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

Walter J. Storkus[1], Yan Lin[1], Lauren Miller[2], Denise Topacio-Hall[2], Geoffrey Lowman[2], Timothy J Looney[3], Lisa H. Butterfield[1], Jennifer L. Taylor[1], Ahmad Tarhini[4], Hussein Tawbi[5], John M. Kirkwood[1].

From the [1]University of Pittsburgh School of Medicine & Hillman Cancer Center (Pittsburgh, PA), [2]Thermofisher (Carlsbad, CA), [3]Thermofisher (San Francisco, CA), [4]Cleveland Clinic Foundation (Cleveland, OH), [5]MD 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.

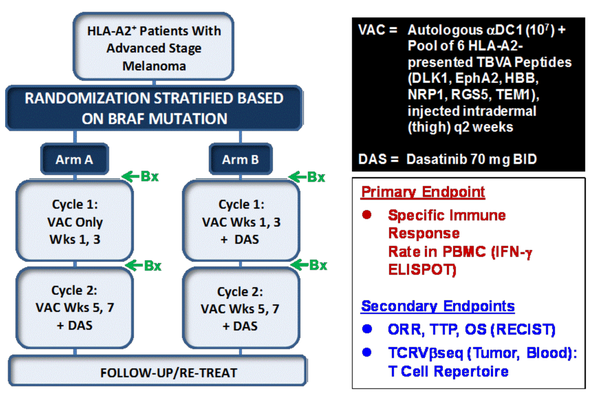
Background

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

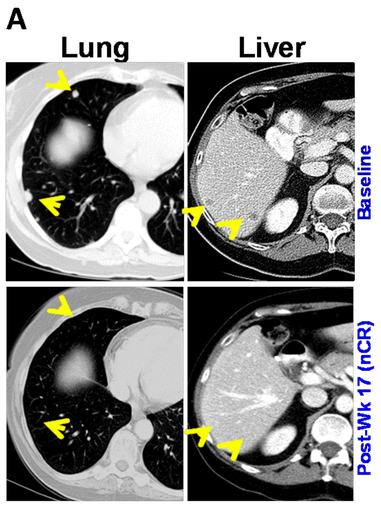
Patient Demographics and Treatment. Sixteen HLA-A2$^+$ patients with advanced-stage cutaneous (11) or uveal (5) melanoma were entered on NCT01876212 (**Fig. 1**), of which 13 were evaluable for primary endpoint analysis in week 5 of study performance. Evaluable patients included 8 males/3 females ranging 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



