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

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

87 **Introduction**

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

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

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

112 These questions have been particularly crucial in patients with mNSCLC. While the combination of
113 chemotherapy plus ICI has revolutionized treatment outcomes, there remains notable unmet medical
114 need in patients with PD-L1–negative tumors and those with primary or acquired resistance to ICI. In
115 preclinical studies, TIL have been isolated and amplified ex vivo from primary NSCLC lesions and
116 demonstrated antitumor activity (9). Additionally, preliminary activity was observed in a phase 1 study
117 of TIL cell therapy in combination with anti-programmed death 1 (anti–PD-1) pathway inhibition in
118 patients with mNSCLC (10). In this context, we report results from a phase 2 multicenter study
119 investigating lifileucel in patients with mNSCLC who were previously treated with ICI therapy.
120

121 **Results**

122 **Patient Disposition and Disease Characteristics**

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

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

133 with anti–PD-1/PD-L1 antibodies, and 27 (96.4%) patients had received ≥1 line of cytotoxic
134 chemotherapy **(Table 1)**. One-quarter (25% [7/28]) of patients in the FAS had disease that was primary
135 refractory to last anti–PD-1/PD-L1 therapy (best response of progressive disease to last anti–PD-1/PD-L1
136 therapy). Patients had multiple tumors (median number of target/non-target lesions, 4.5 [range, 2–11])
137 and median target lesion sum of diameters (SOD) of 79 mm [range, 22–179]). Ten patients (35.7%) had
138 prior brain metastases, and six (21.4%) had liver metastases at baseline (13 [46.4%] had prior brain
139 and/or liver metastases). PD-L1 expression was low (PD-L1 tumor proportion score [TPS] 1%–49%) or
140 negative (PD-L1 TPS <1) in more than half of the tumors (60.7%) **(Table 1)**.
141

142 **Feasibility and Safety of Tumor Tissue Procurement Surgery**

143 Tumor tissue procurement surgery was generally well tolerated **(Supplementary Table 1**), with a median
144 duration of hospitalization after resection of 2 days (range, 1–6). No patient had TIL cell therapy
145 cancelled due to a surgery-related adverse event (AE). In the Tumor Harvest Set (*N* = 39), 7 grade 3/4
146 resection-related AEs occurred in five (12.8%) patients (ie, constipation, hypertension, hypotension,
147 hypoxia, non-cardiac chest pain, pneumothorax, subcutaneous emphysema). The most common site of
148 tumor tissue resection for TIL manufacturing was lung (60.7%); multiple other metastatic sites were
149 successfully used for manufacturing of lifileucel, including lymph node, liver, pleura, adrenal gland, and
150 spleen (**Table 1**).
151

152 **Treatment Administration and Safety**

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

157 The safety profile was consistent with the advanced disease and known profiles of NMA-LD and IL-2
158 (**Table 2, Supplementary Figure 2)** (6,8). All patients experienced grade 3/4 hematologic laboratory
159 abnormalities with first onset date during the period from the start of NMA-LD to 30 days after lifileucel
160 infusion; these events resolved to baseline in 93% of patients with low neutrophils and low leukocytes,
161 82% of patients with low lymphocytes, 85% of patients with low platelets, and 79% of patients with low
162 hemoglobin.

163 Two patients died of treatment-emergent adverse events. A 60-year-old woman died of cardiac failure 6
164 days after last NMA-LD dose, 3 days after lifileucel infusion, and 3 days after last IL-2 dose. The
165 investigator reported this event as not related to any study therapy. Her history of cigarette smoking,
166 apnea syndrome, and recent pulmonary embolism contributed to respiratory insufficiency due to
167 underlying advanced study disease. Fluid overload may have precipitated her heart failure and possibly
168 contributed to respiratory insufficiency. A 61-year-old woman died of multiple organ failure 2 days after
169 last NMA-LD dose, 1 day after lifileucel infusion, and no IL-2 was administered. The investigator reported
170 this event as possibly related to lifileucel, with sepsis reported as an alternative causality. The patient
171 experienced hypotensive episodes and acute respiratory failure and pneumonia (consistent with history
172 of chronic obstructive pulmonary disease) requiring intubation and ventilation before lifileucel infusion.
173 Subsequently, multi-organ failure and hypotension were reported in the setting of pneumonia due to
174 aspiration and possible sepsis.
175

176 **Efficacy**

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

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.

