The utility of AI-powered spatial classification of intratumoral CD8+ immune-cell topology in predicting overall survival in patients with melanoma as part of the CheckMate 067 clinical trial

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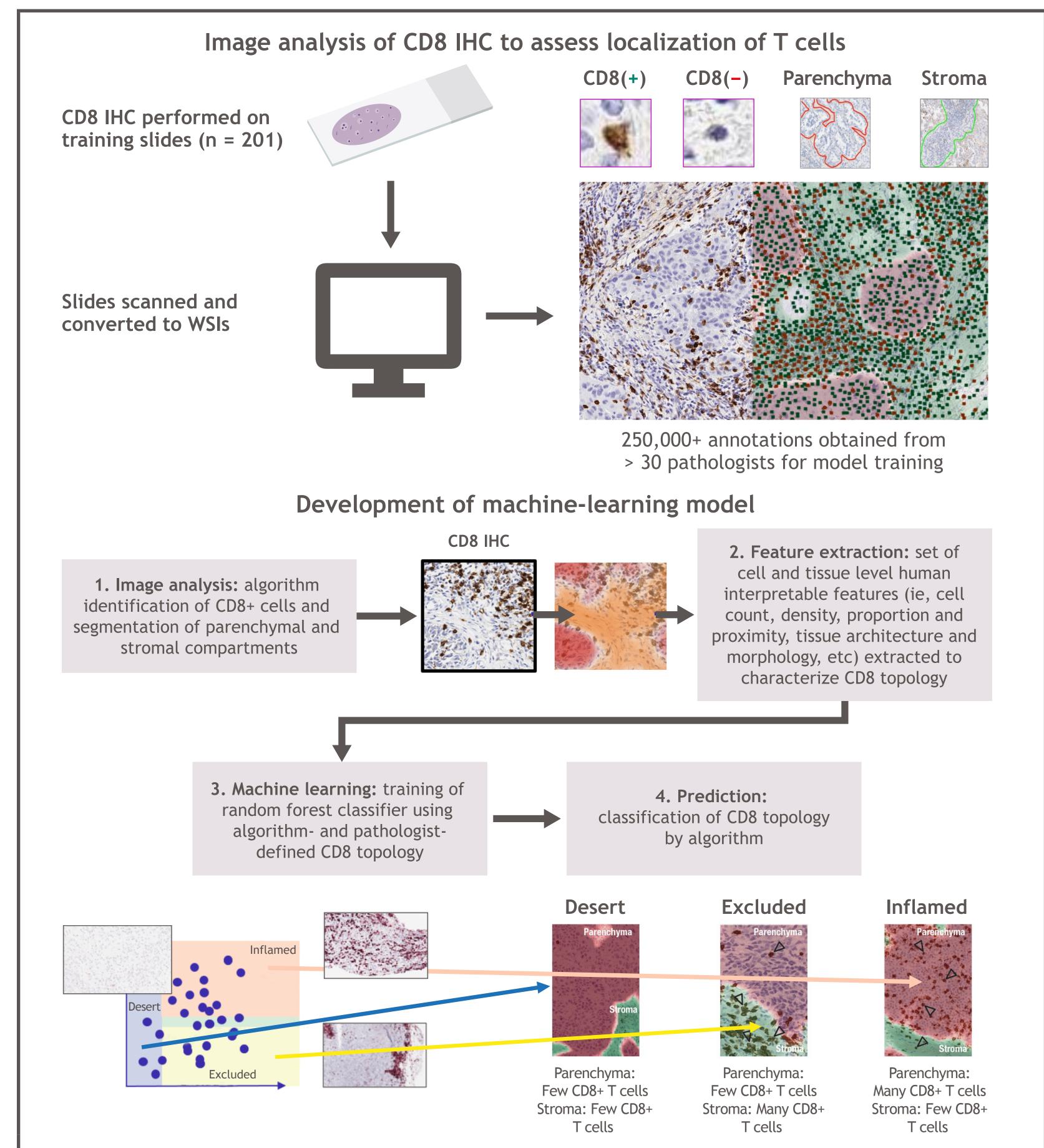
Introduction

