

Peripheral Blood TCRB Chain Convergence Predicts Response to Dendritic Cell-Based Immunotherapy in Advanced-Stage Melanoma Patients



ThermoFisher
SCIENTIFIC

Walter J. Storkus¹, Yan Lin¹, Lauren Miller², Denise Topacio-Hall², Geoffrey Lowman², Timothy J Looney³, Lisa H. Butterfield¹, Jennifer L. Taylor¹, Ahmad Tarhini⁴, Hussein Tawbi⁵, John M. Kirkwood¹.

From the ¹University of Pittsburgh School of Medicine & Hillman Cancer Center (Pittsburgh, PA), ²ThermoFisher (Carlsbad, CA), ³ThermoFisher (San Francisco, CA), ⁴Cleveland Clinic Foundation (Cleveland, OH), ⁵MD Anderson-Cancer Center (Houston, TX). Correspondence to: Walter J. Storkus, Ph.D. (storkuswj@upmc.edu).

Abstract

T cell receptor (TCR) convergence refers to the phenomenon whereby antigen-driven selection enriches for TCRs having a shared antigen specificity but different nucleotide sequences. TCR convergence may be indicative of tumor immunogenicity and thus the sensitivity of a cancer to immunotherapy. Here we used next-generation sequencing of peripheral blood TCRB chain repertoires to evaluate TCR convergence as a predictive biomarker for response to a dendritic cell (DC)/peptide-based vaccine (UPCI 12-048; NCT01876212) targeting tumor-associated blood vessel antigens in 13 evaluable HLA-A2⁺ patients with advanced-stage cutaneous or uveal melanoma. We further evaluated the relationship between TCR convergence and response biomarkers derived from targeted gene expression profiling of pre-treatment (baseline) tumor biopsies.