205 Two responders previously had PR as best response to prior anti–PD-1/PD-L1 blockade, and four
206 responders had progressive disease or SD as best response to prior anti–PD-1/PD-L1 blockade. Upon
207 treatment with lifileucel, one patient with a PD-L1–negative tumor attained a complete metabolic
208 response as assessed by PET/CT scan. Another patient achieving a PR also had a PD-L1–negative tumor.
209 The remaining four patients with PR had PD-L1–positive tumors with TPS between 5% and 90% **(Figure**
210 **1C)**.

211 Baseline tumor samples from 20 patients were available for genomic analysis. Overall, key oncogenic
212 driver mutations were seen in 13 patients (responders and non-responders) – *KRAS* mutations (including
213 one *KRAS* ^{G12C}) were seen in the tumors from 11 patients, and *EGFR* alterations (including *EGFR* gene
214 amplification, as well as exon 19 deletion and T790M mutation) were identified in the tumors from 3
215 patients; one patient's tumor (3B-16) harbored both *KRAS* and *EGFR* mutations (**Figure 3A**). Among the
216 responders, one patient's tumor (3B-17, PR) harbored the *KRAS* ^{G12C} point mutation and *MET*
217 amplification, and one patient's tumor (3B-26, PR) harbored the *KRAS* ^{G12D} mutation. Tumors of patients
218 3B-02 and 3B-25 were not assessed for mutations, and tumors of patients 3B-22 and 3B-28 showed no
219 detectable actionable driver mutations (**Figures 1C and 3A**). The tumor from patient 3B-22 did have
220 mutations in *STK11* and *KEAP1*, which are typically associated with poor outcomes to anti-PD-1/PD-L1
221 therapy (11,12).

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

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

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

233

234 **Phenotype of TIL Infusion Product and T-Cell Clonal Dynamics**

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

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

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

254 Evidence of peripheral TCR repertoire remodeling was observed, with a higher proportion of the TCR
255 repertoire derived from TIL infusion product clones in post-infusion than in pre-infusion blood in nearly
256 all patients, regardless of response (**Figure 4D**).
257

258 **Discussion**

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

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

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

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

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

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

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

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

339 In summary, TIL cell therapy represents a feasible, individualized, and polyclonal potential treatment
340 option for patients with mNSCLC. This is the first study to demonstrate the efficacy and safety of
341 centrally manufactured autologous TIL cell therapy as a single modality in patients with mNSCLC after
342 treatment with anti–PD-1/PD-L1 therapy. These results are encouraging and warrant further
343 investigation of lifileucel in patients with mNSCLC.
344

345 **Methods**

346 **Study Design**

347 IOV-COM-202 (NCT03645928) is a prospective, open-label, multicohort, non-randomized, multicenter
348 phase 2 study evaluating the efficacy and safety of lifileucel in combination with ICI and as a
349 monotherapy in multiple solid tumors. The study consists of 7 cohorts spanning advanced (unresectable
350 or metastatic) melanoma (Cohorts 1A, 1B, 1C); advanced, recurrent, or metastatic head and neck
351 squamous cell carcinomas (HNSCC; Cohort 2A), and mNSCLC (Cohorts 3A, 3B, 3C). Data from cohort 3B,
352 which evaluated lifileucel monotherapy in previously treated (1–3 prior systemic therapies) patients
353 with mNSCLC, are reported here. The treatment schema is shown in **Supplementary Figure** 5).
354 **Supplementary Table 3** provides information about the representativeness of the study population in
355 relation to the population at large.
356

357 Written informed consent was obtained from all patients. The study was conducted in full compliance
358 with the principles of the Declaration of Helsinki (as amended in Tokyo, Venice, Hong Kong, and South
359 Africa), ICH guidelines, and with the laws and regulations of the country in which the research was
360 conducted. Institutional review boards provided initial approval and continuing review of the study.
361

362 **Inclusion Criteria**

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

372

373 **Exclusion Criteria**

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

377

378 **Lifileucel manufacturing and infusion**

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

385

386 **Treatment Regimen**

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

393

394 **Study Endpoints**

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

409

410 **Assessment Schedule**

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

416 TEAEs and SAEs of any attribution were assessed from the time of enrollment until 30 days after the last
417 dose of study treatment (lifileucel infusion); during long-term follow-up, only SAEs related to lifileucel
418 were collected. TEAEs were assessed per the Common Terminology Criteria for Adverse Events (CTCAE)
419 v4.03. AE summaries were based on patient incidence counts and their related percentages, with
420 separate listings for severity and investigator-assessed relationship with study treatment.
421