- The extent of cluster of differentiation 8-positive (CD8+) T-cell infiltration in the tumor microenvironment (TME) is a prognostic biomarker in patients with solid tumors¹⁻⁵
- The spatial pattern and density of CD8+ T-cell infiltration (CD8 topology) can be categorized as "desert" (deficient in CD8+ cells), "excluded" (CD8+ cells at the tumor boundaries and surrounding stroma), and "inflamed" (CD8+ cells within the tumor parenchyma)^{3,4,6,7}
- Understanding the association of CD8 topology with response to immune checkpoint inhibitors in the context of other key biomarkers, such as programmed death ligand 1 (PD-L1) expression, could help identify additional patients who may benefit from such therapies
- Classification of topology by standard manual pathology review is hampered by challenges with manual scoring of CD8 topology related to heterogeneous immune-cell infiltrates and interpathologist variability^{5,6,8,9}
- Digital pathology approaches present an opportunity to quantify CD8 topology in a biologically meaningful, reproducible, and scalable way
- Using an artificial intelligence (AI)-driven algorithm, we retrospectively assessed the value of intratumoral CD8 topology together with PD-L1 expression as a composite biomarker of response to immunotherapy in patients with advanced melanoma in the phase 3 CheckMate 067 clinical trial (NCT01844505)

Methods

- We utilized a deep-learning platform (PathAI, Boston, MA) to quantify CD8+ T cells and recognize parenchymal and stromal compartments of the TME, using cellular and tissue annotations via the PathAI pathologist network
- Samples were classified as desert (deficient in CD8+ cells), excluded (CD8+ cells restricted to stroma or invasive margin), and inflamed (CD8+ cells infiltrating tumor parenchyma)
- The accuracy of the AI-predicted CD8+ cell counts was evaluated using the Frames Validation method, where model counts in 28 held-out test frames from 22 baseline whole slide images (WSIs) (obtained from patients with melanoma enrolled in the CheckMate 067 trial) were compared with a consensus manual count from 5 independent pathologists using Pearson correlation

