3 was amplified and sequenced by NGS, using iRepertoire technology (Huntsville, AL). Unique CDR3 sequences were identified and quantified, using iRepertoire's proprietary algorithms. Further analyses, including normalization and filtering clonotypes for limit of detection, followed by the assessment of clonality, diversity and samples' TCR repertoire overlaps were performed using custom scripts, developed in Python (Python Software Foundation, Fredericksburg, VA).

TIL Infusion Product Characterization by Flow Cytometry

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To characterize the final TIL infusion products, cells were stained for markers, including CD3 BV711, CD8 BV786, CD27 BV605, CD28 BB515 (all from BD Biosciences, San Jose, CA); CD4 PE-Cy7, CD45RA AF700, CCR7 PE (all from BioLegend, San Diego, CA) in one panel, and markers including CD3 BUV395, CD8 BB515 (all from BD Biosciences, San Jose, CA), CD4 VioGreen (Miltenyi, Bergisch Gladbach, Germany), PD-1 BV421, TIM-3 BV650 (BioLegend, San Diego, CA), and LAG3 APC-eFluor 780, TIGIT PerCP-eFluor 710 (eBiosciences, San Diego, CA), in a second panel. Dead cells were excluded using LIVE/DEAD Fixable Blue Dead Cell Stain Kit from ThermoFisher Scientific (Waltham, MA). Cells were acquired on a ZE5 analyzer (BioRad, Hercules, CA) and analyzed using the FlowJo™ software (Tree Star, Ashland, OR).

Statistical Analysis

The ORR and CR rate were summarized using point estimates and two-sided 90% confidence intervals (Cis) based on the Clopper-Pearson exact method. Kaplan-Meier methods were used to summarize time-to-event efficacy endpoints, such as DOR. Safety analyses were descriptive and based on the summarization of TEAEs. Efficacy and safety analyses were based on the FAS, defined as those patients who received lifileucel infusion in Cohort 3B. A sample size of 28 patients would allow an estimation of ORR with a half-width 90% CI <0.17 by the Clopper Pearson exact method.

Data Availability Statement

The data relevant to the study are included within the article and its supplementary data files.

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595 **Table 1**

Characteristic	**Full Analysis Set (N = 28)**
Median age (range), years	61 (40–74)
Sex, n (%)	
Male	14 (50)
Female	14 (50)
Smoking history	
Smoker (current or former)	24 (85.7)
Never	4 (14.3)
Baseline ECOG performance status, n (%)	
0	9 (32.1)
1	19 (67.9)
NSCLC histology, n (%)	
Adenocarcinoma	22 (78.6)
Squamous cell carcinoma	5 (17.9)
Large cell carcinoma	1 (3.6)
PD-L1 status,[a] n (%)	
TPS <1%	6 (21.4)
TPS 1%–49%	11 (39.3)
TPS ≥50%	9 (32.1)
Missing	2 (7.1)
NSCLC stage at study entry	
IIIA	1 (3.6)
IVA	13 (46.4)
IVB	14 (50.0)
Prior brain metastases	10 (35.7)
Prior liver metastases	6 (21.4)
Liver and/or brain metastases, n (%)	13 (46.4)
Median target lesion SOD (range), mm	79 (22–179)
Median number of baseline target and non-target lesions	4.5 (2, 11)
Median number of therapies (range)	2 (1, 6)
Prior adjudicated systemic therapies (by agent type), n (%)	
Immunotherapy	28 (100)
Chemotherapy	27 (96.4)
Monoclonal antibody[b]	8 (28.6)
Targeted therapy[c]	2 (7.1)
Prior adjudicated therapy (by category), n (%)	
Anti–CTLA-4	6 (21.4)
Anti–PD-L1	7 (25.0)
Anti–PD-1	23 (82.1)

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Anti–PD-1 and/or anti–PD-L1	28 (100)
Anti-VEGF	6 (21.4)
EGFR inhibitor	1 (3.6)
Other	4 (14.3)
Primary refractory to last anti–PD-1/PD-L1 therapy	7 (25%)
Resected tumor site, n (%)	
Lung	17 (60.7)
Liver	2 (7.1)
Lymph node	3 (10.7)
Spleen	1 (3.6)
Subcutaneous	1 (3.6)
Other[d]	4 (14.3)