422 **Tumor Tissue Collection for Gene Mutations and Protein Expression Levels**

423 If adequate tissue was available during tumor tissue resection for TIL manufacturing, tissue material for
424 studying gene mutations (e.g., *EGFR, ALK, ROS*), and protein levels (e.g., PD-L1 testing) was obtained at
425 the same time and from the same anatomic location(s) as the material harvested for TIL generation.
426 Tumor samples were processed to obtain formalin-fixed paraffin-embedded (FFPE) samples.
427

428 **Assessment of Tumor PD-L1 status**

429 When available, results of PD-L1 TPS assessment were provided to the sponsor in the screening
430 enrollment packet. In addition to the historical TPS score, if sufficient tumor was available at the time of
431 resection for TIL manufacturing, FFPE tumor blocks were prepared and analyzed for PD-L1 levels (PD-L1
432 22C3 pharmDx Pan Tumor assay, Neogenomics, Fort Myers, FL).

433

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

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

442

443 **TCR Repertoire Analysis**

444 In vivo persistence of T cells comprising lifileucel was assessed by monitoring the presence of TIL
445 product-specific TCR β chain CDR3 sequences in the patient's blood over time, as previously described
446 (32,33). TIL product CDR3 sequences were also assessed in patients' tumors. Briefly, the TCR repertoire
447 of the lifileucel lots and corresponding tumor (FFPE), and pre- and post-infusion peripheral blood
448 mononuclear cell (PBMC) samples from patients who underwent tumor resection for the purpose of
449 lifileucel manufacturing were established by RNA-seq: Total RNA was extracted, using Qiagen's RNeasy
450 Mini Kit protocol. TCR β CDR3 was amplified and sequenced by NGS, using iRepertoire technology
451 (Huntsville, AL). Unique CDR3 sequences were identified and quantified, using iRepertoire's proprietary
452 algorithms. Further analyses, including normalization and filtering clonotypes for limit of detection,
453 followed by the assessment of clonality, diversity and samples' TCR repertoire overlaps were performed
454 using custom scripts, developed in Python (Python Software Foundation, Fredericksburg, VA).

455

456 **TIL Infusion Product Characterization by Flow Cytometry**

457 To characterize the final TIL infusion products, cells were stained for markers, including CD3 BV711, CD8
458 BV786, CD27 BV605, CD28 BB515 (all from BD Biosciences, San Jose, CA); CD4 PE-Cy7, CD45RA AF700,
459 CCR7 PE (all from BioLegend, San Diego, CA) in one panel, and markers including CD3 BUV395, CD8
460 BB515 (all from BD Biosciences, San Jose, CA), CD4 VioGreen (Miltenyi, Bergisch Gladbach, Germany),
461 PD-1 BV421, TIM-3 BV650 (BioLegend, San Diego, CA), and LAG3 APC-eFluor 780, TIGIT PerCP-eFluor 710
462 (eBiosciences, San Diego, CA), in a second panel. Dead cells were excluded using LIVE/DEAD Fixable Blue
463 Dead Cell Stain Kit from ThermoFisher Scientific (Waltham, MA). Cells were acquired on a ZE5 analyzer
464 (BioRad, Hercules, CA) and analyzed using the FlowJo™ software (Tree Star, Ashland, OR).
465

466 **Statistical Analysis**

467 The ORR and CR rate were summarized using point estimates and two-sided 90% confidence intervals
468 (Cis) based on the Clopper-Pearson exact method. Kaplan-Meier methods were used to summarize time-
469 to-event efficacy endpoints, such as DOR. Safety analyses were descriptive and based on the
470 summarization of TEAEs. Efficacy and safety analyses were based on the FAS, defined as those patients
471 who received lifileucel infusion in Cohort 3B. A sample size of 28 patients would allow an estimation of
472 ORR with a half-width 90% CI <0.17 by the Clopper Pearson exact method.
473

474 **Data Availability Statement**

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

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594

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)

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)

596

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)		

598

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)

