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Tumor characteristics associated with benefit from pembrolizumab in advanced non-small cell lung cancer

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Abstract

Purpose—Several biomarkers have been individually associated with response to PD-1 blockade, including PD-L1 and tumor mutational burden (TMB) in non-small cell lung cancer (NSCLC), and CD8 cells in melanoma. We sought to examine the relationship between these distinct variables with response to PD-1 blockade and long term benefit.

Experimental Design—We assessed the association between baseline tumor characteristics (TMB, PD-L1, CD4 and CD8) and clinical features and outcome in 38 patients with advanced NSCLC treated with pembrolizumab (median follow-up of 4.5 years, range 3.8 to 5.5 years).

Results—PD-L1 expression and CD8 infiltration correlated with each other and each significantly associated with objective response rate (ORR) and progression free survival (PFS). TMB was independent of PD-L1 and CD8 expression, and trended towards association with ORR and PFS. There was no association between CD4 infiltration and outcomes. Only PD-L1 expression was correlated with overall survival (OS). Among five patients with long-term survival >3 years with no additional systemic therapy, PD-L1 expression was the only discriminating feature. The increased predictive value for PFS and OS of composite biomarker inclusive of PD-L1, CD8, CD4, and TMB was limited.

Conclusion—In NSCLC patients treated with PD-1 blockade with long term follow up, TMB, PD-L1 and CD8 were each associated with benefit from PD-1 blockade. Pre-treatment PD-L1 expression was correlated with T lymphocyte infiltration as well as OS, while models incorporating TMB and infiltrating CD4 and CD8 lymphocytes did not substantially add to the predictive value of PD-L1 alone for OS.

Introduction

The success of PD-1 checkpoint inhibition in treating patients with non-small cell lung cancers (NSCLC) is an important milestone in the history of cancer therapy [1]. The hallmark of cancer immunotherapy is the durability of the tumour-specific immune response, but this durability has only been achieved in a minority of patients, highlighting the need for biomarkers to predict long term response to therapy. Further, biomarkers can identify factors to help guide the study of the combination of immunotherapies [2].

Tumor PD-L1 expression is correlated with clinical benefit in NSCLC, and is now routinely used as a biomarker in clinical practice [3–8]. Still, PD-L1 is an imperfect biomarker, as many high expressors are non-responders, and responders with negative or low tumor PD-L1 expression are often observed. Tumor mutational burden (TMB) has also been associated with objective response rate (ORR) and progression free survival (PFS) to PD-1 checkpoint inhibitors in NSCLC [9–12]. Application of TMB in clinical practice requires ongoing efforts for harmonization of computation approaches for quantification, solutions for expeditious return of results, cost, and intra- and inter-tumoral heterogeneity. A correlation of TMB with overall survival (OS) in analyses to date is either not seen or limited by relatively short follow-up [11,13].

Studies in melanoma patient-derived tumor specimens revealed that responses to PD-1/L1 blockade rely on pre-therapy tumor infiltration of activated CD8 T effector cells [14]. The role of CD4 T lymphocytes in response to anti-PD1 therapy has not been well studied, with no clear correlation identified to date. In addition, no previous evaluation has examined the relationship between PD-L1, TMB, and infiltrating CD4 and CD8 T-cells in a single patient cohort and the composite power of these biomakers to predict long term outcomes in patients with NSCLC treated with PD-1 checkpoint inhibitors.

Methods

Study Design and Treatment

Patients were identified with advanced NSCLC treated at one of two institutions (University of California, Los Angeles (UCLA) and Memorial Sloan Kettering Cancer Center (MSK)) with pembrolizumab as part of KEYNOTE-001[3]. The study was performed in accordance with the Decleration of Helsinki and informed written consent was obtained from each subject, or each subject's guardian, prior to enrollment on trial. The patient eligibility criteria, study schema, and treatment schedules have been previously described.

All patients were consented to institutional review board approved protocols for tissue banking and sample analysis. Efficacy was determined by investigator assessed immune

related response criteria (irRC), with imaging performed per protocol every nine weeks. Progression-free and overall survival were defined from the date the patient began pembrolizumab. Patients who had not progressed/still alive were censored for PFS at the date of the last scan and for OS at the date of last contact.

Whole Exome Sequencing (WES)

Tissue from 25 patients was used for whole exome sequencing. DNA was extracted and quality controlled from tumor and patient-matched blood or other non-cancerous tissue. Data for 10 patients were performed as previously described [9]. For the additional 15 patients, WES was performed at the UCLA Clinical Microarray Core using the Roche Nimblegen SeqCap EZ Human Exome Library v3.0 targeting 65 Mb of genome. Paired-end (2×100 or 2×150 base-pair) sequencing of the enriched exome libraries was performed on a HiSeq platform (Illumina, San Diego, CA) to a goal mean depth of 150x over targeted regions. Reads were aligned to genome build GRCH37 with bwa 0.7.12, followed by duplicate removal (Picard Tools 1.137), indel realignment, and base recalibration using the Genome Analysis Toolkit (GATK v3.4, Broad Institute) with reference files from the b37 GATK resource bundle.