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597 **Table 2**

TEAES (≥20%) Preferred term	Full Analysis Set (N = 28)		
	Any Grade n (%)	Grade 3/4 n (%)	Grade 5 n (%)
Number of patients reporting ≥1 TEAE	28 (100.0)	27 (96.4)	2 (7.1)[b]
Chills	19 (67.9)	1 (3.6)	0
Hypotension	18 (64.3)	7 (25)	0
Pyrexia	16 (57.1)	1 (3.6)	0
Hypoxia	15 (53.6)	5 (17.9)	0
Alopecia	10 (35.7)	0	0
Diarrhea	10 (35.7)	3 (10.7)	0
Peripheral edema	10 (35.7)	0	0
Decreased appetite	9 (32.1)	3 (10.7)	0
Dyspnea	9 (32.1)	3 (10.7)	0
Fatigue	9 (32.1)	4 (14.3)	0
Febrile neutropenia	8 (28.6)	8 (28.6)	0
Nausea	8 (28.6)	1 (3.6)	0
Hypertension	7 (25.0)	6 (21.4)	0
Hypokalemia	7 (25.0)	2 (7.1)	0
Sinus tachycardia	7 (25.0)	0	0
Vomiting	7 (25.0)	0	0
Constipation	6 (21.4)	0	0
Capillary leak syndrome	6 (21.4)	1 (3.6)	0
Headache	6 (21.4)	0	0
Pleural effusion	6 (21.4)	1 (3.6)	0
Weight decreased	6 (21.4)	0	0
Hematologic laboratory abnormalities **Preferred Term**	**Full Analysis Set (N = 28)** **Grade 3/4**		
Low leukocytes	28 (100)		
Low lymphocytes	28 (100)		
Low neutrophils	28 (100)		
Low platelets	27 (96.4)		
Low hemoglobin	19 (67.9)		

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599 **Table 3**

Response (RECIST v1.1)	**Full Analysis Set (N = 28)**
ORR, n (%) (95% CI)	6/28 (21.4) (8.3–41.0)
BOR, n (%)	
CR[a]	1/28 (3.6)
PR	5/28 (17.9)
SD	12/28 (42.9)
PD	6/28 (21.4)
Non-evaluable	4/28 (14.3)
DOR, months (range)	1.1+ to 26.2+
DOR for patient with CR, months	26.2+
DOR for patients with PR, months	8.7+, 4.2, 2.6, 2.4, 1.1+
Median duration of study follow-up, months (range)	16 (0.1+ to 27.6)

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Table Legends

Table 1. Patient demographics and baseline characteristics

[a]Per central laboratory from tumor tissue resection specimen, except for four patients who had TPS assessed locally using archival tumor sample.
[b]Includes sacituzumab govitecan, bevacizumab, vopratelimab, ramucirumab, BMS-986016, BMS-986253, and daratumumab.
[c]Includes erlotinib and PF-06647020 (clinical trial).
[d]Other resection sites included 1 adrenal gland, 1 pleura, 2 soft tissues.
CTLA-4, cytotoxic T-lymphocyte associated protein 4; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; SOD, sum of diameters; TPS, tumor proportion score; VEGF, vascular endothelial growth factor.

Table 2. Non-hematologic treatment-emergent adverse events in ≥20% of patients[a] and Grade 3/4 hematologic laboratory abnormalities

[a]Among AEs of interest related to ACT, there were no reported cases of ICANS; one patient with fever on the day of TIL infusion was reported to have grade 1 cytokine release syndrome per investigator, although the etiology remains indistinguishable from an infusion reaction.
[b]One grade 5 TEAE was reported by investigator for cardiac failure (not related to any study therapy), with alternative causality reported as disease-related, and another one for multiple organ failure (possibly related to lifileucel), with sepsis reported as alternative causality.
ACT, adoptive cell therapy; AE, adverse event; ICANS, immune effector cell-associated neurotoxicity syndrome; TEAE, treatment-emergent adverse event; TIL, tumor-infiltrating lymphocyte.