600

601 **Table Legends**

602 **Table 1.** Patient demographics and baseline characteristics

603 [a]Per central laboratory from tumor tissue resection specimen, except for four patients who had TPS
604 assessed locally using archival tumor sample.
605 [b]Includes sacituzumab govitecan, bevacizumab, vopratelimab, ramucirumab, BMS-986016, BMS-986253,
606 and daratumumab.
607 [c]Includes erlotinib and PF-06647020 (clinical trial).
608 [d]Other resection sites included 1 adrenal gland, 1 pleura, 2 soft tissues.
609 CTLA-4, cytotoxic T-lymphocyte associated protein 4; ECOG, Eastern Cooperative Oncology Group; EGFR,
610 epidermal growth factor receptor; NSCLC, non-small cell lung cancer; PD-1, programmed cell death
611 protein 1; PD-L1, programmed death-ligand 1; SOD, sum of diameters; TPS, tumor proportion score;
612 VEGF, vascular endothelial growth factor.
613

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

616 [a]Among AEs of interest related to ACT, there were no reported cases of ICANS; one patient with fever on
617 the day of TIL infusion was reported to have grade 1 cytokine release syndrome per investigator,
618 although the etiology remains indistinguishable from an infusion reaction.
619 [b]One grade 5 TEAE was reported by investigator for cardiac failure (not related to any study therapy),
620 with alternative causality reported as disease-related, and another one for multiple organ failure
621 (possibly related to lifileucel), with sepsis reported as alternative causality.
622 ACT, adoptive cell therapy; AE, adverse event; ICANS, immune effector cell-associated neurotoxicity
623 syndrome; TEAE, treatment-emergent adverse event; TIL, tumor-infiltrating lymphocyte.
624

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

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

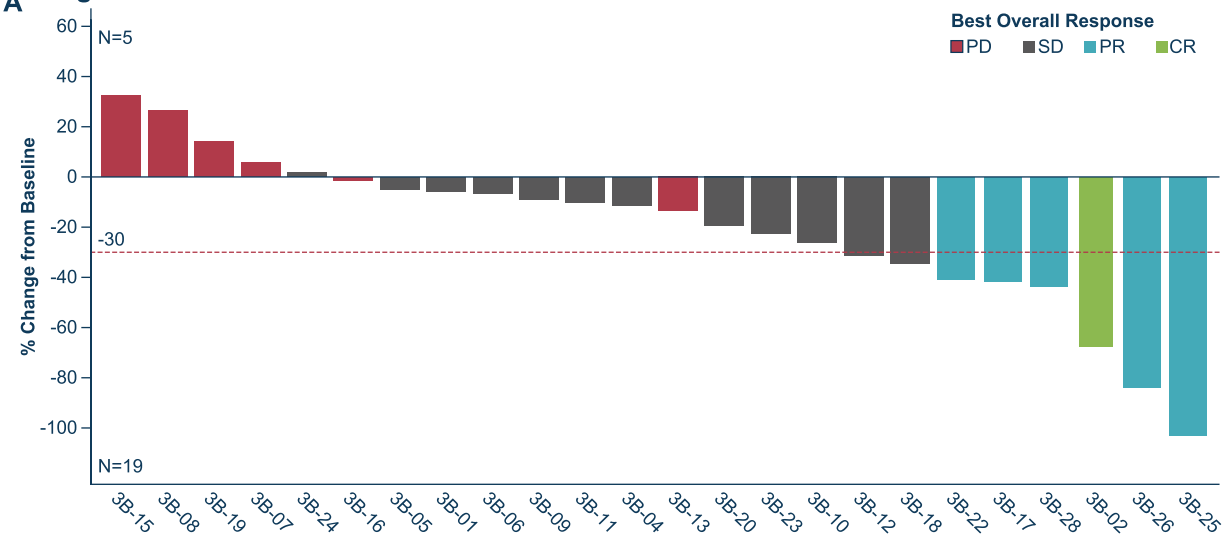
677 data showing exclusive and shared clones between tumor and TIL infusion product. Comparison of TCR
678 clones in the TIL infusion product with TCR clones in the baseline tumor. Total number of CDR3vβ clones
679 are shown on the Y-axis. Unique CDR3vβ clone counts, assessed by RNA sequencing of the TCR
680 repertoire, are identified as shared (dark blue) or specific to individual tumor FFPE samples (light blue)
681 or the respective TIL product lots (green) are plotted for all patients. (**C**) TCR clonal expansion and
682 persistence. Tumor samples collected at the time of resection were analyzed and compared with the TIL
683 products infused and blood samples from pre- and post-infusion timepoints. Overlapping (shared) and
684 unique clonotypes between the tumor sample and the TIL infusion product were analyzed. (**D**) TIL
685 clones in pre-infusion (Day -7) and post-infusion blood (Day 42). The TIL clonotypes were also assessed
686 for their contribution to the total TCR repertoire in the pre- and post- infusion blood.
687 TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte; uCDR3, unique complementarity-determining
688 region 3.
689