Tumor content was assessed from sequencing data using Sequenza (v2.1.2, http://www.cbs.dtu.dk/biotools/sequenza/). Those below Sequenza's minimum sensitivity of 30% tumor cellularity were assessed for tumor content by immunohistochemistry. Cases were only included if they met sufficient overall quality criteria for coverage (>50x tumour, and >30x normal) and tumour content (>10%).

Mutation calling from mapped BAM files of all 25 samples was performed by a uniform pipeline incorporating MuTect (v1.1.7), Varscan (v2.3.9), and a Fisher's exact test of alternate read-counts between tumor/normal samples from calls made by the GATK Haplotypecaller (v3.4, jointly genotyped at the cohort level from gvcfs), as previously described [15,16]. Variant sites were considered if identified by 2 out of 3 programs, covered by a minimum 10 reads in both the tumor and normal sample, and if the variant allele was supported by at least 4 reads. Functional consequence of mutations was determined using Oncotator (Broad Institute, v1.5, Dec112014 data corpus). Only non-synonymous mutations (Nonsense, Missense, Splice_Site, Frameshift indels, In-frame indels, Start_Codon indels or SNPs, and Stoploss/Nonstop variants) were counted toward tumor mutational burden to minimize differences between exon-capture kits. A final filter was applied to exclude variants at sites of known germline variation with a population allele frequency >0.0005 in the Exome Aggregation Consortium (ExAC) database v0.3.1.

Human leukocyte antigen (HLA) typing was performed on 25 patients in the correlative cohort. Of these patients, five had HLA typing previously performed [17]. For the remaining 20 patients, HLA zygosity was determined by inference of HLA alleles from whole exome sequencing by ATHLATES as previously described [17].

Immunohistochemistry (IHC) analyses

Patients with adequate, non-lymph node pre-treatment FFPE tissue samples were stained with haematoxylin and eosin, anti-CD4, anti-CD8, anti-PD-L1, anti-CD45, and anti-FOXP3

at the UCLA Anatomic Pathology Immunohistochemistry and Histology Laboratory (CLIA-certified). Readers were blinded to patient outcomes. Antibodies used included rabbit polyclonal CD4 (Clone SP35, 1:100 dilution, low pH retrieval, Cell Marque), CD8 (clone C8/144B, 1/100, low pH retrieval, DAKO), PD-L1 (SP142, 1/200 dilution with High pH retrieval Spring Biosciences, Pleasanton, CA), CD45 (Clone 2B11+PD7/26, 1:600 dilution, low pH retrieval, DAKO), and FOXP3 (Cat # 14–4776-82, 1:200 dilution, high pH retrieval, eBioscience). IHC was optimized and performed on Leica Bond III autostainer using Bond ancillary reagents and Refine Polymer Detection system. Slides were examined for the presence of CD4, CD8 and PD-L1 within the tumor parenchyma. All slides were scanned at an absolute magnification of ×200 (resolution of 0.5 μm per pixel). The percentage of positively IHC stained cellularity against all nucleated cells including both tumor cells and stromal cells / immune infiltrates (% positive cells/all nucleated cells) was calculated using the Halo platform (Indica Labs, Corrales, NM)[18]. While the primary analysis was based on all nucleated cells, a secondary analysis evaluated whether the PD-L1 staining was tumor predominant or immune infiltrate predominant by calculating the percentage of positively stained cells in the tumor by H&E using 50% as the cutoff (i.e. if more than 50% of the PD-L1 expression comes from the tumor cells, then it's defined as tumor predominant). A subset of specimens for which slides were available were also evaluated with CD45 to verify the identity of cells described as immune cells by H&E was CD45 positive, Tumors from lymph nodes were not used in this analysis due to the inability to differentiate anti-tumour vs resident immune cells and the high background PD-L1 expression. Of note, PD-L1 expression reported in this analysis is distinct from the PD-L1 testing performed as part of KEYNOTE-001; testing was re-done here to permit analysis of multiple marker expression from a single tissue sample, stained at the same time. Additionally, it should be recognized that SP142 was used for the PD-L1 IHC analysis performed using an optimized semi-manual staining procedure, different from the commercial kit developed for this antibody.

Statistical Analysis

Patient characteristics were summarized descriptively using median (min/max or Q1/Q3) or frequencies (percentages). Survival curves were plotted using the Kaplan-Meier method and compared between groups using the log-rank test. Comparisons between response groups and TMB, PD-L1, CD8, CD4 were assessed using the Wilcoxon test for two groups or the Kruskal-Wallis test for 3 groups. The objective response rate was reported as proportion along with Clopper-Pearson exact CIs. The chi-square or Fisher's exact test were used to test for differences between groups for categorical variables. Assocations between continuous or ordinal measures were assessed using the Spearman rank correlation coefficients. These analyses were exploratory and not powered for statistical comparison across subgroups. Using the planned significance level of 0.05, each group of primary analyses was estimated to have a false discovery rate no more than 12%.

