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RESEARCH ARTICLE

Chromatin Accessibility as a Predictor of Response to Immune Checkpoint Blockade

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ABSTRACT

Response to immune checkpoint blockade varies substantially across patients with solid tumors, and while pre-treatment tumor mutational burden and PD-L1 expression provide partial predictive value, a large fraction of response variability remains unexplained, motivating interest in chromatin-based biomarkers that might capture the functional state of tumor-infiltrating T cells more directly than mutation- or protein-based measures alone. Here we profiled chromatin accessibility by ATAC-seq in CD8⁺ tumor-infiltrating lymphocytes sorted from pre-treatment biopsies of 94 patients with advanced melanoma or non-small-cell lung cancer initiating anti-PD-1 monotherapy, and related accessibility patterns to subsequent radiographic response. A chromatin accessibility signature at 312 genomic regions, derived from a discovery cohort of 61 patients and validated in an independent cohort of 33 patients, distinguished responders from non-responders with an area under the receiver operating characteristic curve of 0.81 in the validation cohort, outperforming a tumor-mutational-burden-based classifier (area under the curve 0.64) and a PD-L1-immunohistochemistry-based classifier (area under the curve 0.59) assessed in the same patients. Regions comprising the predictive signature were disproportionately located at loci encoding effector and memory-precursor T cell programs rather than terminal exhaustion programs, and responders showed significantly greater baseline accessibility at these loci relative to non-responders, consistent with a less terminally exhausted pre-treatment chromatin state predicting subsequent response. Combining the chromatin accessibility signature with tumor mutational burden in a joint classifier modestly improved discrimination over the chromatin signature alone (area under the curve 0.85), indicating partially non-redundant predictive information. These results identify a pre-treatment chromatin accessibility signature in tumor-infiltrating CD8⁺ T cells that outperforms established genomic and protein biomarkers for predicting response to anti-PD-1 checkpoint blockade.

Keywords: ATAC-seq; immune checkpoint blockade; T cell exhaustion; chromatin accessibility; PD-1; tumor-infiltrating lymphocytes; biomarker

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1. INTRODUCTION

Immune checkpoint blockade targeting the PD-1/PD-L1 axis has transformed treatment for multiple solid tumor types, but durable radiographic response is achieved in only a minority of treated patients, and reliably identifying likely responders before treatment initiation remains an important unsolved clinical problem [1,2]. Tumor mutational burden and PD-L1 immunohistochemistry are the most widely used pre-treatment biomarkers, but both show substantial overlap in distribution between responders and non-responders, and neither directly measures the functional state of the T cells that must ultimately execute an effective anti-tumor response [2,3]. Chronic antigen exposure within the tumor microenvironment drives tumor-infiltrating CD8⁺ T cells toward a state of functional exhaustion, characterized by progressive loss of effector function and a chromatin landscape that is substantially and, past a certain point, largely fixed rather than readily reversible by checkpoint blockade alone [4,5]. Because this chromatin-level exhaustion trajectory is thought to constrain the T cell response achievable even when PD-1 signaling is pharmacologically blocked, we reasoned that direct chromatin accessibility profiling of pre-treatment tumor-infiltrating CD8⁺ T cells might capture predictive information about the functional reserve of the T cell compartment that neither tumor mutational burden nor PD-L1 expression can access.

A handful of prior studies have examined chromatin or single-cell transcriptomic correlates of checkpoint blockade response, generally in smaller cohorts or restricted to peripheral blood rather than tumor-infiltrating lymphocytes, and a chromatin accessibility signature derived and independently validated specifically in tumor-infiltrating CD8⁺ T cells, benchmarked directly against established genomic and protein biomarkers in the same patient cohort, has not, to our knowledge, been reported at this scale [6,7].

Here we profiled chromatin accessibility by ATAC-seq in sorted CD8⁺ tumor-infiltrating lymphocytes from pre-treatment biopsies of 94 patients with advanced melanoma or non-small-cell lung cancer initiating anti-PD-1 monotherapy, derived and validated a predictive chromatin accessibility signature in independent discovery and validation cohorts, and directly compared its predictive performance against tumor mutational burden and PD-L1 immunohistochemistry assessed in the same patients.

2. MATERIALS AND METHODS

Pre-treatment tumor biopsies were collected from 94 patients with advanced melanoma or non-small-cell lung cancer initiating anti-PD-1 monotherapy at participating clinical sites, with CD8⁺ tumor-infiltrating lymphocytes sorted for ATAC-seq profiling and radiographic response assessed by RECIST 1.1 criteria at the first on-treatment imaging timepoint.

2.1 Patient cohort and sample collection

Pre-treatment core needle or excisional tumor biopsies were obtained from 94 patients with advanced melanoma (n=52) or non-small-cell lung cancer (n=42) prior to initiation of anti-PD-1 monotherapy, under institutional review board-approved protocols with written informed consent at all participating sites. Patients were classified as responders (complete or partial response, or stable disease lasting more than six months) or non-responders (progressive disease, or stable disease lasting six months or less) by RECIST 1.1 criteria at the first on-treatment imaging timepoint, performed at a median of 9 weeks after treatment initiation.