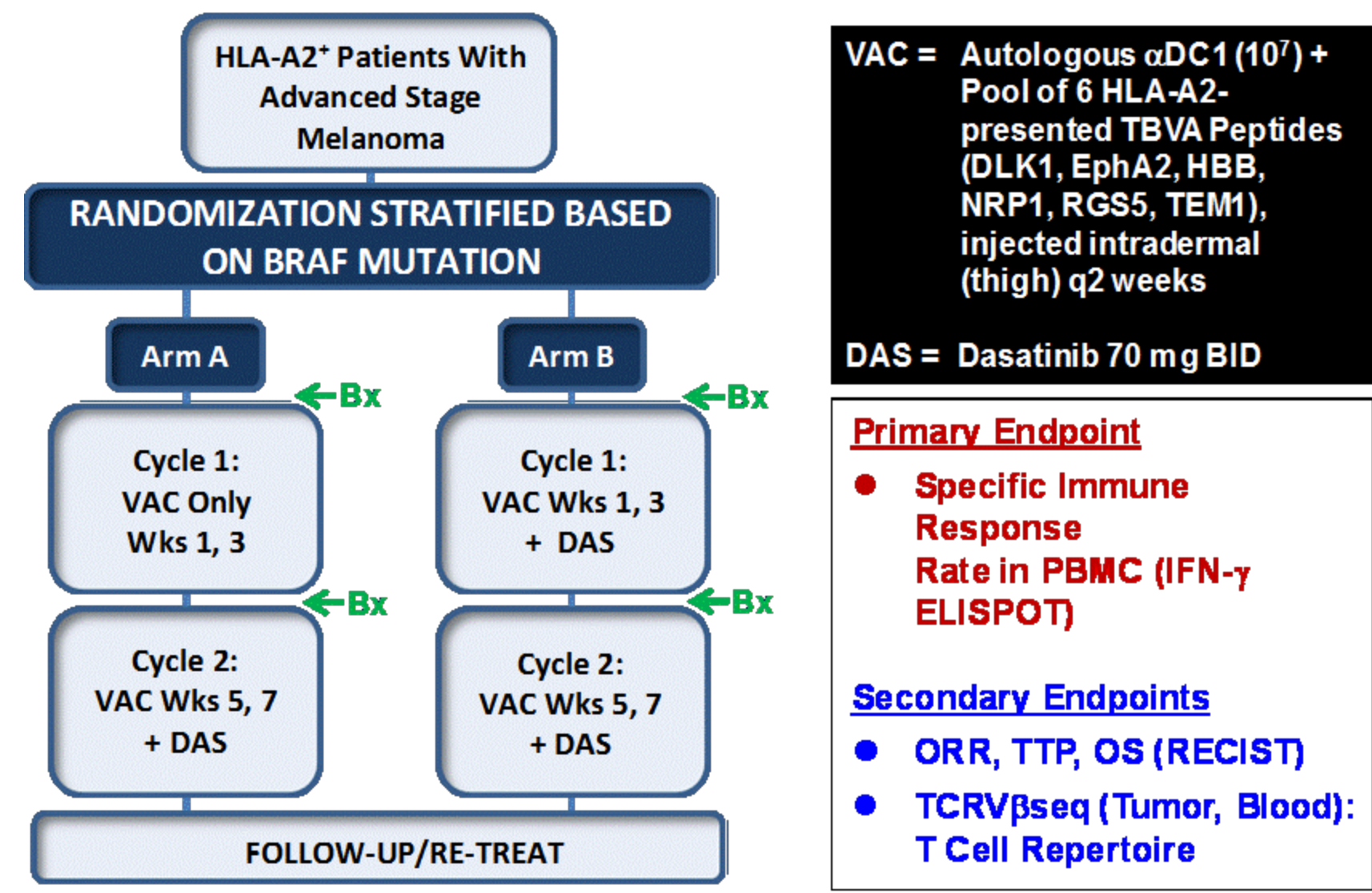
Background

Based on preclinical modeling supporting the therapeutic efficacy of DC/peptide-based vaccines targeting tumor-associated blood vessels and the ability of the tyrosine kinase inhibitor dasatinib to favorably condition the host for improved host responsiveness to vaccination and to better direct/sustain vaccine-induced TIL, we developed an open-label, single-center, prospective randomized Phase II clinical trial at the University of Pittsburgh Hillman Cancer Center for HLA-A2⁺ patients with advanced-stage melanoma (NCT01876212). Patients received ID injections of Type-1-polarized, autologous DC loaded with a mixture of peptides derived from six tumor-associated vascular antigens (DLK1, EPHA2, HBB, NRP1, RGS5, TEM1)-derived peptides combined +/- daily oral administration of dasatinib (70 mg BID) applied as an immune adjuvant/conditioning agent. The primary objective of this study is to evaluate the effects of the combination immunotherapy on specific CD8⁺ T cell response rates (in IFN- γ ELISPOT assays). Secondary objectives included the evaluation of the objective clinical responses (RECIST) and exploratory immunological endpoints, including TCRBseq analysis of patient T cell repertoires in PBMC and tumor biopsies at baseline and post-treatment to define potential predictive biomarkers.

Methods

Patient Demographics and Treatment. Sixteen HLA-A2⁺ patients with advanced-stage cutaneous (11) or uveal (5) melanoma were entered on NCT01876212 (Fig. 1), of which 13 were evaluable for primary endpoint analysis in week 5 of study performance. Evaluable patients included 8 males/3 females ranging in age from 42-83 years old, with 8 cutaneous disease patients and 5 uveal melanoma patients. Sites of disseminated disease included skin, liver, lung, lymph node, soft tissue. All patients had progressed on prior immune checkpoint blockade protocols (Fig. 2). In advance of treatment, patient apheresis was performed to isolate monocytes used to develop autologous Type-1-polarized DC (by the Immunologic Monitoring and Cellular Products Laboratory (IMCPL) of the Hillman Cancer Center), that were subsequently loaded with a mixture of peptides derived from the 6 aforementioned tumor-associated vascular antigens combined +/- daily oral administration of dasatinib (applied as an immune adjuvant/conditioning agent). On the day of treatment, vaccines were thawed and delivered intradermally (arm, thigh) every 2 weeks until disease progression. Peripheral blood and tumor was isolated at baseline and at various timepoints during treatment (week 5 only for tumor). Objective clinical responses (OCR) were determined by PET-CT imaging.

Figure 1. Schema and Endpoint Analyses (NCT01876212)



TCRBseq Analysis. Total RNA was extracted from peripheral blood leukocytes (PBL) from the 13 evaluable HLA-A2⁺ patients, which included 6 responders (4 PR, 2 SD) and 7 non-responders (i.e. progressive disease; PD). TCRB chain repertoire libraries were constructed by multiplex PCR utilizing FR1 and constant gene targeting primers via the Oncomine TCRB-LR assay, then sequenced using the Ion Torrent S5 to a target depth of 1.5M raw reads per library. To evaluate T cell repertoire convergence we searched for instances where TCRB chains were identical in amino acid space but had distinct nucleotide sequences owing to N-addition and exonucleotide chewback within the V-D and D-J junctions of the CDR3. Targeted gene expression profiling of pre- and post-treatment tumor biopsies was performed via the Oncomine Immune Response Research Assay (OIRRA) using total RNA input.