Univariable and multivariable Cox regression models for progression free and overall survival were constructed using TMB, CD8, PD-L1, and CD4 with all combinations of markers (including interaction terms) to identify the most predictive model. In order to assess the prognostic ability of each of these models, the survival c-statistic for survival models was computed [19]. Similar methodlogy was carried out for the binary outcome (PR vs

SD/PD per irRC) using logistic regression models. These regression analyses were exploratory given the limited sample size.

All tests were two-sided; P values < 0.05 were considered statistically significant. The false discovery rates associated with the primary analyses was estimated using the Benjamini-Hochberg step-up procedure. Statistical analyses were performed using GraphPad Prism and R v3.3.3 software (www.r-project.org).

RESULTS

Demographics of the correlative cohort

We identified 38 patients (33 from UCLA; 5 from MSKCC) with available baseline fresh or archival tumor adequate for WES and IHC studies including PD-L1, CD8 and CD4. Best response in this cohort was partial response in 16 patients (42%), stable disease in 10 (26%) and progressive disease in 12 (32%) (Table 1). Thirty two patients had tissue for PD-L1, CD8 and CD4 evaluation and 25 patients had TMB determined. Twenty-one patients had a complete set of all four parameters evaluated.

Demographic features of the correlative cohort are largely representative of the clinical cohort [all NSCLC patients treated in KEYNOTE-001 trial at UCLA (N=97, Table 1)] except a higher percentage of responders. The population treated at UCLA was generally similar to the overall study population with the exception of a greater percentage of *EGFR*-mutation positive patients treated at UCLA (31%) compared to the total study population (15%) [20]. Median follow-up in the UCLA clinical cohort was 4.4 years (range 38 days-1995 days), based on an internal database lock on December 31, 2017. A total of 12 patients had an OS duration ≥ 3 years (up to 65.6 months). Of those, 7 long term benefiters survived > 3 years from the initial dose of pembrolizumab with no additional systemic therapy after pembrolizumab (Table 1), while 5 received additional systemic therapy after pembrolizumab.

The median PD-L1 expression was 26.5% (range of 0.5–98). PD-L1 was ≥ 50% in 9 of 32 patients (28%), similar to other reports [5]. PD-L1 level was correlated with prior smoking history (p=0.04) and squamous histology (p=0.025), but not *EGFR* mutational status (p=0.271) (Figure 1, Supplemental Table 1). Median TMB was 104 (range 9–1616) and TMB was significantly correlated with smoking status (p=0.008) but not with other clinical features. The median CD8 and CD4 T cell infiltration was 4.5% and 3.0% respectively (ranges 0–23% and 0–31%). CD8 infiltration was numerically higher in patients who were smokers (p=0.06, Figure 1) and those who were treatment naïve (p=0.03, Supplemental Table 3). Specifically, when CD8 was analyzed as categorical variable, patients with at least one prior line of therapy were more likely to have low (<5%) CD8 than treatment naïve patients (65.4% vs 16.7%, p=0.030, Supplemental Table 3).

Individual variables and clinical benefit

ORR by irRC—PD-L1 expression was significantly higher in the responders compared to those with SD/PD group (median 66% vs 15%, p=0.002). Although the primary PD-L1 analysis assessed all nucleated cells, two patterns were seen based on whether the majority

of PD-L1 positive cells were in the tumor or immune infiltrating cells. To confirm the accuracy of this aseessment, a subset were evaluated by CD45 staining to confirm the assessment of immune cells as shown in Figure 1B. There was an association beween responses and the majority of PD-L1 expression being on tumor cells (p=0.007, Fisher's exact test, Figure 1C). A trend was observed of higher TMB in responders compared to those with SD/PD (median 189 vs 55, p=0.08). Of the 25 patients with WES data available for analysis, only 2 exhibited HLA homozygosity and there was no correlation found between HLA zygosity and response (data not shown). CD8 cell infiltration was significantly higher in responders compared to patients in the SD/PD group (median 8% vs 3%, p=0.02). CD4 infiltration was not different between responders and non-responders (median 8% vs 2%, p=0.17) (Figure 1A and 2, Supplemental Figure 1, Supplemental Table 2). Additional FOXP3 staining in 6 available cases with CD4 infiltration suggested Tregs are less than 5% of the CD4+ cells in these cases (data not shown).

PFS and OS—Consistent with prior reports, baseline tumor PD-L1 expression was associated with improved PFS (p=0.002), as well as with improved OS assessed either as a continuous or categorical variable (PD-L1<50% vs 50%) (p=0.03, Table 2, Figure 2 and 3, Supplemental Table 3 and Supplemental Figure 1 and 2). When assessed as a continuous variable, TMB showed a trend towards improved PFS (HR 1 (0.99–1.00), P=0.065). When assessed by quartiles, increasing TMB correlated with improved PFS (p=0.02, Table 2 and Supplemental Table 4), similar to previous publications [9,12], which was mostly driven by the top quartile (Supplemental Figure 2). However, in this limited dataset, TMB was not associated with OS (Table 2, Figure 2). Baseline tumor CD8 (but not CD4) infiltration was significantly higher in patients with longer PFS when assessed as a continuous variable (p=0.026, Table 2, Figure 2) and with longer OS when assessed as a categorical variable (p=0.048, Supplemental Table 4).