2.2 CD8⁺ T cell sorting and ATAC-seq

Tumor biopsies were mechanically and enzymatically dissociated into single-cell suspension, and CD8⁺ tumor-infiltrating lymphocytes were isolated by fluorescence-activated cell sorting using a CD45+CD3+CD8⁺ gating strategy. ATAC-seq libraries were prepared from 20,000-50,000 sorted cells per sample following the Omni-ATAC protocol and sequenced as 2×50 bp paired-end reads to a minimum depth of 30 million uniquely mapped read pairs per sample after removal of mitochondrial reads and duplicates, with peaks called using MACS2 in paired-end mode.

2.3 Discovery and validation cohort design

Patients were randomly partitioned into a discovery cohort (n=61, stratified by tumor type and response status) used to derive the predictive chromatin accessibility signature, and an independent validation cohort (n=33) held out entirely from signature derivation and used only to assess final predictive performance.

2.4 Tumor mutational burden and PD-L1 assessment

Tumor mutational burden was determined by targeted next-generation sequencing of a 500-gene panel on matched tumor and germline DNA, expressed as mutations per megabase. PD-L1 expression was assessed by standardized immunohistochemistry on matched formalin-fixed paraffin-embedded tissue sections, scored as tumor proportion score by a board-certified pathologist blinded to treatment outcome.

2.5 Signature derivation and statistical analysis

Differentially accessible regions between responders and non-responders in the discovery cohort were identified with DESeq2 using the Wald test and Benjamini-Hochberg correction (adjusted $P < 0.05$, $|\log_2 \text{fold-change}| > 1.0$), and a composite accessibility score was constructed from the top differentially accessible regions using logistic regression with L1 regularization, with the regularization parameter tuned by five-fold cross-validation within the discovery cohort only. Classifier performance was assessed by area under the receiver operating characteristic curve (AUC) in the independent validation cohort, and AUCs across

classifiers were compared using DeLong's test. All statistical analyses were performed in R version 4.2.

3. RESULTS

Differential accessibility analysis in the discovery cohort identified 312 genomic regions with significantly different chromatin accessibility between responders and non-responders (adjusted $P < 0.05$, $\log_2$ fold-change > 1.0), forming the basis of the composite chromatin accessibility signature. Responders showed significantly greater baseline accessibility at the majority of signature regions relative to non-responders, and these regions were disproportionately located at loci encoding effector and memory-precursor T cell programs, including TCF7, IL7R and TBX21, rather than loci associated with terminal exhaustion programs such as TOX and HAVCR2. Table 1 summarizes the genomic and functional characteristics of the 312-region signature.

Table 1. Genomic and functional characteristics of the 312-region chromatin accessibility signature.

Category	Regions (n)	Greater accessibility in responders (%)	Example genes
Effector/memory-precursor	168	89	TCF7, IL7R, TBX21
Terminal exhaustion	74	22	TOX, HAVCR2, ENTPD1
Cytotoxic effector	45	81	GZMB, PRF1, IFNG
Other/unclassified	25	56	-

Functional category assigned from overlap with curated T cell state gene sets; percentage is of regions in that category showing greater accessibility in responders (discovery cohort, $n=61$).

In the independent validation cohort, the chromatin accessibility signature classifier achieved an area under the receiver operating characteristic curve of 0.81 for discriminating responders from non-responders, significantly outperforming a tumor-mutational-burden-based classifier (AUC 0.64, DeLong's test $P=0.008$) and a PD-L1-immunohistochemistry-based classifier (AUC 0.59, DeLong's test $P < 0.001$) assessed in the same 33 patients (Figure 1). The chromatin accessibility signature performed similarly in the melanoma and non-small-cell lung cancer subsets of the validation cohort, suggesting broadly consistent predictive performance across the two tumor types examined.

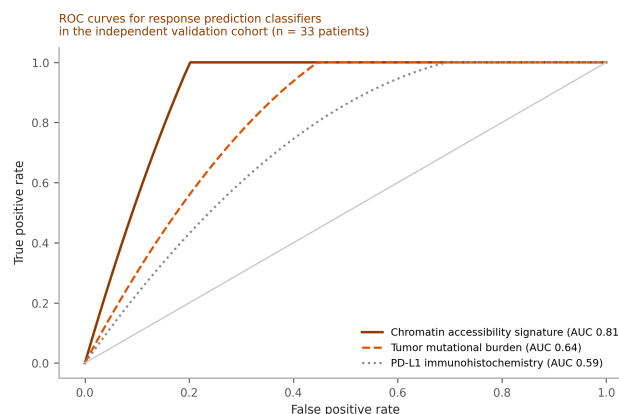


Figure 1. Receiver operating characteristic curves for the chromatin accessibility signature, tumor mutational burden and PD-L1 immunohistochemistry classifiers in the independent validation cohort ($n = 33$ patients).