Figure 1. Overview of development of the AI algorithm to predict CD8 topology

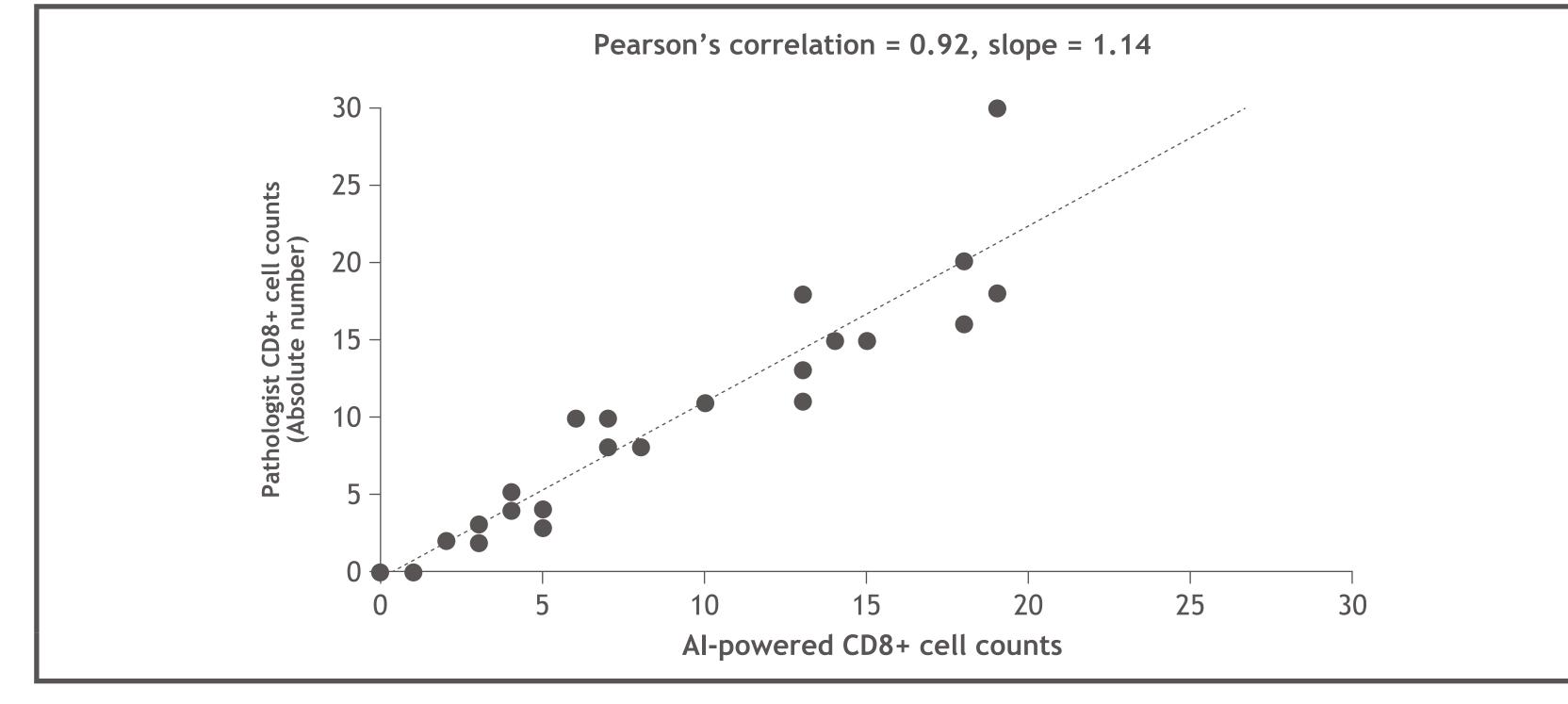


- The features extracted from these annotations were used to train a random forest classifier to predict CD8 topology using parenchymal and stromal CD8+ immune-cell measurements
- To validate AI-predicted CD8 topology classifications, pathologists manually classified CD8 topology on an independent set of digital WSIs from 40 CD8 immunohistochemistry (IHC) slides (C8/144B, Agilent, Santa Clara, CA) obtained from resection/core needle biopsies of patients with advanced melanoma enrolled in the CheckMate 038 clinical trial
- Inter-pathologist concordance was compared with concordance between pathologists and the AI algorithm in classifying CD8 topology
- We then generated a composite biomarker that consisted of AI-classified CD8 topology combined with PD-L1 expression on tumor cells (TCs) using a cutoff of 1% as a reference (determined as part of the original CheckMate 067 trial¹⁰)
- Associations between overall survival (OS) in patients with previously untreated metastatic melanoma who received nivolumab plus ipilimumab (NIVO + IPI; n = 102), NIVO monotherapy (n = 107), or IPI monotherapy (n = 106) in CheckMate 067, and AI-defined CD8 topology alone or in combination with PD-L1 expression were estimated using Cox proportional hazards models
- PD-L1 expression on TCs was determined using the Dako PD-L1 IHC 28-8 pharmDx assay (Agilent, Santa Clara, CA)
 - Manual scoring of PD-L1 expression on TCs was derived from the percentage of TCs with complete or partial PD-L1 expression at any level of intensity divided by the number of total TCs
- Tumor samples with PD-L1 expression ≥ 1% were classified as PD-L1-positive, and those with PD-L1 expression < 1% were classified as PD-L1-negative
- Kaplan-Meier curves were used to illustrate comparisons of survival in samples identified as immune-excluded or -inflamed and/or PD-L1-positive or PD-L1-negative
- Patients with an CD8-desert phenotype were not included in the survival analyses due to low sample size

