Tumor mutational burden (TMB) in immune checkpoint inhibitor (ICI)-naïve and -experienced patients with metastatic melanoma treated with lifileucel, a tumor-infiltrating lymphocyte (TIL) cell therapy

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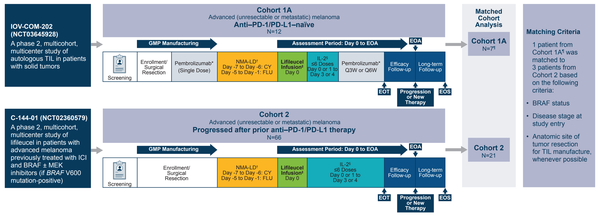
Background

- Cutaneous melanoma is characterized by high tumor mutational burden (TMB; ≥10 somatic mutations per megabase [mut/Mb] of coding DNA), which is associated with increased expression of tumor-specific neoantigens that can be recognized by T cells[1]
- In melanoma, a high TMB genotype is also associated with an increased response rate to immune checkpoint inhibitors (ICI)[2,3]; however, the TMB in tumors that progress or recur after ICI is not well defined
- Lifileucel is a one-time, autologous tumor-infiltrating lymphocyte (TIL) cell therapy under investigation for treatment of patients with advanced (unresectable or metastatic) melanoma in both post-ICI and ICI-naïve settings and has demonstrated encouraging efficacy and an acceptable and consistent safety profile in previous studies[4-6]
- We conducted a retrospective matched cohort comparison analysis of prospectively enrolled patients with advanced melanoma treated with lifileucel in 2 trials (**Figure 1**)
  - IOV-COM-202 trial, Cohort 1A (ICI-naïve patients)
  - C-144-01 trial, Cohort 2 (patients previously treated with ICI and BRAF ± MEK inhibitors [if *BRAF* V600 mutation-positive])
- Herein, we aimed to investigate the potential association between prior ICI therapy, TMB, and response to lifileucel

Methods

Figure 1. Matched Cohort Comparison Analysis



CY, cyclophosphamide; EOA, end of assessment; EOS, end of study; EOT, end of treatment; FLU, fludarabine; GMP, Good Manufacturing Practice; NMA-LD, nonmyeloablative lymphodepletion; PD-1, programmed cell death protein-1; PD-L1, programmed death ligand-1; Q3W, once every 3 weeks; Q6W, once every 6 weeks.

*In Cohort 1A, 1 dose of pembrolizumab (200 mg or 400 mg) administered after tumor resection but before NMA-LD, and then continued pembrolizumab every 3 weeks (200 mg) or 6 weeks (400 mg) for ≤24 months after NMA-LD. †Cyclophosphamide (60 mg/kg) administered once daily for 2 days followed by fludarabine (25 mg/m^2) once daily for 5 days. ‡Lifileucel infusion (1 × 10^9 to 150 × 10^9 cells). §≤6 IL-2 doses (600,000 IU/kg) every 8–12 hours (3–24 h after the completion of lifileucel infusion). ¶Based on number of patients with available data.

Clinical Assessments

- **Response** was assessed by investigators per RECIST v1.1
  - Data cutoff date: January 20, 2022 for Cohort 1A and September 15, 2021 for Cohort 2

