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T-cell receptor beta variable gene polymorphism predicts immune-related adverse events during checkpoint blockade immunotherapy

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ABSTRACT

Background Immune checkpoint inhibitors have revolutionized cancer treatment. However, they are associated with a unique spectrum of side effects, called immune-related adverse events (irAEs), which can cause significant morbidity and quickly progress to severe or life-threatening events if not treated promptly. Identifying predictive biomarkers for irAEs before immunotherapy initiation is therefore a critical area of research. Polymorphisms within the T-cell receptor beta (TCRB) variable (TRBV) gene have been implicated in autoimmune disease and may be mechanistically linked to irAEs. However, the repetitive nature of the TCRB locus and incomplete genome assembly has hampered the evaluation of TRBV polymorphisms in the past.

Patients and methods We used a novel method for long-amplicon next generation sequencing of rearranged TCRB chains from peripheral blood total RNA to evaluate the link between TRBV polymorphisms and irAEs in patients treated with immunotherapy for cancer. We employed multiplex PCR to create amplicons spanning the three beta chain complementarity-determining regions (CDR) regions to enable detection of polymorphism within the germline-encoded framework and CDR1 and CDR2 regions in addition to CDR3 profiling. Resultant amplicons were sequenced via the Ion Torrent and TRBV allele profiles constructed for each individual was correlated with irAE annotations to identify haplotypes associated with severe irAEs (≥ grade 3).

Results Our study included 81 patients who had irAEs when treated with immunotherapy for cancer. By using principal component analysis of the 81 TRBV allele profiles followed by k-means clustering, we identified six major TRBV haplotypes. Strikingly, we found that one-third of this cohort possessed a TRBV allele haplotype that appeared to be protective against severe irAEs.

Conclusion The data suggest that long-amplicon TCRB repertoire sequencing can potentially identify TRBV haplotype groups that correlate with the risk of severe irAEs. Germline-encoded TRBV polymorphisms may serve as a predictive biomarker of severe irAEs.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The polymorphic nature of T cell receptor and inherited abnormalities of T cell receptor genes were associated with autoimmune disease. Thus, we reasoned that inherited abnormalities in the T cell receptor beta (TCRB) variable (TRBV) gene may result in aberrant T cell function and thus increase the probability of autoantigen recognition. Although structural features and a link to chronic autoimmune disease support the concept that germline-encoded TRBV polymorphism could be a key determinant of immune-related adverse events (irAEs), prior efforts using short-read whole-genome sequencing failed to identify germline variants associated with irAEs, though the role of TRBV polymorphism has not been assessed owing to challenges in analyzing the repetitive TCRB locus using these techniques.

WHAT THIS STUDY ADDS

⇒ This represents the first next-generation sequencing (NGS)-based method to permit haplotype-level resolution of the TRB locus. In this study, using the novel method we had developed to detect TRBV polymorphism by long-amplicon NGS of rearranged TCRB chains from peripheral blood leukocytes, we were able to identify six major TRBV haplotypes in 81 patients who had irAEs following administration of immune checkpoint inhibitors for treatment of cancer. Strikingly, one-third of the patients possess a TRBV allele haplotype that appears protective against grade 3 or higher irAEs.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Germline-encoded TRBV polymorphism may serve as a predictive biomarker of irAEs to identify patients at risk for severe irAEs (≥grade 3). Risk-assessment-based stratification will enable patients to receive personalized irAE-monitoring and treatment plans and adopt proactive measures to mitigate the risk of irAEs. Such measures will allow for prevention and early recognition of irAEs, which is critical for optimal irAE management, while improving patient outcomes.

INTRODUCTION

Immune checkpoint inhibitors (ICIs) have created a paradigm shift in cancer treatment[1] by reversing tumor-induced inhibition of the immune system, thereby unleashing potent cytotoxic T-cell mediated anti-tumor responses. T-cell responses mediating durable progression-free survival may also promote T-cell destruction of healthy tissue in some patients to produce a unique spectrum of side effects called immune-related adverse events (irAEs). These irAEs may affect multiple organs[2] and if not treated promptly may rapidly progress, causing significant morbidity, permanent damage, or even death.[3 4] Despite the consistent relapse-free survival (RFS) benefit with ICIs in the adjuvant setting, the incidence of and morbidity of irAEs associated with ICI treatment is significant, indicating a critical need to identify patients at risk for severe irAEs (≥grade 3).