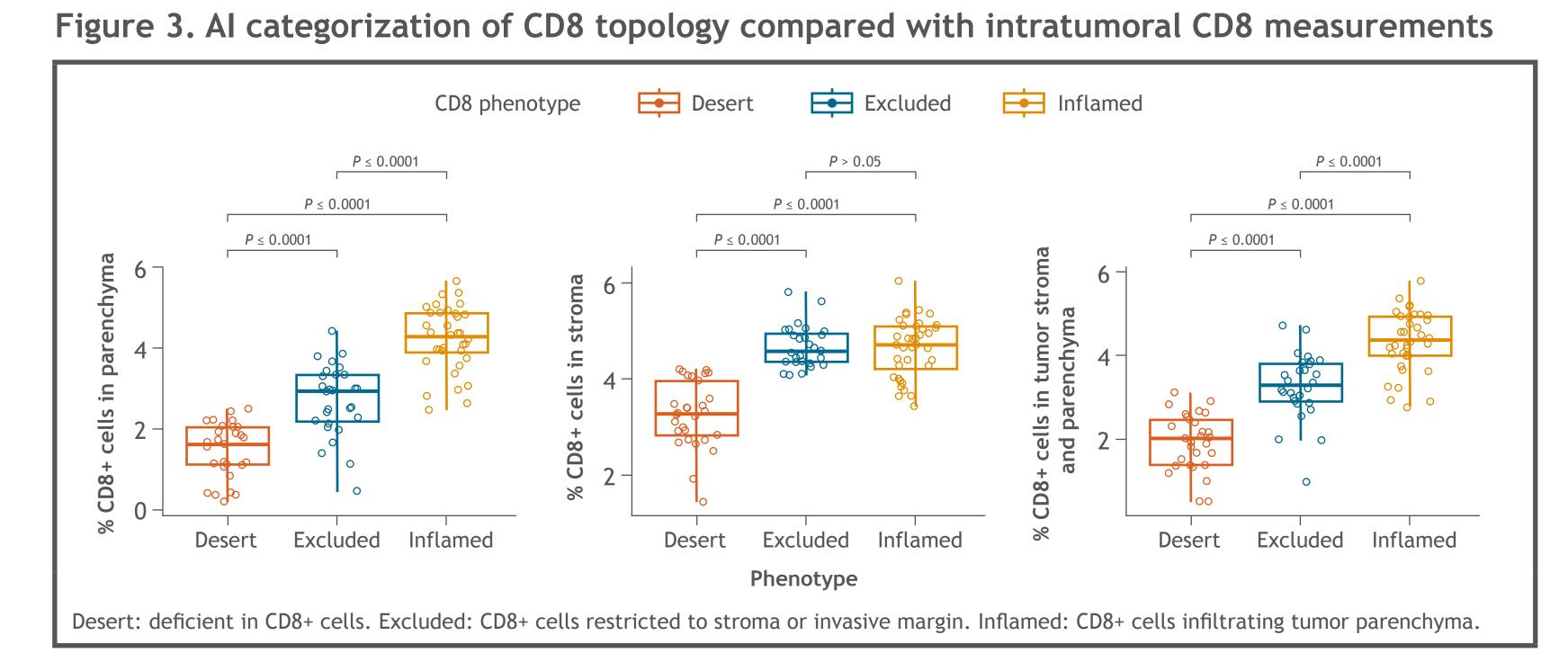
Results

- The correlation between AI-powered and pathologist quantification of CD8+ cells was high (Figure 2)
- Classifications of CD8 topology by the AI algorithm were concordant with manual scoring performed and determined by a consensus of pathologists (Cohen's κ, 0.79) and non-inferior to the agreement between 2 pathologists (Cohen's k, 0.65)

Figure 2. Al categorization of CD8+ cell counts compared with a consensus of pathologists

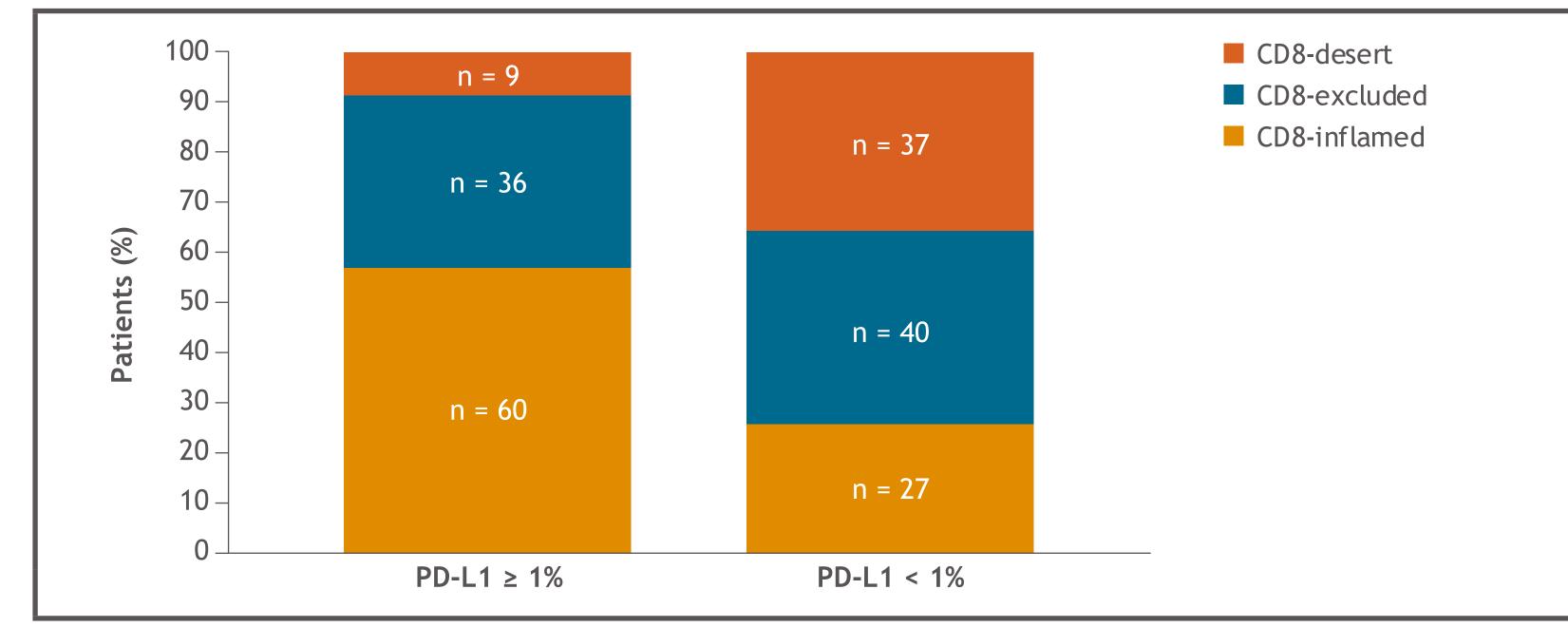


- Measurements of the percentage of CD8 positivity in the stromal and parenchymal compartments were similar to AI-predicted CD8 categorizations (Figure 3)
- Pathologist review of AI-predicted topology found these categorizations to be accurate