Translational Assessments

- **TMB** of the resected tumor was measured using the ImmunoID NeXT Platform™ (Personalis®, Menlo Park, CA, USA). Whole exome sequencing was performed on DNA from formalin-fixed paraffin-embedded (FFPE) tumor samples. Matched peripheral blood mononuclear cell (PBMC) DNA was sequenced as germline controls. Single nucleotide variant (SNV) and short insertion and deletion (indel) calling was performed using Personalis's proprietary methods. TMB was calculated based on SNVs and indels
  - High TMB was defined as ≥10 somatic mut/Mb
- **Tumor neoantigen burden (TNB)** was predicted from SNVs, indels, and fusions detected by exome and transcriptome sequencing using SHERPA™ (Personalis), which integrates human leukocyte antigen (HLA) class I peptide-binding affinity and presentation for neoantigen prediction
- **Neoantigen presentation score (NEOPS™)**, a Personalis-proprietary composite biomarker assessment, is an adjusted neoantigen burden metric for which neoantigens deemed to preferentially bind to major histocompatibility complex (MHC) class I molecules impacted by somatic alterations such as damaging antigen-presentation mutations and HLA loss of heterozygosity (LOH) are removed from consideration[7]
- For **genes of interest**, somatic SNVs and copy number alterations (CNAs) were identified by Sentieon and Personalis tools. Gene variant effects were predicted using the variant annotation and effect prediction tool, SnpEff.[8] SNVs and CNAs were filtered by preferred transcript (with the most clinical evidence in cancer and most cited in COSMIC) and the following criteria: a) present at <1% allele frequency in all control population datasets (such as 1000Genomes, Exome Aggregation Consortium [ExAC] and Exome Sequencing Project [ESP]), b) present at ≥5% tumor allele frequency in the sample, c) moderate or high effect on protein function, and d) present in the Personalis Research Cancer Gene List
- Expression of **gene signatures** previously reported to be associated with response to immunotherapy was explored, as follows:
  - For interferon γ (IFNγ) (6-gene) and expanded immune (18-gene) signature, effector T cell, IFNγ/effector T cell, chemokine, transforming growth factor β (TGFβ), antigen-processing machinery (APM), and β-catenin gene sets, $\log_2$(transcripts per million [TPM] counts + 1) was used; a single gene set score for each gene set and patient was calculated using the z-score method in the Gene Set Variation Analysis (GSVA) R package[9]
  - Melanoma plasticity signature (MPS) score was calculated using the methods described previously[10] using fragments per kilobase of transcript per million reads mapped (FPKM) values of the 45 genes in the signature, with gene weights multiplied by 1 for upregulated genes and -1 for downregulated genes; the values were added and converted to z-scores for the cohort
  - Tumor immune dysfunction and exclusion (TIDE) prediction was carried out on the TIDE platform (https://tide.dfci.harvard.edu). TPM values were transformed to $\log_2$(TPM + 1) values and normalized using the recommended method. For each gene, the mean $\log_2$(TPM + 1) for that gene was subtracted from the $\log_2$(TPM + 1) for each sample. The prior treatment parameter was selected as a subset of the sample that had prior treatment with ICI
  - Tertiary lymphoid structure (TLS) score was calculated as the mean $\log_2$(TPM counts + 1) of genes
  - GSVA scores were converted to z-scores for each gene set
- **T-cell receptor (TCR) repertoire** was analyzed using bulk RNA sequencing data, as follows:
  - Unique complementarity-determining region 3 (uCDR3) sequences (clonotypes) identified in the FFPE tumor (collected at the time of resection) and TIL products were analyzed for their contribution to the total TCR repertoire of the pre- and post-infusion blood (PBMC) samples
  - Shannon Entropy Index was calculated to describe the diversity of the CDR3 population; values can range from 0 (monoclonal sample) to $\log_2$(R) (evenly distributed, polyclonal sample with R unique clones)
  - Simpson Clonality Index (inversely related to diversity [Shannon Entropy Index]) was calculated to describe the mono- or polyclonality of a sample; values can range from 0 (evenly distributed, polyclonal sample) to 1 (monoclonal sample)

Statistical Analysis

- Logistic regression of Cohort 1A and Cohort 2 combined was used to analyze the correlation of TMB, TNB, and NEOPS with response, with cohort as the confounding factor
- Pearson correlation was used to assess the correlation of TMB, TNB, and NEOPS with best change in target lesion sum of diameters (SOD)
- Wilcoxon rank sum test was used to assess correlation of gene signature scores and response

Results

Table 1. Baseline Characteristics of Cohort 1A (IOV-COM-202) and Cohort 2 (C-144-01) Matched Subsets

Characteristic	Cohort 1A (N=7)	Cohort 2 (N=21)
Sex, n (%)		
Female	2 (28.6)	5 (23.8)
Male	5 (71.4)	16 (76.2)
Median age, years (range)	52.5 (45–61)	55.0 (30–70)
Median no. of prior therapies (min, max)	0 (0, 2)	3 (1, 9)
Anti–CTLA-4, n (%)	1 (14.3)	15 (71.4)
Anti–PD-1/PD-L1, n (%)	Not applicable	21 (100)
BRAF/MEK inhibitor, n (%)	2 (28.6)	5 (23.8)
Primary refractory to prior anti–PD-1 or anti–PD-L1, n (%)	Not applicable	12 (57.1)
Mean no. of baseline target and non-target lesions (SD)	4.9 (1.35)	5.5 (2.16)
Mean target lesion SOD, mm (SD)	114.3 (111.98)	111.62 (76.18)
PD-L1 TPS per central laboratory, n (%)		
PD-L1–positive (TPS ≥5%)	3 (42.9)	9 (42.9)
PD-L1–negative (TPS <5%)	3 (42.9)	11 (52.4)