Relationship between biomarker variables—Consistent with previous data [10–12], TMB and PD-L1 were not correlated with each other (Spearman rho 0.19, p=0.406) (Supplemental Table 1, Supplemental Figure 3). No significant correlation was found between TMB and infiltrating CD4 or CD8 lymphocytes. PD-L1 was correlated with CD8 (Spearman rho 0.66, p<0.001) and CD4 expression (Spearman rho 0.48, p=0.005).

Multivariable modeling—In univariate/multivariable analysis of PFS, OS, and ORR using combinations of TMB, CD8, PD-L1 and CD4, univariate PD-L1 expression had the highest c-index for benefit prediction either when all 38 correlative tumors were evaluated or when only the 21 tumors with all 4 parameters available were assessed (Table 3). Additional combinations of two, three, or four variables (data not shown) and two variables with interaction modeling did not significantly increase the predictive value as compared to single variable PD-L1(Table 3).

Characterization of long term survivors—Five patients had long term benefit, defined as survival > 3 years from the initial dose of pembrolizumab with no additional systemic therapy. PD-L1 expression 50% was seen in 4 out of these 5 patients (80%, three was evaluated at UCLA, and one was evaluated by Merck proportion score) and the fifth had

39% PD-L1 expression. Out of all correlative analyses assessed, only baseline PD-L1 expression level was significantly higher in the long term benefiter group compared to the remaining patients in the correlative cohort (median of 72% vs 16%, p=0.029, Figure 2, Supplemental Table 5).

Discussion

Checkpoint inhibitors unleash a patient's immune system to fight cancer and have transformed the the management landscape of NSCLC. It is an exciting proof of principle that cancer immunotherapy can be effective and durable beyond indications traditionally considered immunotherapy-sensitive [21]. However, only a minority of patients benefit, and data on long term benefit is particularly lacking, as correlative analyses are typically generated on patients with immature follow-up based on surrogate endpoints, rather than OS.

T-cell based anti-tumour response can be influenced by many factors in different immune compartments, including tumour foreignness from the normal counterpart, sensitivity to effectors, the tumour immune suppressive contexture, T cell priming and activation as well as exhaustion status [21,22]. Developing reliable biomarkers to predict response to checkpoint inhibitors is critical in selecting the most effective therapy to maximaize clinical benefit. Our study in a NSCLC cohort treated with pembrolizumab on KEYNOTE-001 with long term follow up, suggest that baseline PD-L1 expression correlates with lymphocyte infiltration in NSCLC, and in our data, is the most reliable biomarker to predict survival with PD-1 checkpoint blockade.

High PD-L1 expression in tumors can be either constitutive due to a genetic alteration, or induced by IFN-gamma released by activated immune cells [23]. The latter scenario is a strong indication that an active anti-tumor immune response blocked by the PD-1 checkpoint is occurring. Indeed in our study, high PD-L1 expression is signfiantly correlated with ORR, PFS, OS, squamous histology, and history of smoking. It is also highly correlated with the tumor infiltrating lymphocytes (both CD4 and CD8 cells), indicating an active adaptive immune response. Although our primary PD-L1 analysis looked at all nucleated cells, to address questions regarding PD-L1 staining in infiltrating immune cells vs. tumor cells, we sought differences in response based on location of PD-L1 staining, showing that tumor PD-L1 statining was the most relevant in our dataset. In addition, PD-L1 is the only parameter that correlated with long term benefit from pembrolizumab, and multivariate modeling did not indicate much improved predictive value with the addition of other parameters. Of note, all of the long term benefiters had high pre-treatment PD-L1 expression (>50% in 4 cases and 39% in the remaining 1 case).

The efficacy of PD-1 checkpoint inhibitors is hypothesized to rely on a pre-existing anti-tumor response that is specifically blocked by the PD-1 checkpoint [23,24]. PD-L1, and to some extent infiltrating CD4 and CD8 lymphocytes, serve as a surrogate marker for this scenario. TMB assesses the neoantigens that could potentially be recognized by a patients' immune system. Indeed, the median TMB of different histologies in which anti-PD1/L1 therapies have been approved has a nearly linear relationship with their corresponding

response rates [18,25]. Both PD-L1 and TMB have been associated with ORR and PFS in NSCLC, but these two biomarkers are assessing different things as evidenced in our study by the observed lack of association of TMB with PD-L1 expression or infiltrating CD4 and CD8 lymphocytes. The lack of correlation of TMB with an active immune response could be responsible for the inability to use TMB to predict OS to date, although other data sets assessing TMB have immature follow-up for OS[1113].