- Of 105 patients with PD-L1 expression ≥ 1%, 36 (34%) had phenotypes that were classified as CD8-excluded and 60 (57%) had phenotypes that were classified as CD8-inflamed (Figure 4)
- Of 104 patients with PD-L1 expression < 1%, 40 (38%) had phenotypes that were classified as CD8-excluded and 27 (26%) had phenotypes that were classified as CD8-inflamed
- Due to the low numbers of patients with PD-L1 expression ≥ 1% who had phenotypes classified as CD8-desert (9%), it was not possible to perform the OS analysis with this group

Figure 4. Classification of patients by CD8 topology and TC PD-L1 expression



- In patients with PD-L1 expression ≥ 1%, no statistically significant difference in OS was observed between the CD8-excluded and CD8-inflamed phenotypes across the different treatment arms (Figure 5)
- Among patients with PD-L1 expression < 1%, those with a CD8-excluded phenotype benefited significantly from NIVO + IPI therapy (P = 0.002) compared with those with a CD8-inflamed phenotype, whereas the difference between phenotypes was not significant for those treated with NIVO monotherapy (P = 0.41) (Figure 5, Table 1)

Figure 5. OS by CD8 topology (inflamed vs excluded)^a and PD-L1 expression (<1% vs ≥ 1%)