Table 3. Efficacy outcomes by investigator assessment
[a]Metabolic CR.
+, censored; BOR, best overall response; CI, confidence interval; CR, complete response; DOR, duration of response; ORR, objective response rate; PD, progressive disease; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease.

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Figure Legends

Figure 1. Efficacy outcomes as assessed by investigator (RECIST v1.1) in the Full Analysis Set (**A**) Best percentage change from baseline in target lesion sum of diameters. For Patient 3B-02, the best overall response of CR was based on investigator assessment of a complete metabolic response via negative FDG-PET scan. (**B**) Percentage change from baseline in target lesion sum of diameters.★ denotes metabolic CR. (**C**) Time to initial response, time on efficacy assessment for confirmed responders. Each bar is presented for each patient starting from date of lifileucel infusion to the date of new anti-cancer therapy, end of assessment, death, or data cutoff date, whichever occurs earlier. *Tumor sample from patient 3B-28 was assessed only by site-reported testing for actionable driver mutations.
CR, complete response; FDG-PET, fluorodeoxyglucose-positron emission tomography; ICI, immune checkpoint inhibitor; PD, progressive disease; PD-L1, programmed death ligand 1; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease; TPS, tumor proportion score.

Figure 2. CT scans from before and 6 weeks after TIL treatment in a 41-year-old man with stage IV mucinous lung adenocarcinoma. Tumor harbors KRAS G12D mutation, TMB of 3.3 mt/Mb and PD-L1 expression of 0%. Prior progression on 3 lines of therapy including carboplatin, paclitaxel, and pembrolizumab after initial response. CT scan demonstrated 81% PR by RECIST v1.1 criteria at 12 weeks after TIL infusion. TMB, tumor mutational burden. PR, partial response. TIL, tumor-infiltrating lymphocytes

Figure 3. Mutation landscape and TMB. (**A**) Mutation landscape showing genes of interest*. *Based on mutation profiling of patient tumor samples collected during the study. Percentage of patients with alterations in each gene is shown on the Y-axis. †Actionable driver oncogenes include *KRAS*, *EGFR*, *RET*, *BRAF*, *MET*, *ALK*, and *ROS1*. Mutations of genes of interest are colored by alteration type. Percentage of patients with alterations in each gene is shown on the left. Stacked bar of number of patients with each alteration type in each gene is shown on the right. Response (responder, non-responder), and best overall response (CR, PR, SD, PD, NE) are shown on the top. Bar plot of tumor mutational burden is shown on the top**.** (**B**) TMB distribution at baseline. TMB values (mut/Mb sequenced regions of interest) obtained from the PGDx targeted sequencing assay are shown as a violin plot. (**C**) TMB exome equivalent by patient response. TMB values (mut/Mb sequenced regions of interest) from PGDx were divided by a factor of 1.647 using a calibration approach by Vega et al (13) to derive TMB exome equivalent values. Derived TMB values grouped by response are shown in the boxplot, with *P*-value from Kruskal-Wallis test.
BOR, best overall response; CR, complete response; NE, non-evaluable; PD, progressive disease; PR, partial response; ROI, region of interest; SD, stable disease; TMB, tumor mutational burden.

Figure 4. Diversity, clonality, proportion of overlapping clones, or persistence of patient-specific TCR clones (**A**) TCR repertoire profile across samples. *Denotes statistically significant difference between the samples. Violin plots of the Simpson Clonality Index are shown for the tumor, TIL products, and pre- and post-infusion blood. Pre-infusion blood samples were collected at Day -7 and post-infusion blood samples at Day 42. Simpson Clonality Index reflects mono- or poly-clonality of a sample and is inversely related to diversity (Shannon Entropy Index). Values can range from 0 (evenly distributed, polyclonal sample) to 1 (monoclonal sample). Significantly differing populations were determined with the correction for multiple comparisons by controlling false discovery using two-stage linear step-up procedure of Benjamini, Krieger, and Yekutieli and are indicated with *(P<0.05). (**B**) Individual patient