Limitations of our study, in addition to relatively small sample size, include a cohort with higher ORR relative to unselected NSCLC population and a lower TMB than has been seen in other studies. Though partly explained by more conservative mutation calling methods, another possible reason could be the presence of more never smokers, particularly patients with *EGFR* mutant tumors. This could decrease the generalizability of our data to a more typical NSCLC patient population. Yet, as inferior outcomes have been noted with *EGFR* mutant disease treated with a PD-1 inhibitor as compared to *EGFR* wild type disease [26], the lack of OS correlation with TMB despite the inclusion of *EGFR* mutation positive patients could also be considered an expected outcome. Another potential limitation is that our study utilized the SP142 antibody to assess PD-L1, rather than the 22C3 antibody, which is more commonly used in clinical practice. Of note our staining methods used a semi-manual procedure optimized in our laboratory, and the (at the time) commercially available SP142 antibody did not perform less well than 22C3 using this procedure. Our protocol is different from the commercial kits using automated strainers, which was utilized in the Blueprint Programmed Death Ligand 1 (PD-L1) Immunohistochemistry (IHC) Assay Comparison Project [27]. In addition, we previously evaluated the relationship between the percentage of PD-L1–stained tumor cells with 22C3 and SP142, as well as the association between the PD-L1 levels identified by each antibody and clinical outcomes in patients from this same correlative cohort, finding excellent concordance between both antibodies, which somewhat mitigates concern regarding the use of SP142[28]. Also, while in cutaneous melanoma, pre-treatment high CD8 infiltration in the tumor invasive margin correlates with clinical response to anti-PD1 therapy [14], we could not adequately assess invasive margins as the majority of the samples were from core biopsies. Despite our efforts, we were not able to see a strong value to adding the other markers to PD-L1 to predict OS. In the case of infiltrating CD4 and CD8 lymphocytes, this was because the markers were highly correlated with PD-L1. In the case of TMB, this could be due to the small sample size, and of note, increasing predictive value of the composite of PD-L1 plus TMB has been reported in other larger studies for outcomes including ORR and PFS [10,12,13]. Interestinly, although CD4 lymphocyte infiltration was associated with CD8 and PD-L1 expression, it did not show correlation with response or survival to pembrolizumab. It is possible that the functionality of CD4 does not depend on numbers, or the primary location of functionality is not in the tumors. Further studies with multiplex immunofluorescent staining or nanostring are required to further illucidate the tumor milieu and the mechanism of response to PD-1 blockade.

In conclusion, long term follow up of NSCLC patients treated with pembrolizumab demonstrated the robustness of pre-therapy PD-L1 expression to predict OS, including long term benefit. Models incorporating TMB and infiltrating CD4 and CD8 lymphocytes did not substantially add to the predictive value of PD-L1 alone for OS. Whether the addition of

other therapies, such as chemotherapy or immunotherapies including CTLA-4 inhibitors, to PD-1 checkpoint inhibitors will change the relative predictive benefit from these biomarkers will be learned from emerging data in ongoing or recently completed clinical trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Conflict of Interest

EG receives clinical research funding from AztraZeneca, Bristol-Myers Squibb, Eli Lilly, Genentech, Merck, Iovance, Dynavax, Mirati, Novartis, Neon. Advisory Board funds from Dracen and EMD Serono

AR receives honoraria from consulting with Merck

MDH receives research funding from Bristol-Myers Squibb; is paid consultant to Merck, Bristol-Myers Squibb, AztraZeneca, Genentech/Roche, Janssen, Nektar, Syndax, Mirati, and Shattuck Labs; and a patent has been filed by MSK related to the use of tumor mutation burden to predict response to immunotherapy (PCT/US2015/062208).

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28. Lisberg A, Hu-Lieskovan S, Grogan T, et al.: P2.09–05 Evaluation of PD-L1-Stained Tumor Cells via the 22C3 and SP-142 Antibodies in Cohort of Patients Treated on KEYNOTE-001, 2018