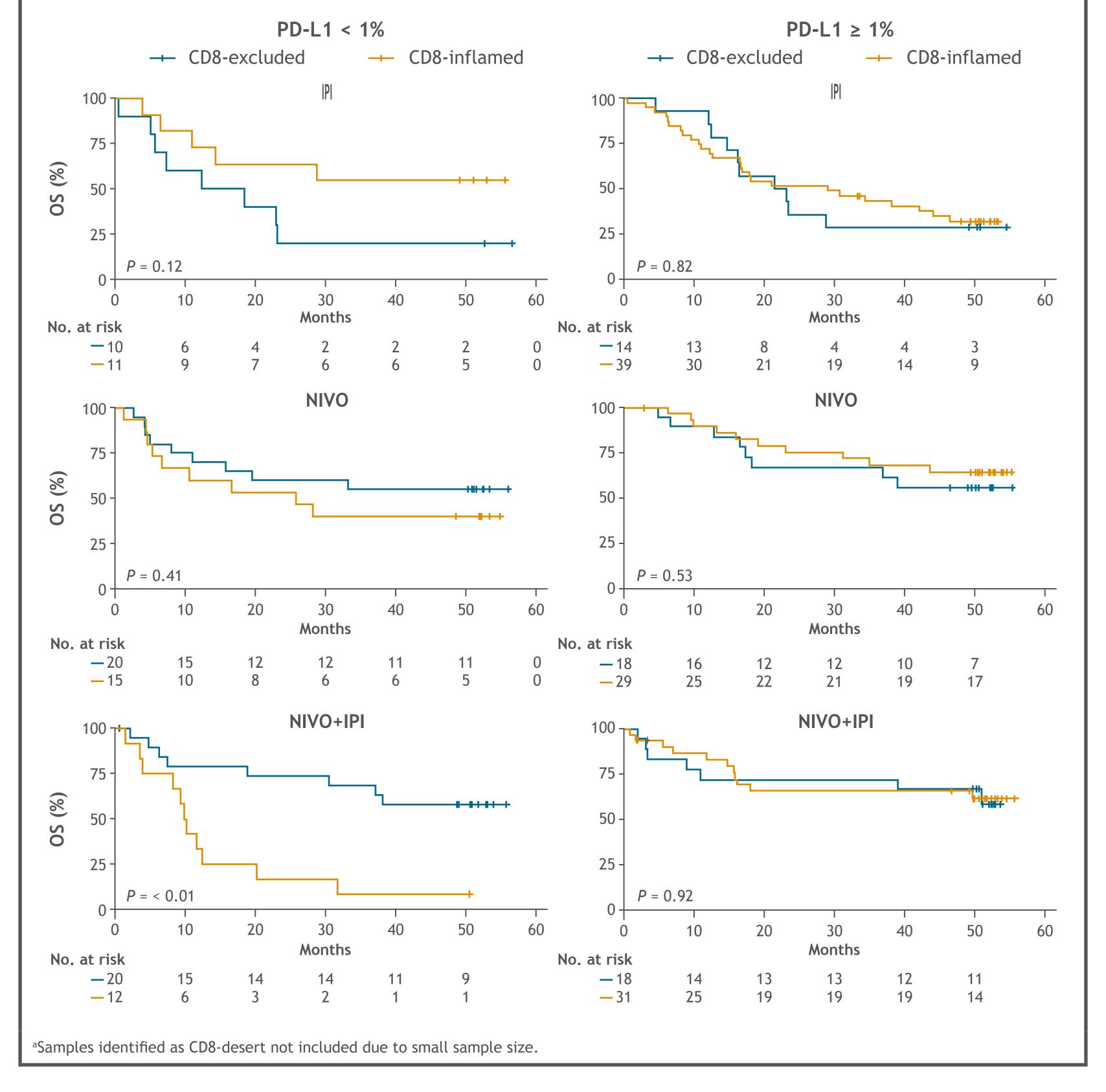
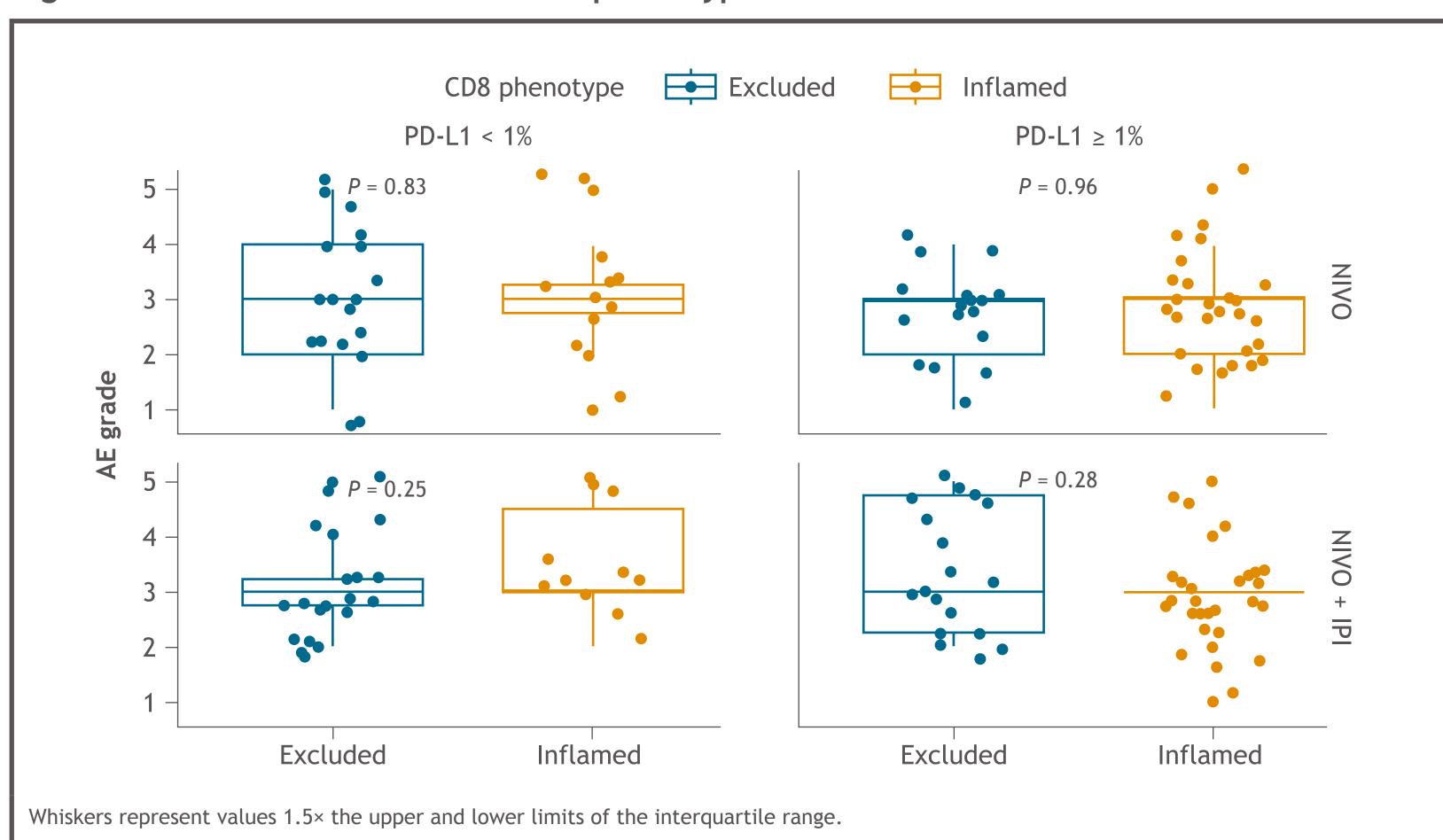