Results

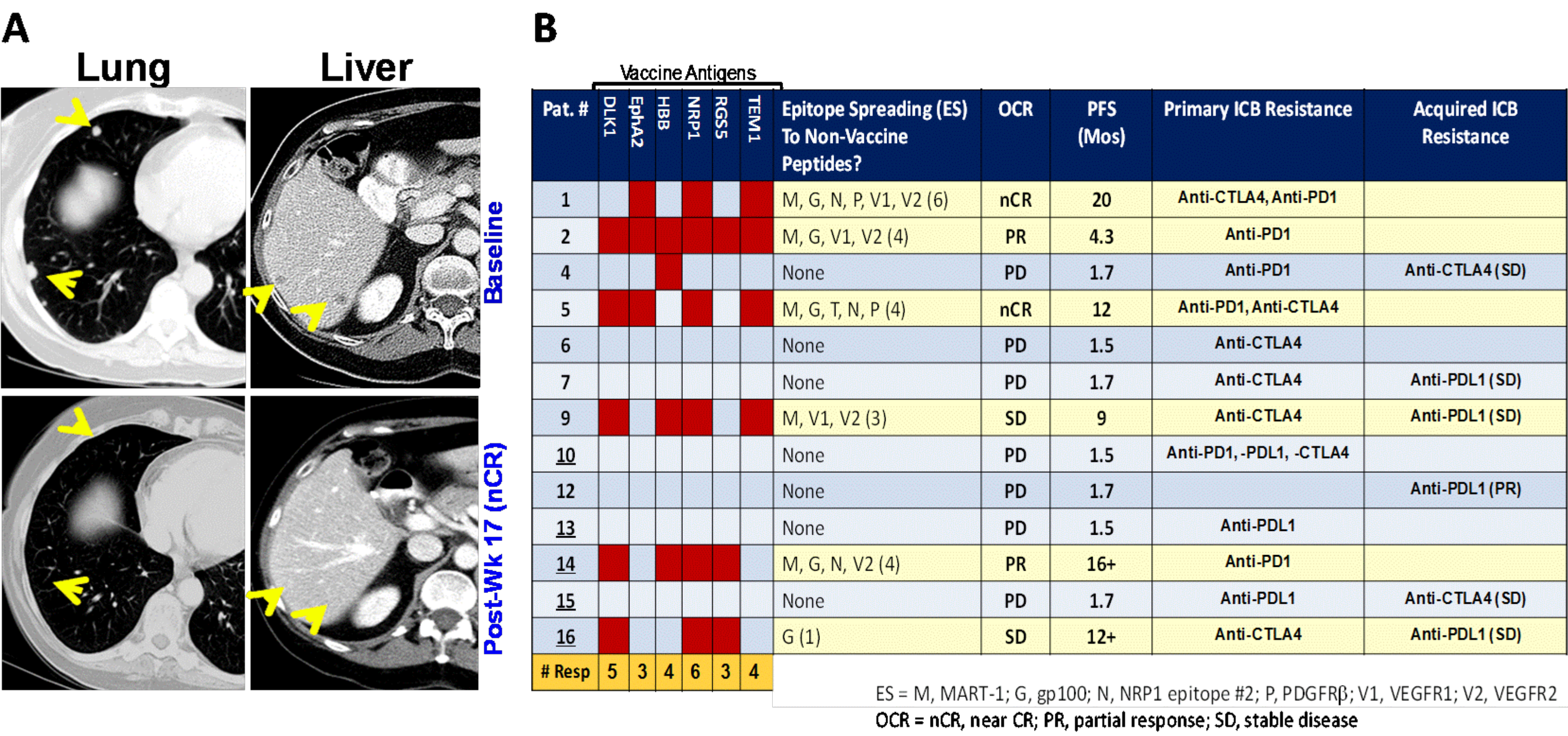


Figure 3. Baseline TCRB Convergence in the Peripheral T Cell Repertoire Is Predictive of OCR in Patients Treated on UPCI 12-048/NCT01876212. RNA was isolated from patient PBMC prior to treatment, and TCRB sequencing performed using the ThermoFisher Ion Torrent™ platform. In **A**, an example of TCRB convergence is cited from patient #5 (near-CR). In **B**, the aggregate frequency of convergent TCRB sequences is reported for evaluable patients based on treatment outcomes, with 6 patients exhibiting OCR and 7 patients with progressive disease post-therapy. Panel **C** reports baseline aggregate frequencies of convergent TCRB versus time-to-progression, while panel **D** correlates aggregate frequencies of convergent TCRB with PD-L1 transcript expression levels at baseline amongst the 13 evaluable PD/OCR patients. For panels **B-D**, each symbol (dots) represent an individual patient.