Statement of Translational Relevance

Our analysis of baseline tumor characteristics (tumor mutational burden, PD-L1 expression, CD4 and CD8 infiltration) in 38 patients with non-small cell lung cancer (NSCLC) treated by pembrolizumab with long term follow-up indicated a significant correlation of tumor PD-L1 expression with tumor infiltrating lymphocytes, response, and preogression-free/overall survival, especially among long term survivors (overall survival longer than 3 years without need of subsequent systemic therapy). Although the cohort is small, it is the largest reported cohort that evaluates all four tumor characteristics in the same treated with anti-PD-1 therapy with long-term follow up. This data helps to advance our understanding of response to PD-1 checkpoint inhibitors and guide selection of patients most likely to experience long term benefit from therapy.
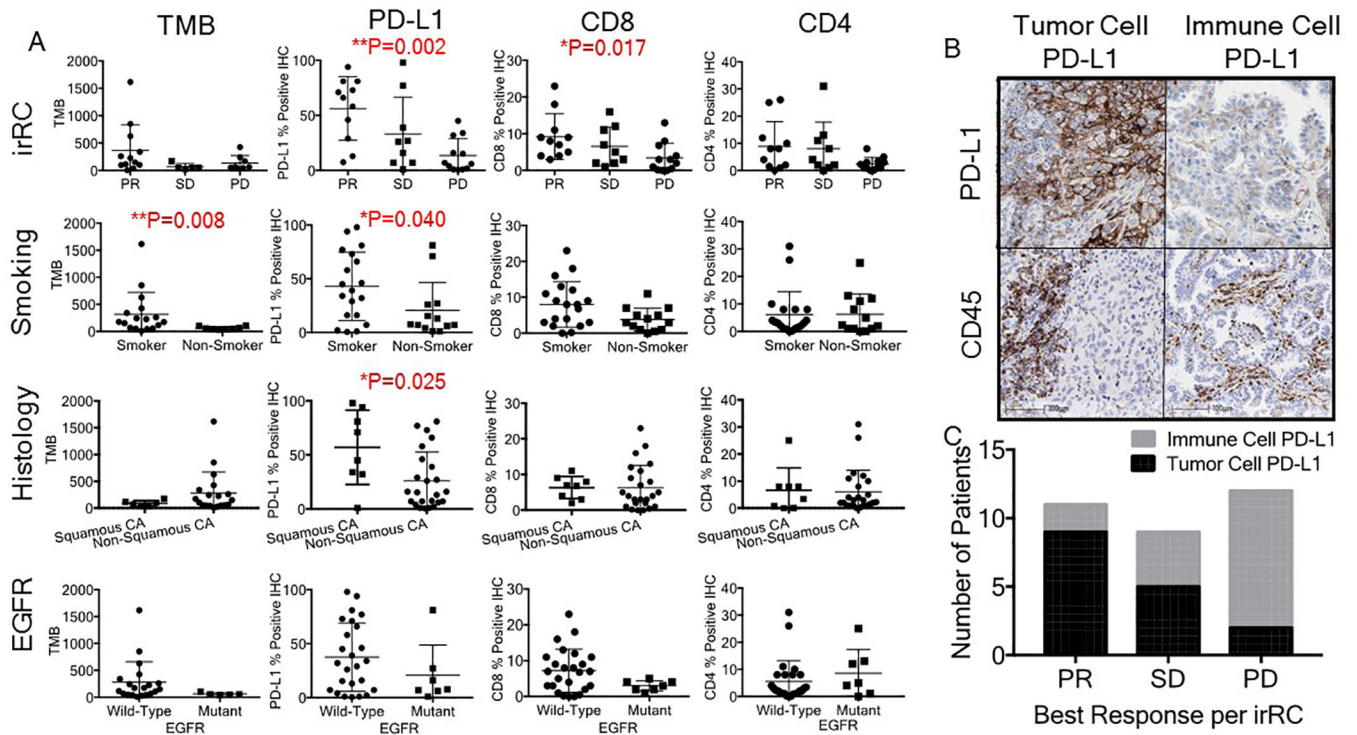
Figure 1.