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data showing exclusive and shared clones between tumor and TIL infusion product. Comparison of TCR clones in the TIL infusion product with TCR clones in the baseline tumor. Total number of CDR3vβ clones are shown on the Y-axis. Unique CDR3vβ clone counts, assessed by RNA sequencing of the TCR repertoire, are identified as shared (dark blue) or specific to individual tumor FFPE samples (light blue) or the respective TIL product lots (green) are plotted for all patients. (**C**) TCR clonal expansion and persistence. Tumor samples collected at the time of resection were analyzed and compared with the TIL products infused and blood samples from pre- and post-infusion timepoints. Overlapping (shared) and unique clonotypes between the tumor sample and the TIL infusion product were analyzed. (**D**) TIL clones in pre-infusion (Day -7) and post-infusion blood (Day 42). The TIL clonotypes were also assessed for their contribution to the total TCR repertoire in the pre- and post- infusion blood.
TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte; uCDR3, unique complementarity-determining region 3.

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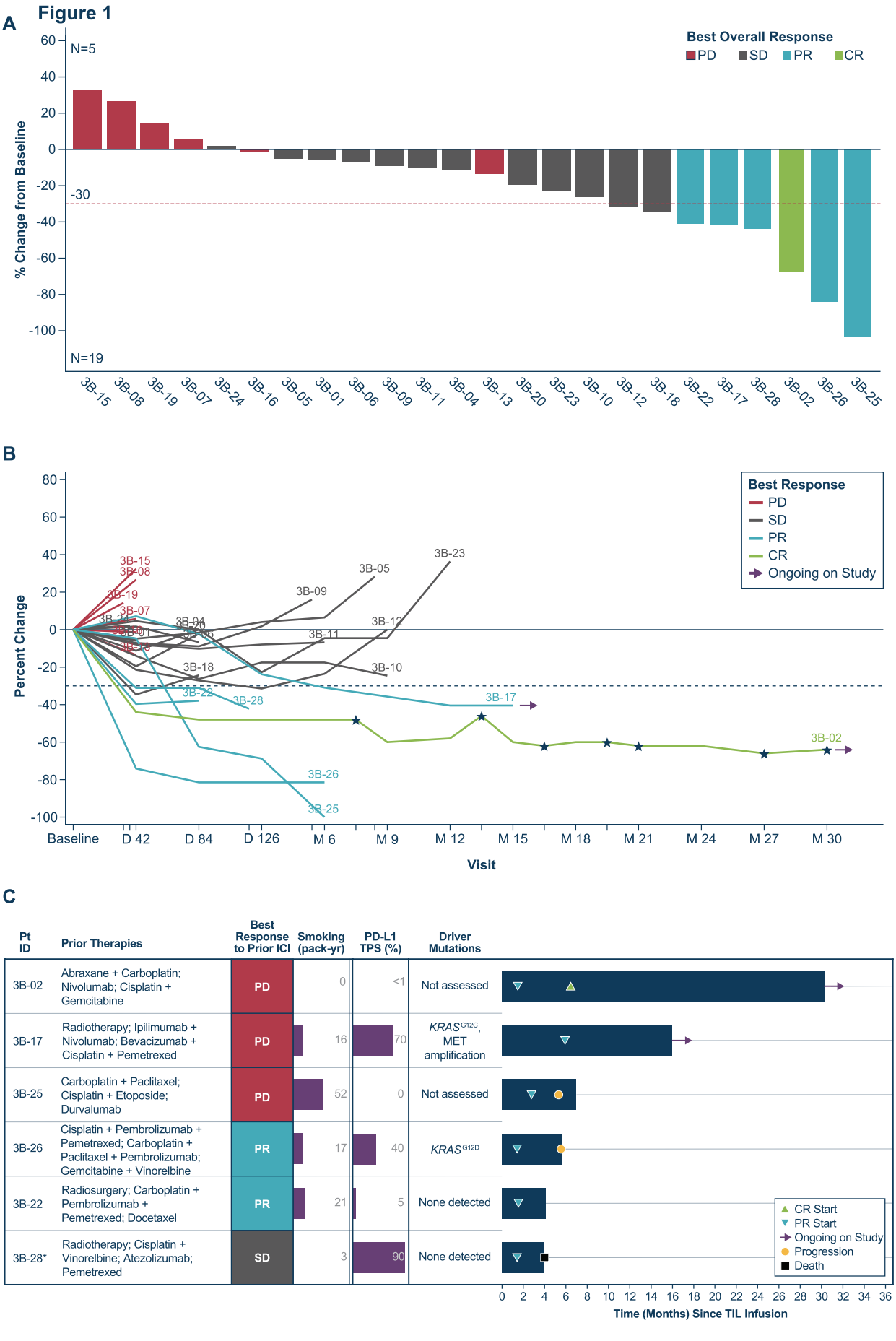
Figure 1