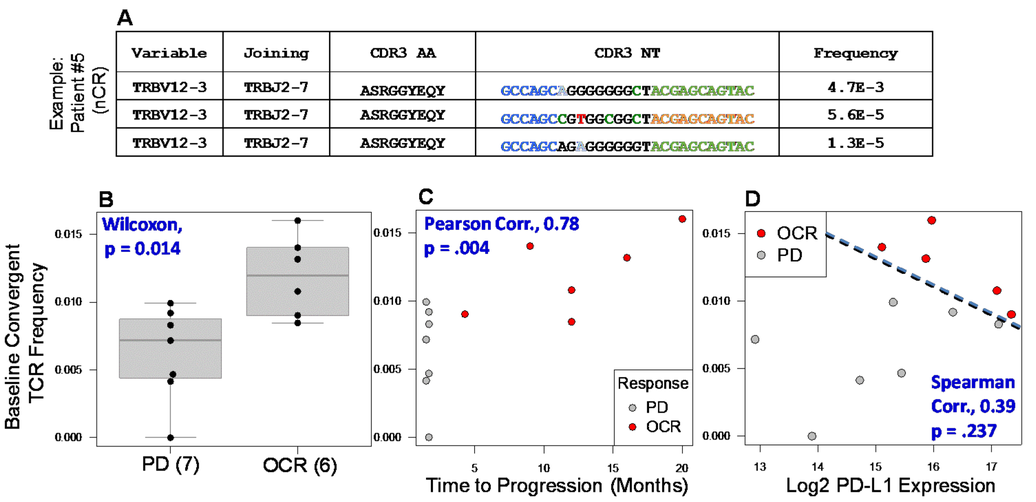
B

Pat. #	Vaccine Antigens: DLK1	EphA2	HBB	NRP1	RGS5	TEM1	Epitope Spreading (ES) To Non-Vaccine Peptides?	OCR	PFS (Mos)	Primary ICB Resistance	Acquired ICB Resistance
1		■		■	■		M, G, N, P, V1, V2 (6)	nCR	20	Anti-CTLA4, Anti-PD1	
2	■	■	■	■	■		M, G, V1, V2 (4)	PR	4.3	Anti-PD1	
4			■				None	PD	1.7	Anti-PD1	Anti-CTLA4 (SD)
5	■	■		■		■	M, G, T, N, P (4)	nCR	12	Anti-PD1, Anti-CTLA4	
6							None	PD	1.5	Anti-CTLA4	
7							None	PD	1.7	Anti-CTLA4	Anti-PDL1 (SD)
9	■		■	■		■	M, V1, V2 (3)	SD	9	Anti-CTLA4	Anti-PDL1 (SD)
10							None	PD	1.5	Anti-PD1, -PDL1, -CTLA4	
12							None	PD	1.7		Anti-PDL1 (PR)
13							None	PD	1.5	Anti-PDL1	
14	■		■	■	■		M, G, N, V2 (4)	PR	16+	Anti-PD1	
15							None	PD	1.7	Anti-PDL1	Anti-CTLA4 (SD)
16	■			■	■		G (1)	SD	12+	Anti-CTLA4	Anti-PDL1 (SD)
# Resp	5	3	4	6	3	4					

ES = M, MART-1; G, gp100; N, NRP1 epitope #2; P, PDGFRβ; V1, VEGFR1; V2, VEGFR2
OCR = nCR, near CR; PR, partial response; SD, stable disease

Figure 2. Summary of Immunological and Clinical Response Data. In **A**, PET-CT imaging of pulmonary and liver metastases is provided for patient #5 (near-CR) at baseline vs. 17 weeks post entry on trial. In **B**, tabulated data for the 13 evaluable patients treated on trial are presented, including peripheral blood CD8$^+$ T cell response (IFN-γ ELISPOT) to peptide epitopes in the vaccine formulation and peptide epitopes not included in the vaccine, but contained in melanoma lineage antigens or in melanoma-associated vascular antigens; best clinical response (OCR); progression free survival (in months); prior resistance (primary vs. acquired) to immune checkpoint blockade (ICB); and time since last treatment with ICB at initiation of treatment on NCT01876212).

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.



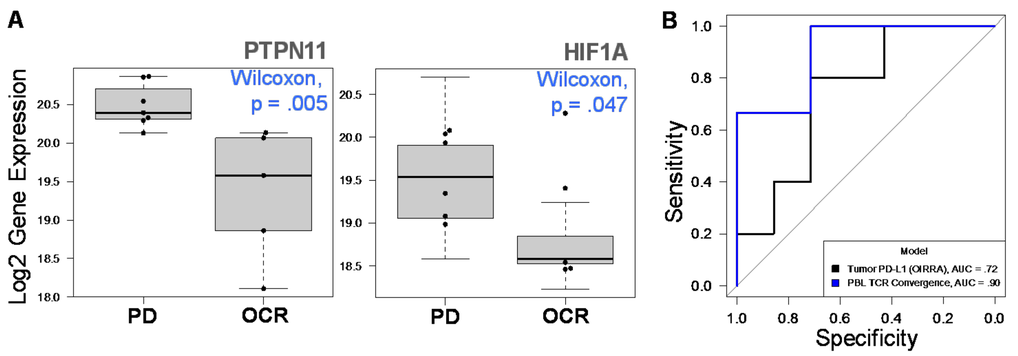


Figure 4. Tumor/Blood Biomarkers Associated with Clinical Outcomes. In **A**, targeted gene expression profiling (OIRRA) of tumor biopsy materials revealed reduced transcript levels of PTPN11 and HIF1A in post-treatment biopsies from OCR vs. PD patients. In **B**, ROC curves for prediction of clinical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

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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).