Correlative factors and clinical characteristics. A, Scatter plot of correlative factors against clinical characteristics. B, Examples of PD-L1 expression on tumor vs. immune cells. C, Baseline PD-L1 expression in tumor vs. immune cells by best response per irRC (P = 0.007, Fisher exact test).
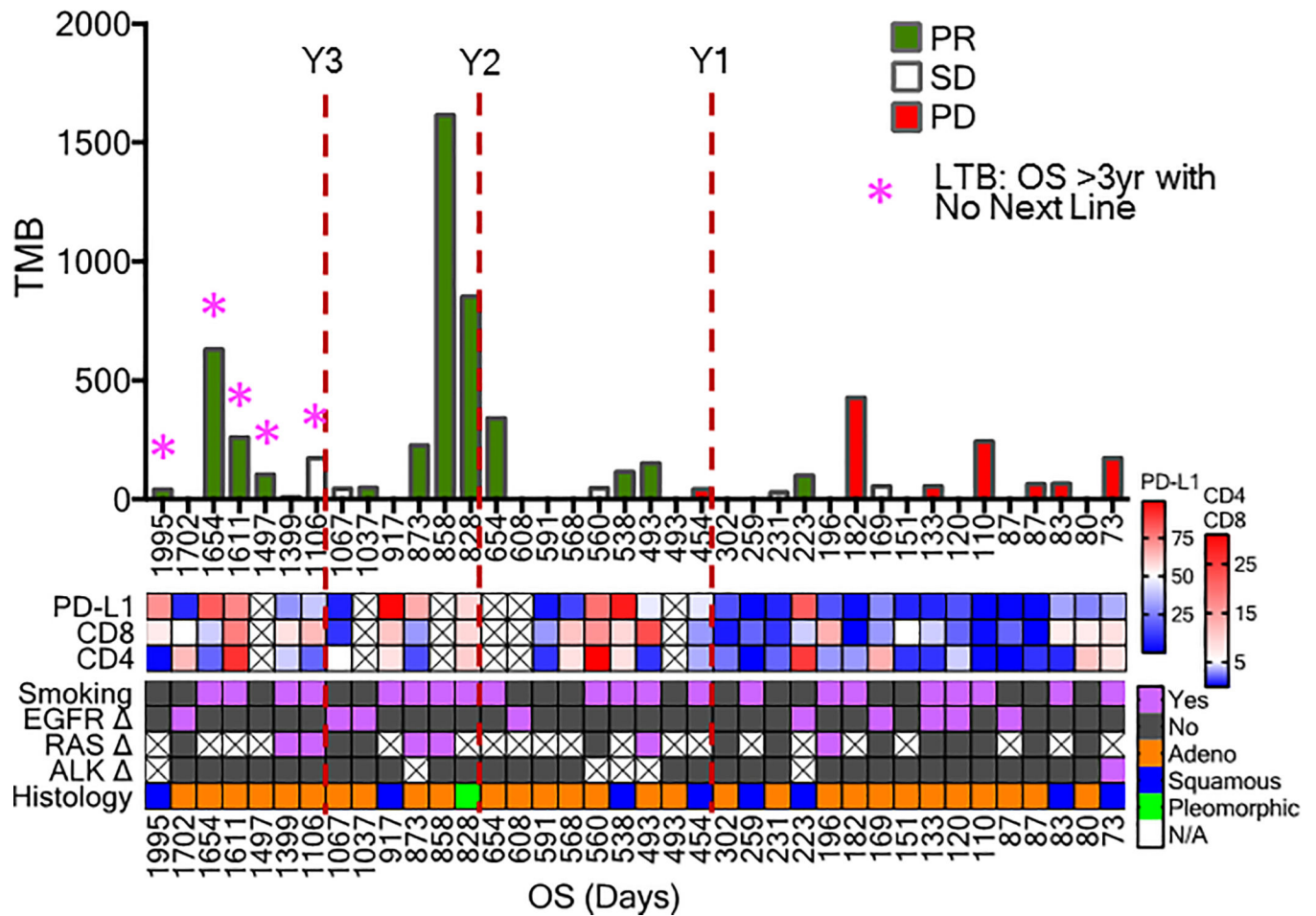
Figure 2.
Schematic presentation of the analyzed parameters in the correlative cohort per overall survival (OS). TMB: total mutational burden. PR: partial response. SD: stable disease. PD: progressive disease. Δ: mutation. LTB: Long term benefiter. Y1: year one. Y2: year two. Y3: year 3.
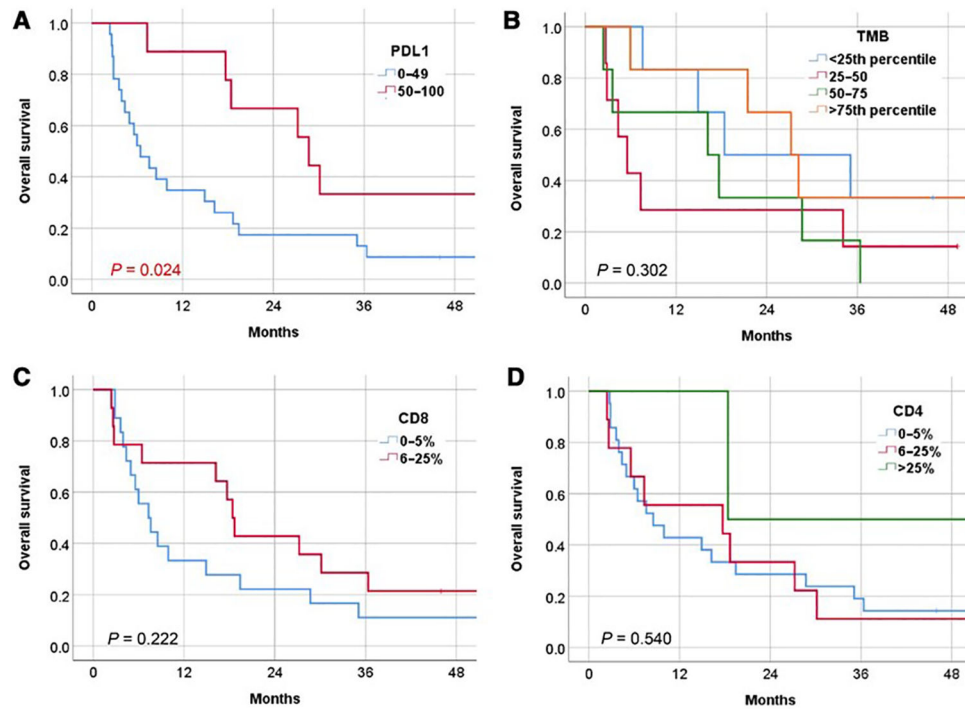
Figure 3.
OS and subgroup survival analysis of the correlative cohort (N = 38) by log-rank (Mantel–Cox) test. A, OS By PD-L1 categories (0%–49% n = 23 vs. >49% n = 9, P = 0.024). B, OS By TMB percentile (<25th percentile n = 6 vs. 25–50th percentile n = 7 vs. 50–75th percentile n = 6 vs. >75th percentile n = 6, P = 0.302). C, OS By CD8 categories (0%–5% n = 18 vs. 5%–25% n = 14, P = 0.222). D, OS By CD4 categories (0%–5% n = 21 vs. 5%–25% n = 9 vs. >25% n = 2, P = 0.540).