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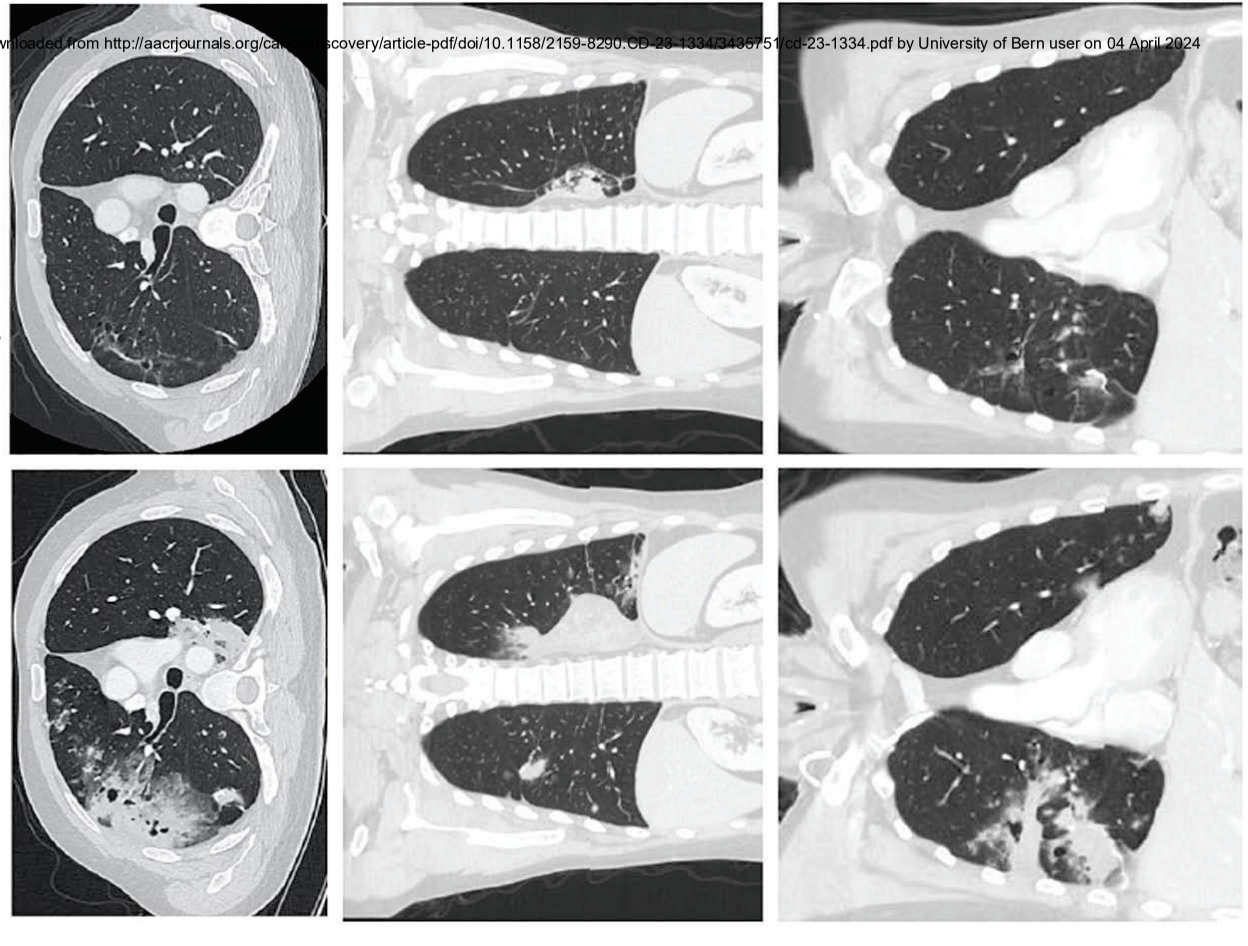