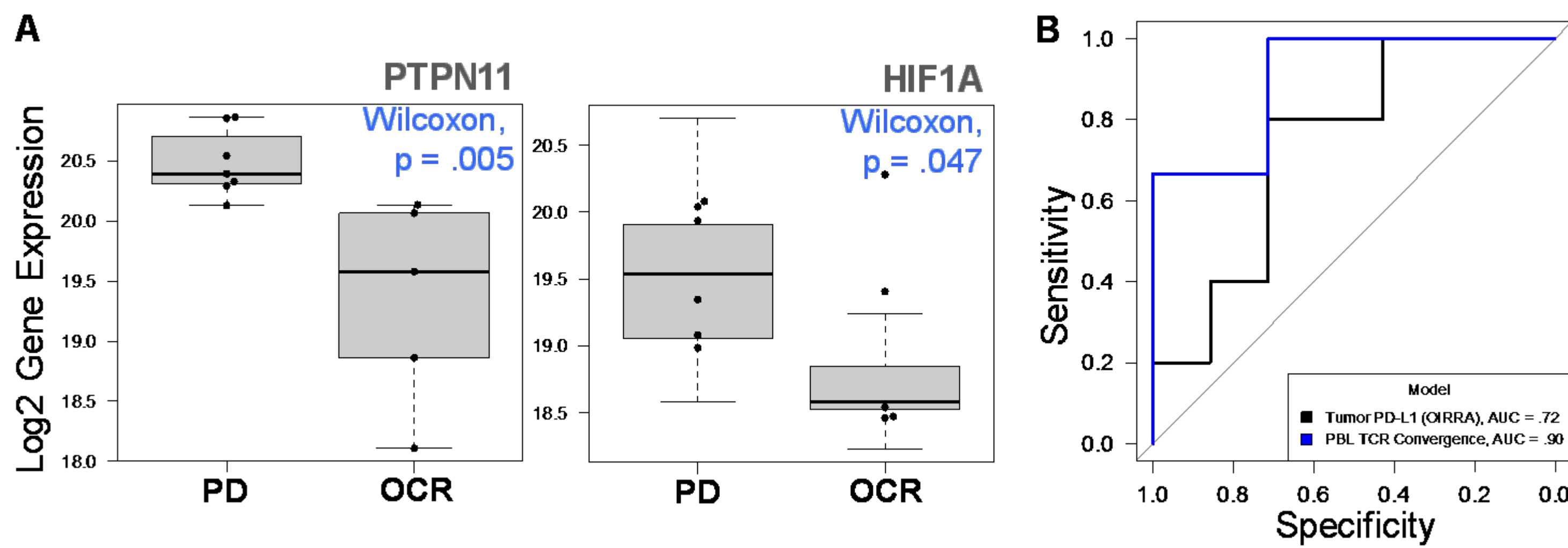


Figure 4. Tumor/Blood Biomarkers Associated with Clinical Outcomes. In **A**, targeted gene expression profiling (OIRRA) of tumor biopsy materials revealed reduced transcript levels of PTPN11 and HIF1A in post-treatment biopsies from OCR vs. PD patients. In **B**, ROC curves for prediction of clinical response based on tumor expression of PD-L1 (OIRRA) and TCRB convergence at baseline.

Summary of Results:

- Sequencing of TCRB libraries yielded on average 20k clonotypes per individual with mean evenness (normalized Shannon entropy) of .84.
- TCR convergence was elevated in pretreatment PBL of responders compared to non-responders (mean frequency .012 vs .006; $p = .014$, Wilcoxon), discriminated responders from non-responders with high accuracy (AUROC = .90), and closely correlated with time to progression following treatment (Pearson correlation = .78; $p = .004$).
- Targeted gene expression profiling of tumor revealed elevated PD-L1 expression in pre-treatment responders compared to non-responders and reduced PTPN11 and HIF1A in post-treatment biopsies from responders compared to non-responders.
- Combining pre-treatment PBL TCR convergence values with tumor PD-L1 expression values improved the prediction of response.

Conclusions and Significance

These data suggest that peripheral blood TCRB convergence may serve as a useful biomarker for response to (dendritic cell-based) immunotherapy when used alone or in combination with established biomarkers (such as PD-L1 expression in tumor) derived from transcriptional profiling of the tumor microenvironment.

Ongoing and future studies will further clarify the prognostic and/or predictive utility of TCRB convergence as an immune repertoire biomarker.

Acknowledgments

This work was supported by NIH R01 CA168118 (WJS). Trial Registration: NCT01876212. This study was closed to accrual on 7/11/18; primary endpoint: Immunologic response based on IFN- γ ELISPOT from peripheral blood CD8⁺ T cells; secondary endpoints include OCR (RECIST), analysis of TCRBseq from peripheral blood/tumor at baseline and 5 weeks post-treatment. Ethics Approval: This study was approved by the University of Pittsburgh IRB (#PRO12060479).