Further, as the number of cancer indications for which ICIs are recommended continues to grow, driven by trends towards expanded use in both neoadjuvant[5 6] and adjuvant settings,[7] the healthcare burden of irAEs will also proportionally increase. Given that effective management of irAEs is dependent on early recognition and prompt intervention,[4 8–10] a critical need exists to identify patients at risk for severe irAEs. To date, however, robust biomarkers for predicting at-risk patients are lacking.

Antigen specificity of the T-cell receptor (TCR) is determined in part by the sequence of complementarity-determining regions (CDRs) and framework regions encoded by the T-cell receptor beta (TCRB) variable (*TRBV*) gene.[11] Germline-encoded variation in this region may predispose an individual to aberrant T-cell function and modulate TCR interaction with human leukocyte antigen (HLA), increasing the likelihood of autoantigen recognition. Consistent with this notion, germline genetic factors such as *TRBV* polymorphisms have been implicated in autoimmune diseases[12 13] and changes in TCR affinity for HLA may result from single amino acid substitutions in the germline-encoded portions of the TCRB chain.[14–17] The observation that severe irAEs may manifest as acute forms of chronic autoimmune disease,[18–20] add support to the concept that germline-encoded *TRBV* polymorphism could be a key determinant of irAEs. To date, germline variants predictive of irAEs associated with immunotherapy have not been identified using whole genome sequencing (WGS) or microarrays,[21] though such studies have not assessed the role of TRBV polymorphism owing to the repetitive nature of the TRBV locus, which hinders analysis by traditional microarray and short read WGS methods.[14] Identifying such biomarkers could enable patients to receive a personalized irAE monitoring care plans including proactive measures to mitigate the risk of irAEs, and also select the most appropriate ICI therapy given the patient's risk profile.

To circumvent the challenge of measuring *TRBV* polymorphisms by WGS, we developed a method for the detection of *TRBV* polymorphisms by next-generation sequencing (NGS) of rearranged TCRB chains from peripheral blood leukocytes. This represents the first NGS-based method to permit haplotype-level resolution of the TRB locus. In this study, we sought to evaluate the link between *TRBV* polymorphisms and severe irAEs using peripheral blood samples collected from 81 individuals who experienced irAEs of varying severity during ICI treatment.

PATIENTS AND METHODS

Cohort selection and description

We performed haplotype analysis of the TRB locus by employing our novel method for long-amplicon NGS of rearranged TCRB chains from peripheral blood total RNA of 81 patients with cancer who had irAEs during treatment with ICIs.[22]

Our study consisted of two cohorts of clinical samples with the National Cancer Institute Common Terminology Criteria for Adverse Events-graded irAEs following the administration of ICIs for cancer, either as standard of care or on an early phase clinical trial, (hereafter described as cohort 1, N=*54*, Roswell Park Comprehensive Cancer Center; cohort 2, N=*27*, The University of Texas MD Anderson Cancer Center), for a total of 81 subjects. We included a homogeneous population of white patients in this study to facilitate identification of haplotypes and interpretation of results. Summary statistics on age, gender, race, cancer type, treatment, and irAEs for both cohorts are included in table 1.

Library preparation and sequencing

Total RNA was extracted from the buffy coat fraction of centrifuged peripheral blood (cohort 1) or whole blood (cohort 2). RNA was converted to complementary DNA (cDNA) (SuperScript VILO cDNA Synthesis Kit, Thermo Fisher Scientific), then 25 ng cDNA (Cohort 1) or 50 ng cDNA (Cohort 2) was used for library preparation. Libraries prepared using the Ion Torrent Oncomine TCR Beta-LR (long read) assay (Thermo Fisher Scientific)[23] were sequenced on the Ion GeneStudio S5 System using Ion 530 chips to achieve ~1.5M raw reads per library (approximately 8 samples per chip). This targeted assay generated ~330 bp TCRB amplicons spanning the three beta chain CDR regions (see online supplemental file 1). Cohort 1 library preparation and sequencing was performed by OmniSeq, while cohort 2 library preparation and sequencing was performed by the MD Anderson Sequencing Core Facility. Sequenced data was uploaded to Ion Reporter (V.5.12) for clonotyping and analysis of secondary repertoire features including measurement of the evenness of clone sizes (normalized Shannon entropy) and clone richness (number of unique clonotypes in a sample).[23] Ion Reporter clone summary files containing the annotated sequence and frequency of each clonotype detected in a sample were used as input for downstream detection of novel alleles and TRBV allele haplotyping.[23]