CTLA-4, cytotoxic T-lymphocyte–associated protein 4; TPS, tumor proportion score.

- Baseline characteristics of the patients included in each matched subset are presented in **Table 1**
- Consistent with prior reports from the full cohorts,[5,11] the matched subsets showed that objective response rate (ORR) was higher in ICI-naïve patients in Cohort 1A than in ICI-experienced patients in Cohort 2
  - ORR was 71.4% in Cohort 1A (5 of 7 patients; 2 complete responses) and 38.1% in Cohort 2 (8 of 21 patients; 1 complete response)
- Safety in the matched subsets was consistent with prior reports from the full cohorts[4,5]

Figure 2. TMB Distribution in ICI-Naïve vs ICI-Experienced Patients

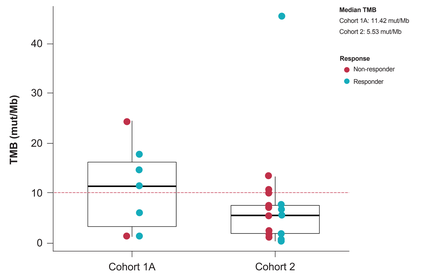


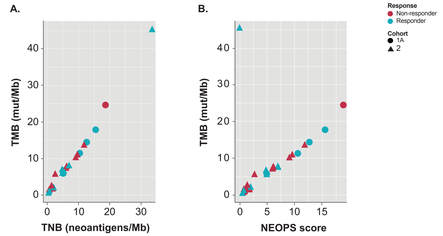
Table 2. Response to Lifileucel by TMB

	Cohort 1A (ICI-Naïve) (N=7)	Cohort 2 (ICI-Experienced) (N=18)*
High TMB,† n (%)	4 (57.1)	3 (16.7)
	p=0.127	
ORR, n/N$_1$ (%)		
Low TMB	2/3 (66.7)	6/15 (40.0)
High TMB†	3/4 (75.0)	1/3 (33.3)

*3 patients in Cohort 2 were missing DNA analyses due to insufficient tissue. †≥10 mut/Mb.

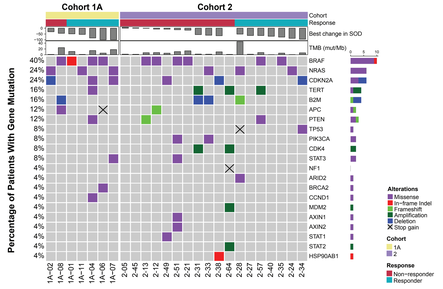
- Among responders, 60% in Cohort 1A and 14.3% in Cohort 2 had high TMB (**Figure 2**)
- A higher proportion of patients who were ICI-naïve had high TMB than those who were ICI-experienced; ORR was similar in low- and high-TMB groups (**Figure 2**, **Table 2**)
- In logistic regression analysis adjusted for cohort, TMB was not associated with response to lifileucel (odds ratio, 1.0; 95% CI, 0.9–1.1; *p*=0.58)

Figure 3. Correlation of TMB With TNB and NEOPS Scores



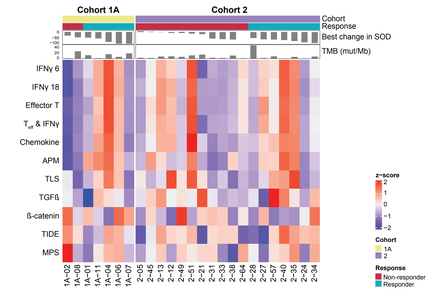
- TMB correlated with predicted TNB (**Figure 3A**) and NEOPS scores (**Figure 3B**) in ICI-naïve and ICI-experienced patients
- TMB, predicted TNB, or NEOPS score did not correlate with response to lifileucel treatment or with best change in target lesion SOD following lifileucel treatment in both cohorts (data not shown; all *p*>0.05)