690

691

Figure 1

A



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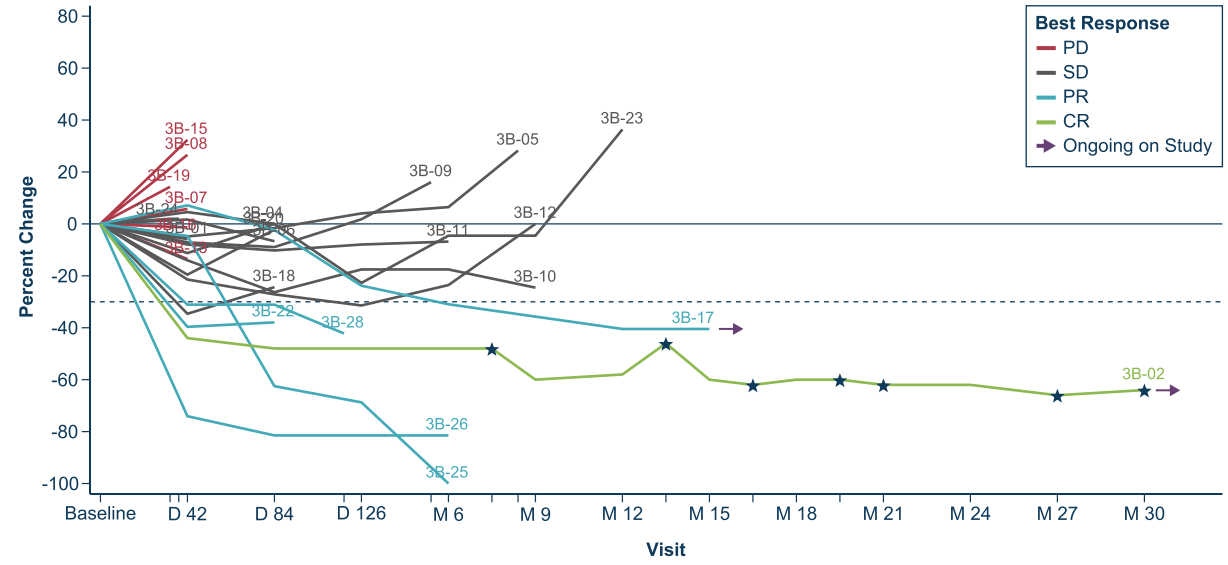