Figure 2

Figure 3

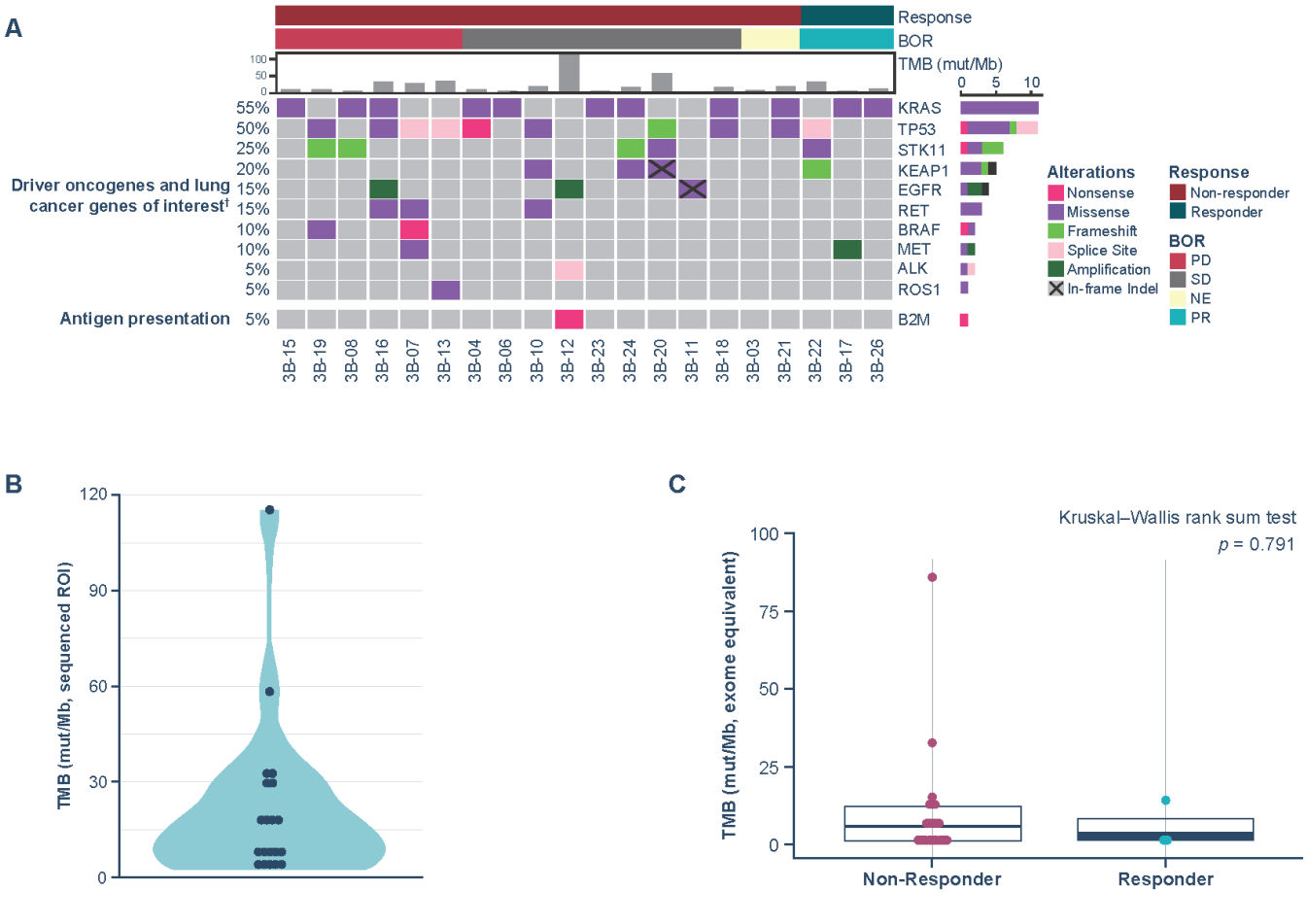

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