Table 1. OS by CD8 topology in patients with PD-L1 expression < 1%

Treatment arm	IPI		NIVO + IPI		NIVO	
Phenotype (n)	PD-L1 < 1%, CD8-excluded (10)	PD-L1 < 1%, CD8-inflamed (11)	PD-L1 < 1%, CD8-excluded (20)	PD-L1 < 1%, CD8-inflamed (12)	PD-L1 < 1%, CD8-excluded (20)	PD-L1 < 1%, CD8-inflamed (15)
Median OS, months	15.4	> 50.0	> 50.0	10.1	> 50.0	25.8
HR (95% CI)	2.44 (0.79-7.56); <i>P</i> = 0.12		0.23 (0.09-0.61); <i>P</i> < 0.01		0.68 (0.27-1.70); <i>P</i> = 0.41	

- HR, hazard ratio.
- In patients treated with NIVO + IPI with a CD8-inflamed phenotype and PD-L1 expression < 1%, a greater incidence of adverse events (AEs) occurred than in patients with an excluded phenotype (Figure 6); however, this is limited by a small sample size
- Among patients with PD-L1 expression < 1%, severe AEs (SAEs; defined as grade ≥ 3) were less frequent in patients with a CD8-excluded phenotype than in those with a CD8-inflamed phenotype (data not shown)

Figure 6. Association between tumor phenotype and AE incidence



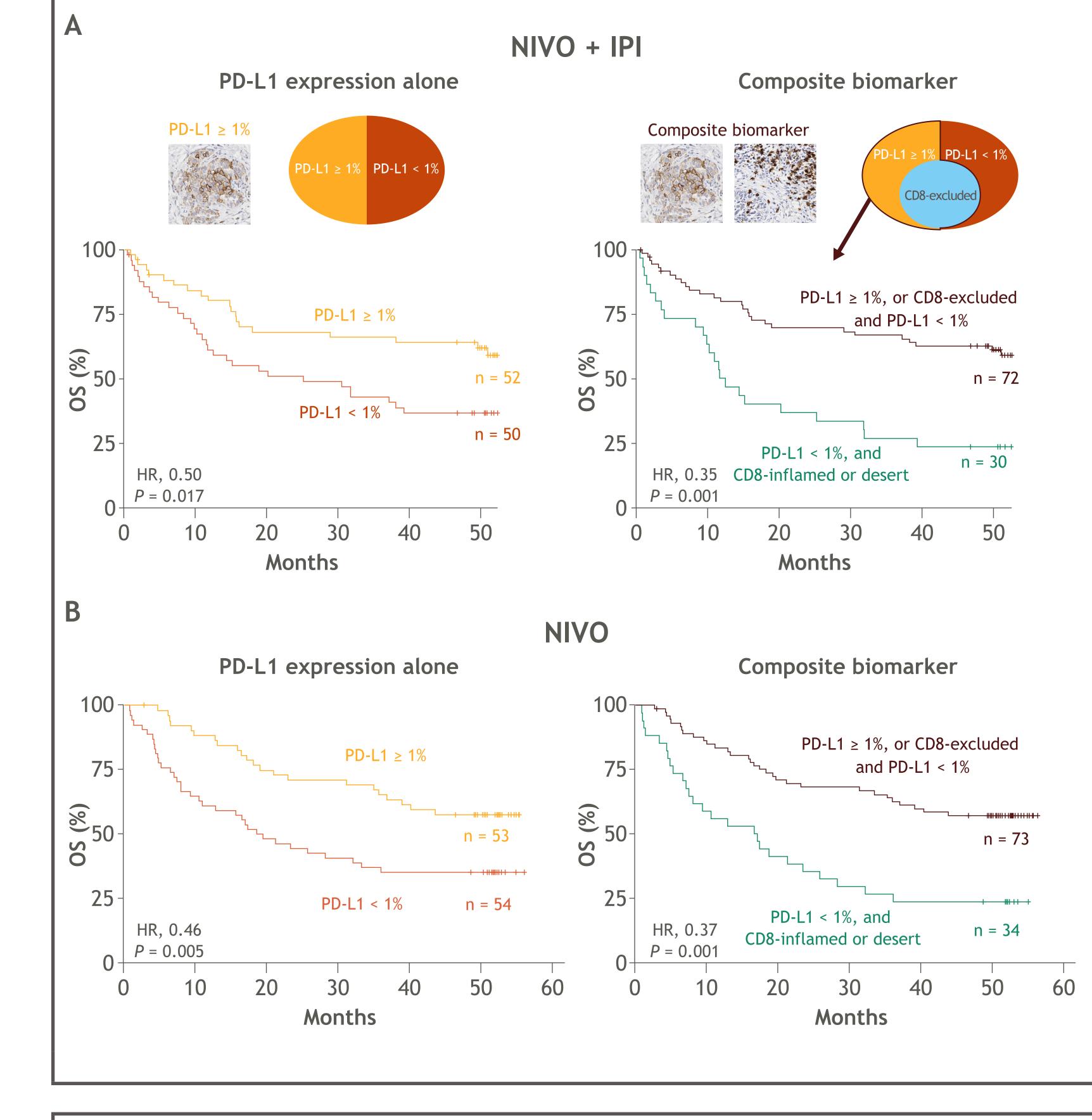
- To investigate whether combining two sets of patients could result in greater clinical benefit for patients treated with NIVO ± IPI, we introduced a composite biomarker that combines patients with PD-L1 expression < 1% and classified as CD8-excluded phenotype and those with PD-L1 expression ≥ 1%, accounting for 68%-71% of tumors (Table 2)
- Patients identified as positive based on the composite biomarker demonstrated a stronger association with survival than those identified as biomarker-negative (PD-L1 < 1%, and CD8-inflamed or CD8-desert) compared with using PD-L1 expression alone in both the NIVO + IPI and NIVO monotherapy groups (Figures 7A and 7B)