C

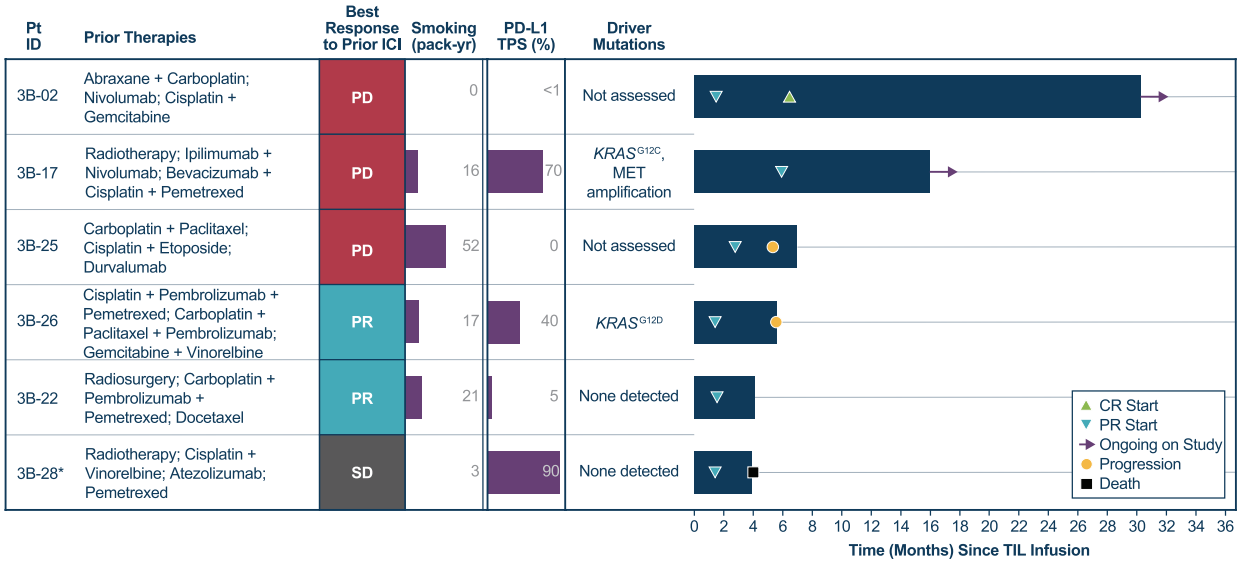

Figure 2

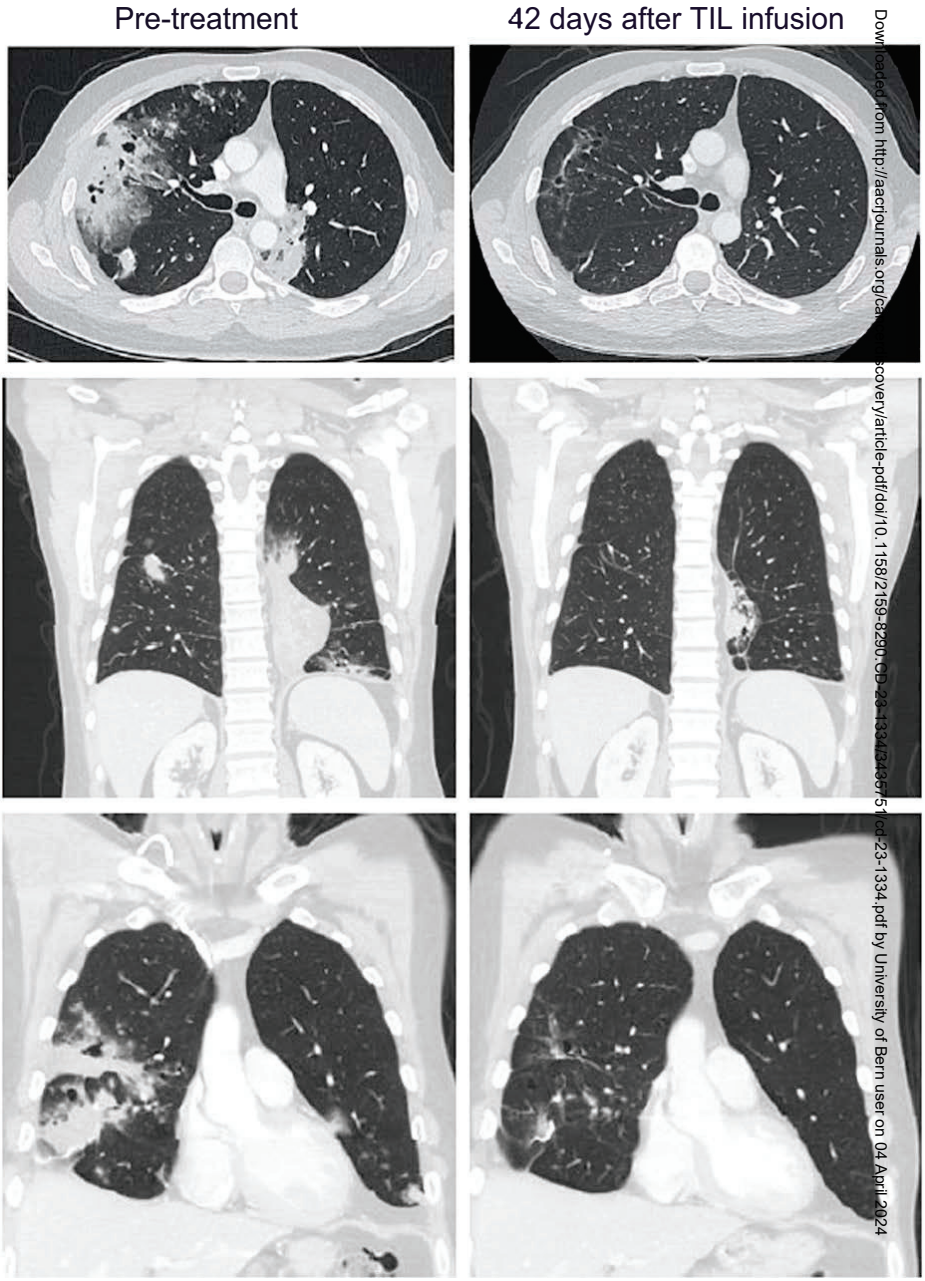