Figure 4. Gene Mutations of Interest



- No association was observed between single-gene mutations and response
- Deletions in β-2-microglobulin (B2M), a known mechanism of resistance, were identified in non-responders in both cohorts (**Figure 4**)

Figure 5. IFNγ Signature and Other Published Signatures[10,12-21]



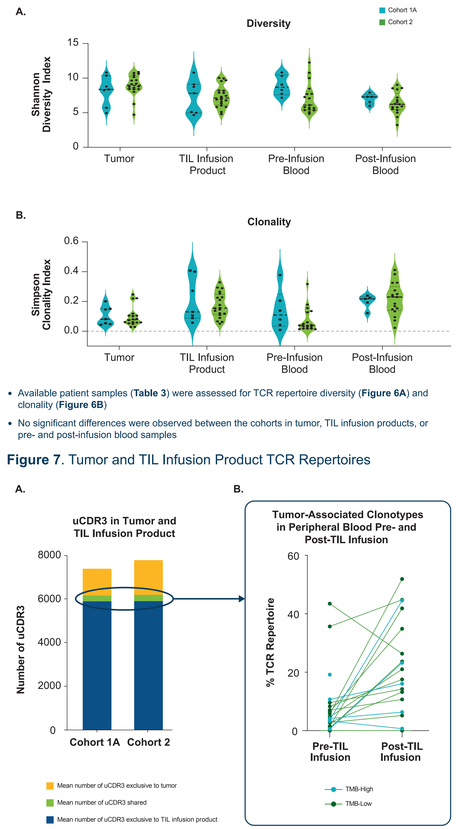
- Response to lifileucel was seen in patients with inflamed and non-inflamed tumors in both cohorts
- No significant correlation with response was observed with signatures of inflammation, TGFβ, β-catenin, TLS, MPS, or TIDE predictions in either cohort (**Figure 5**)

Table 3. Number of Patients Analyzed for TCR Repertoire

	Cohort 1A	Cohort 2
Tumor	7	18
TIL infusion product	7	20
Pre-infusion blood	6	17
Post-infusion* blood	5	17

*Day 42 for Cohort 2; Day 28 for Cohort 1A.

Figure 6. TCR Repertoire Profile



- Available patient samples (**Table 3**) were assessed for TCR repertoire diversity (**Figure 6A**) and clonality (**Figure 6B**)
- No significant differences were observed between the cohorts in tumor, TIL infusion products, or pre- and post-infusion blood samples

Figure 7. Tumor and TIL Infusion Product TCR Repertoires

- Unique CDR3 sequences (uCDR3 clonotypes) identified from the tumor and TIL infusion products are shown in yellow and blue, respectively; clonotypes identified in both samples are indicated in green and reflect tumor-associated clonotypes captured in the TIL products (**Figure 7A**)
- The clonotypes identified in both tumor and TIL infusion products were stratified by high and low TMB; after infusion, TCR repertoires shifted to resemble the tumor-associated clones captured in TIL (**Figure 7B**)

Conclusions

- Lifileucel TIL cell therapy produced clinical responses across the TMB spectrum, regardless of prior ICI exposure
  - A higher proportion of patients who were ICI-naïve had high TMB than those who were ICI-experienced
  - In a multivariate analysis adjusted for cohort, TMB was not associated with response to lifileucel
- TMB correlated with predicted TNB and NEOPS scores in ICI-naïve and -experienced patients
- No association was observed between single-gene mutations and response
- Response to lifileucel was seen in patients with inflamed and non-inflamed tumors in both cohorts
- After TIL infusion, TCR repertoires persisted and shifted to be composed of more tumor-associated clonotypes, regardless of TMB

Lifileucel TIL cell therapy provides potential benefit to patients with melanoma regardless of ICI exposure and independent of tumor biomarkers of mutational burden, single-gene mutations, or inflammation

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Acknowledgments

- The authors would like to thank the patients and their families, as well as the investigators and study site team members who are participating in the study
- This study is sponsored by Iovance Biotherapeutics, Inc. (San Carlos, CA)
- Editorial support was provided by Second City Science and funded by Iovance

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