Table 1.

Patient characteristics of all patients, patients included in the correlative cohort, and long-term benefiters (5 of the 7 LTBs are in the correlative cohort)

Characteristics		UCLA Clinical Cohort (N=97)		Correlative Cohort (N=38)		Long Term Benefiters (N=7)	
Age-year							
	Median	65		67.5		59	
	Range	(32–83)		(48–82)		(51–68)	
Gender - no. (%)							
	Male	50	52%	22	58%	6	86%
	Female	47	48%	16	42%	1	14%
Previous Therapy Lines - no. (%)							
	0	13	13%	8	21%	2	29%
	1–3	61	63%	20	53%	5	71%
	>3	23	24%	10	26%	0	0%
Smoking Status - no. (%)							
	Ever	54	56%	21	55%	6	86%
	Never	43	44%	17	45%	1	14%
PD-L1 Proportion Score by Merck - no. (%)							
	Unknown	21		4		3	
	<1	21	22%	10	29%	0	0%
	1–49	38	39%	15	44%	1	25%
	50	17	17%	9	27%	3	75%
Histology - no. (%)							
	Non-squamous	78	80%	30	79%	5	71%
	Squamous	19	20%	8	21%	2	29%
Targetable Mutations - no. (%)							
	EGFR mutation	30	31%	9	24%	0	0%
	ALK Translocation	2	2%	1	3%	0	0%
Best response - no. (%)							
	PR	20	21%	16	42%	6	86%
	SD	28	29%	10	26%	1	14%
	PD	47	48%	12	32%	0	0%

Table 2.

Hazard Ratio of PFS, OS and ORR in all categories of clinical and tumor characteristics

Characteristics	PFS		OS		ORR (PR vs SD/PD)	
	HR (95% CI)	p-value	HR (95% CI)	p-value	OR (95% CI)	p-value
TMB	1.00 (0.99–1.00)	0.065	1.00 (1.00–1.00)	0.775	1.00 (0.99–1.00)	0.127
PD-L1	0.98 (0.96–0.99)	**0.002**	0.99 (0.97–1.00)	**0.032**	0.96 (0.93–0.99)	**0.007**
CD8	0.90 (0.83–0.99)	**0.026**	0.94 (0.87–1.02)	0.131	0.86 (0.74–1.00)	0.052
CD4	0.96 (0.90–1.01)	0.120	0.98 (0.93–1.03)	0.397	0.94 (0.85–1.03)	0.184
Age	1.00 (0.96–1.04)	0.992	0.99 (0.95–1.03)	0.487	0.98 (0.91–1.05)	0.498
Male	0.97 (0.46–2.02)	0.930	0.92 (0.46–1.87)	0.825	1.12 (0.31–4.14)	0.861
EGFR Mutations	1.56 (0.68–3.61)	0.298	1.08 (0.48–2.43)	0.846	1.38 (0.28–6.70)	0.694
ALK	--	--	--	--	--	--
EGFR or ALK	1.85 (0.82–4.18)	0.138	1.27 (0.58–2.77)	0.548	1.71 (0.36–8.15)	0.500
Ever smoker	0.83 (0.40–1.71)	0.613	1.02 (0.51–2.06)	0.955	0.93 (0.26–3.41)	0.917
Squamous	1.52 (0.65–3.59)	0.338	1.36 (0.58–3.15)	0.480	1.28 (0.26–6.33)	0.767
>=One Line Therapy	1.19 (0.51–2.79)	0.689	1.46 (0.60–3.58)	0.403	2.88 (0.57–14.44)	0.199

Table 3.

Univariate / multivariate models for PFS, OS, and best response (BR) using combinations of TMB, PD-L1, CD8, and CD4

		PFS	OS	ORR (PR vs SD/PD)
		c-stat	c-stat	c-stat
Univariate models (n=25 or n=32)				
	TMB (n=25)	0.64	0.51	0.71
	CD8 (n=32)	0.64	0.59	0.76
	PD-L1 (n=32)	**0.69**	**0.62**	**0.82**
	CD4 (n=32)	0.58	0.54	0.65
Univariate models (have both, n=21)		c-stat	c-stat	
	TMB (n=21)	0.63	0.54	0.65
	CD8 (n=21)	0.64	0.61	0.77
	PD-L1 (n=21)	**0.75**	**0.66**	**0.91**
	CD4 (n=21)	0.55	0.52	0.57
Two variable models (n=21 for all)		c-stat	c-stat	c-stat
	TMB	0.75	0.66	0.92
	PD-L1			
	TMB	0.71	0.62	0.79
	CD8			
	PDL1	0.71	0.63	0.89
	CD8			
	TMB	0.65	0.55	0.62
	CD4			
	CD8	0.64	0.59	0.78
	CD4			
	PD-L1	0.71	0.62	0.95
	CD4			