Figure 3

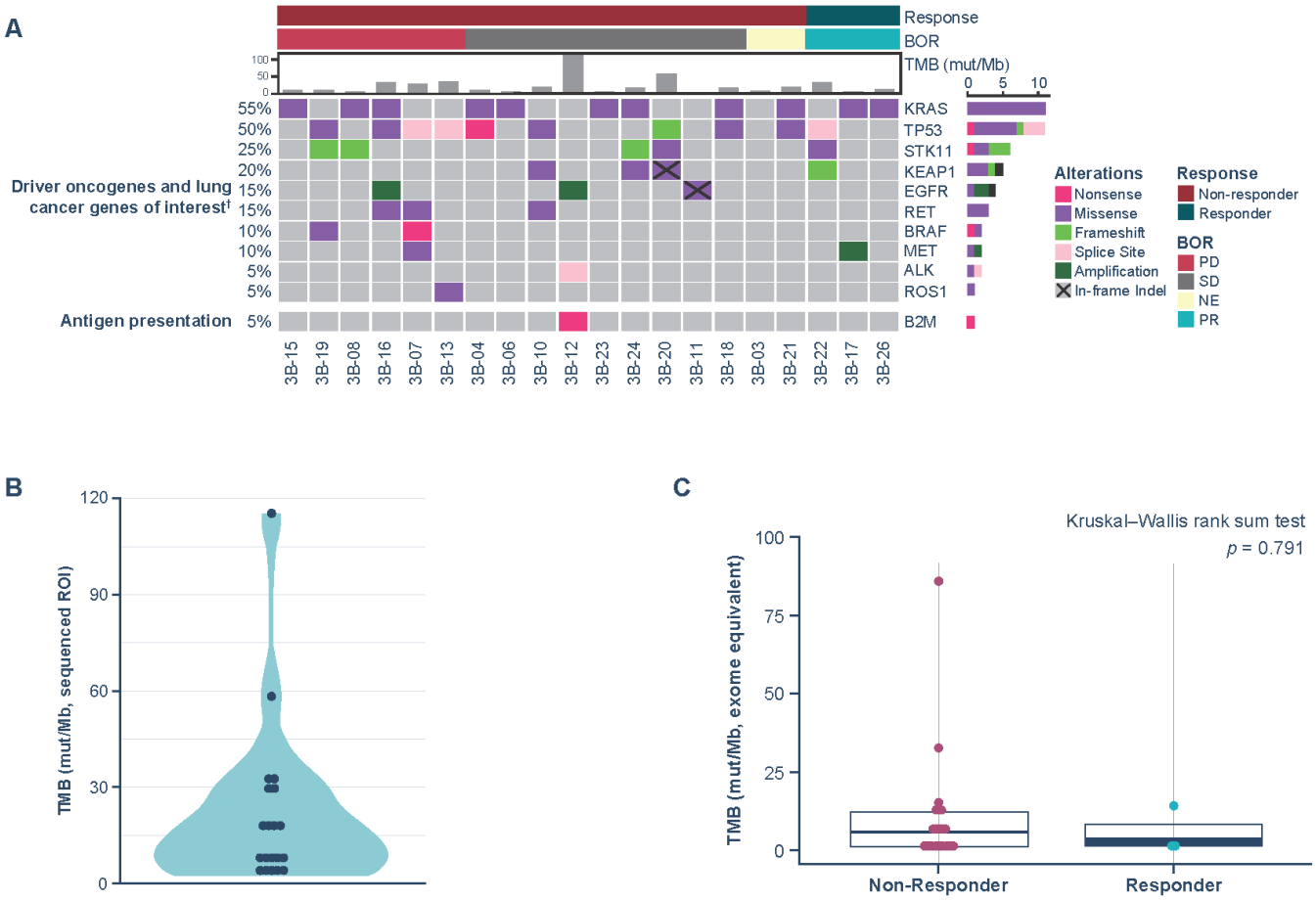

Figure 4