Table 2. HRs comparing OS in patients identified as biomarker-positive using an Al-driven composite biomarker compared with PD-I 1 expression alone

	PD-L1	≥ 1%	Composite biomarker (PD-L1 ≥ 1%, or CD8-excluded and PD-L1 < 1%)		
Biomarker	Number of patients identified as positive (%)	OS HRª (95% CI)	Number of patients identified as positive (%)	OS HR ^b (95% CI)	
NIVO + IPI (n = 102)	52 (51%)	0.50 (0.29-0.89); P = 0.017	72 (71%)	0.35 (0.20-0.61) P < 0.001	
NIVO (n = 107)	53 (50%)	0.46 (0.27-0.79); P = 0.005	73 (68%)	0.37 (0.22-0.62) P < 0.001	

^aHRs represent patients with PD-L1 expression ≥ 1% compared with patients with PD-L1 < 1%; ^bHRs represent patients with PD-L1 ≥ 1%, or CD8-excluded and PD-L1 < 1% compared with patients with PD-L1 expression < 1% and CD8-inflamed or CD8-desert

Figure 7. OS by PD-L1 expression or using composite biomarker in patients treated with NIVO + IPI (A) or NIVO monotherapy (B)



Conclusions

- This study demonstrates the utility of combining AI-powered CD8 topology classifications with TC PD-L1 expression as a composite biomarker associated with immunotherapy response in patients with advanced melanoma
- In patients with melanoma whose tumors had PD-L1 expression < 1% treated with NIVO + IPI, median OS was significantly longer in patients with CD8-excluded tumors than in those with an inflamed phenotype
- In patients treated with NIVO + IPI, there was a trend toward a lower frequency of AEs in patients whose tumors had a PD-L1 expression < 1% and in patients with a CD8-excluded compared with a CD8-inflamed phenotype
- Use of a composite biomarker, consisting of AI-driven intratumoral CD8-topology classifications and PD-L1 expression, identified a larger subset of patients who benefited from treatment with NIVO
- monotherapy or NIVO + IPI, compared with classification using PD-L1 status alone • Further studies are underway to identify mechanisms underlying responses to NIVO + IPI in the subgroups
- of patients analyzed in this study

References

- 1. Geng Y, et al. Cell Physiol Biochem 2015;37:1560-1571.
- 2. Manabe K, et al. *J Dermatol* 2021;48:1186-1192.
- 3. Hendry S, et al. *Adv Anat Pathol* 2017;24:235-251.
- 4. Desbois M, et al. *Nat Commun* 2020;11:5583.
- Amgad M, et al. NPJ Breast Cancer 2020;6:16.
- 8. Brunnström H, et al. *Mod Pathol* 2017;1411-1421. 9. Tsao MS, et al. *J Thorac Oncol* 2018;13:1302-1311. 10. Larkin J, et al. *N Engl J Med* 2015;373:23-34.

7. Braun DA, et al. *Nat Med* 2020;26:909-918.

6. Kather JN, et al. *Elife* 2018;7:e36967.

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