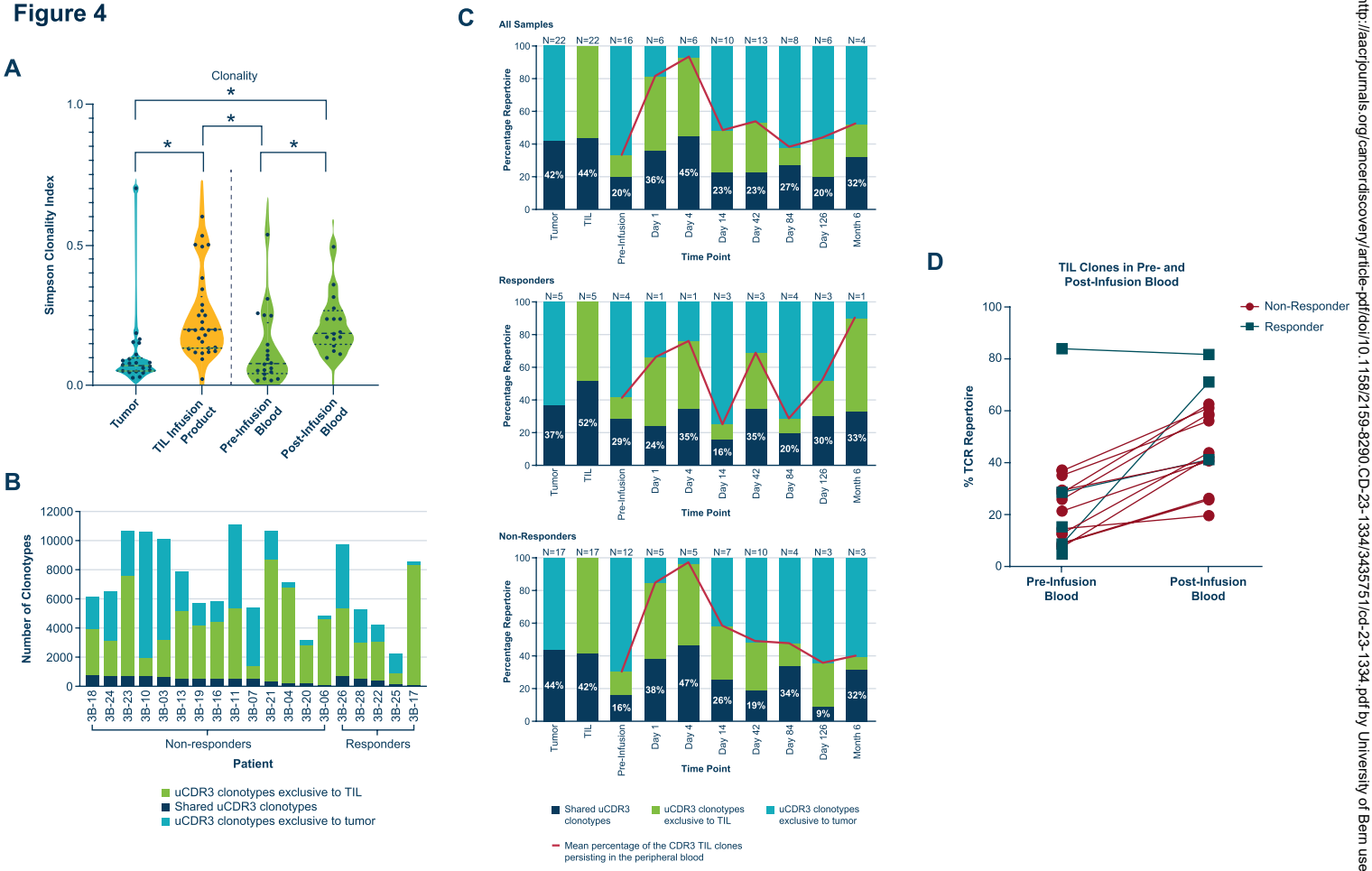
Figure 4



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