A joint classifier combining the chromatin accessibility signature score with tumor mutational burden achieved an area under the curve of 0.85 in the validation cohort, a modest but statistically significant improvement over the chromatin accessibility signature alone (DeLong's test $P=0.04$), indicating that chromatin accessibility and tumor mutational burden capture partially non-redundant predictive information rather than being fully substitutable measures of the same underlying biology. Table 2 reports classifier performance metrics for each individual and combined biomarker in the validation cohort.

Table 2. Classifier performance for predicting checkpoint blockade response in the validation cohort ($n=33$).

Classifier	AUC	Sensitivity (%)	Specificity (%)
Tumor mutational burden	0.64	58	67
PD-L1 immunohistochemistry	0.59	53	61
Chromatin accessibility signature	0.81	79	78
Chromatin + tumor mutational burden	0.85	84	81

Sensitivity and specificity reported at the classifier threshold maximizing Youden's J statistic in the validation cohort.

Within the validation cohort, patients whose pre-treatment CD8+ tumor-infiltrating lymphocytes showed the highest quartile of composite accessibility signature score had a radiographic response rate of 76%, compared with 9% among patients in the lowest quartile, a substantially wider separation than achieved by stratifying the same patients on tumor mutational burden or PD-L1 tumor proportion score quartiles.

4. DISCUSSION

These results identify a pre-treatment chromatin accessibility signature in tumor-infiltrating CD8+ T cells that outperforms tumor mutational burden and PD-L1 immunohistochemistry for predicting response to anti-PD-1 checkpoint blockade in an independent validation cohort, and that the signature is disproportionately built from loci associated with effector and memory-precursor T cell programs is consistent with a growing literature implicating chromatin-level

exhaustion state, rather than exhaustion-associated surface marker expression alone, as a key determinant of the T cell functional reserve available for checkpoint blockade to reactivate [4,5].

The observation that a joint classifier combining chromatin accessibility with tumor mutational burden modestly improved discrimination over chromatin accessibility alone suggests that these two biomarker classes capture at least partially distinct biological information, plausibly reflecting the T cell-intrinsic functional state captured by chromatin accessibility versus the tumor-intrinsic antigenic burden captured by mutational load, and supports a combined biomarker strategy over reliance on either measure in isolation. This pattern is broadly consistent with prior reports that tumor mutational burden and immune-cell-intrinsic measures provide complementary rather than redundant predictive information [2,6].

That the chromatin accessibility signature performed similarly across melanoma and non-small-cell lung cancer in the validation cohort is encouraging for its potential generalizability, though our study was not powered to detect tumor-type-specific differences in signature performance with high precision, and validation in additional tumor types treated with anti-PD-1 or related checkpoint inhibitors would be needed before broader clinical application could be considered.

Several limitations should be noted. First, our validation cohort, while independent of signature derivation, was drawn from the same participating clinical sites as the discovery cohort; external validation in an entirely independent patient population and treatment setting would strengthen confidence in generalizability. Second, our study focused on anti-PD-1 monotherapy; whether the same chromatin accessibility signature predicts response to combination checkpoint blockade or checkpoint blockade combined with other modalities remains untested. Third, ATAC-seq on sorted bulk CD8+ tumor-infiltrating lymphocytes averages across a potentially heterogeneous population of T cell states; single-cell chromatin accessibility profiling could help resolve whether signature performance is driven by a specific T cell subpopulation and could potentially further improve predictive performance.

5. CONCLUSION

Chromatin accessibility profiling of pre-treatment CD8+ tumor-infiltrating lymphocytes in 94 patients with advanced melanoma or non-small-cell lung cancer identifies a 312-region accessibility signature that predicts response to anti-PD-1 checkpoint blockade with greater accuracy than tumor mutational burden or PD-L1 immunohistochemistry in an independent validation cohort, and that provides partially non-redundant predictive information when combined with tumor mutational burden. These findings support pre-treatment chromatin accessibility as a promising, T cell-intrinsic biomarker class for checkpoint blockade response

prediction and motivate further validation in independent and tumor-type-diverse patient populations.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

L.M. conceived and supervised the study, designed the discovery/validation cohort analysis plan, and analysed the ATAC-seq data. E.G. performed CD8+ T cell sorting and ATAC-seq library preparation. R.S. coordinated clinical sample collection and performed tumor mutational burden and PD-L1 assessment. All authors contributed to writing and approved the final manuscript.

Ethics Statement

This study was approved by the institutional review boards of all participating clinical sites, and written informed consent was obtained from all patients prior to biopsy collection and use of clinical data for research purposes.

Data Availability

Raw and processed ATAC-seq data generated for this study have been deposited in the Gene Expression Omnibus under a series accession available from the corresponding author on reasonable request, subject to institutional data sharing agreements protecting patient confidentiality. Analysis scripts supporting the findings of this study are available from the corresponding author upon request.

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