Identification of haplotype groups in 81 patient sample set

The standard Ion Reporter workflow assigns sequence reads to variable, diversity and joining genes of T-cell

Table 1 Description of cohort 1 and 2 samples

		Cohort 1				Cohort 2			
Category	**Subdefinition**	**Overall**	**Grade 0–2 irAE**	**Grade 3–4 irAE**	**P value***	**Overall**	**Grade 0–2 irAE**	**Grade 3–4 irAE**	**P value***
Number of individuals		54	44	10	NA	27	21	6	NA
Age									
	Median (range)	65 (34–84)	64 (42–84)	67 (34–76)	0.85	60 (24–80)	59 (24–80)	66 (49–75)	0.14
Sex									
	M	34	27	7	0.73	12	9	3	1
	F	20	17	3		15	12	3	
Race									
	White	54	44	10	NA	27	21	6	NA
Tumor type									
	Melanoma	25	19	6	0.25	2	1	1	0.22
	Non-small cell lung cancer	13	13	0		0	0	0	
	Renal carcinoma	9	7	2		2	1	1	
	Urothelial carcinoma	5	4	1		1	0	1	
	Unknown	1	1	0		0	0	0	
	Gastric	1	0	1		0	0	0	
	Sarcoma					4	2	2	
	Skin (non-melanoma)					4	4	0	
	Cervical cancer					3	3	0	
	Colorectal cancer					3	3	0	
	Ovarian cancer					2	2	0	
	Parotid gland					2	2	0	
	Adrenal cancer					1	1	0	
	Endometrial cancer					1	1	0	
	Pancreatic cancer					1	0	1	
	Paraganglioma					1	1	0	
Treatment									
	Anti-PD-1 (monotherapy)	30	26	4	0.31	12	11	1	0.03
	Anti-CTLA-4 (monotherapy)	24	18	6		0	0	0	
	Anti-PD-1/PD-L1-based combination					12	9	3	

Continued

Table 1 Continued

Category	Subdefinition	Cohort 1				Cohort 2			
		Overall	Grade 0–2 irAE	Grade 3–4 irAE	P value*	Overall	Grade 0–2 irAE	Grade 3–4 irAE	P value*
	Anti-CTLA-4-based combination					2	0	2	
	T-cell agonist					1	1	0	
Repertoire features									
	Reported reads per sample (thousands)	568 (94–1,718)	567 (94–1,247)	533 (159–1,718)	0.73	580 (120–1,408)	568 (120–1,321)	1,109 (309–1,408)	0.19
	Clones detected (thousands)	32 (5–70)	32 (5–70)	30 (14–62)	0.63	26 (6–49)	28 (6–49)	20 (7–27)	0.19
	Clone size evenness	0.86 (0.46–0.96)	0.84 (0.56–0.96)	0.88 (0.46–0.94)	0.87	0.90 (0.62–0.97)	0.92 (0.70–0.97)	0.84 (0.62–0.93)	0.16

*P values were calculated using Fisher's exact test for categorical data or two-sided Student's t-test for continuous data.
CTLA-4, cytotoxic T-lymphocyte-associated protein 4; irAE, immune-related adverse event; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1.

receptors found in the international ImMunoGeneTics (IMGT) database, eliminates sequences having PCR or sequencing derived errors, then reports rearrangements in a clone summary file. We leveraged the information from the clone summary file to determine the set of variable gene (V-gene) alleles present in each sample within the cohort as previously described.[22 23] This data was used to generate a V-gene allele matrix, where each row of the matrix represents a different sample, and each column of the matrix represents a different V-gene allele (figure 1A). For each sample/V-gene allele combination, red indicates the presence of an allele and blue indicates allele absence. Thus, each row of the resultant matrix represents the V-gene allele profile (presence/absence of each of the 104 different V-gene alleles) of a unique sample. The R prcomp function was used to extract principal components of the cross-sample TRBV allele variation, then the first two principal components (online supplemental file 1) were used to project the samples into two-dimensional space.[23] The two-dimensional projection revealed the presence of distinct patient sample clusters (online supplemental file 1) corresponding to unique sets of co-inherited variable genes (ie, allele haplotypes). The first two principal component values were therefore used as input for k-means clustering of patient samples into six haplotype groups via the R kmeans function with centers=6, nstart=500, iter.max=1000, and algorithm="Lloyd". The optimal number of clusters was determined using the "elbow" method and plotting the within cluster sum of squares over cluster centers from 1 to 15 (online supplemental file 1). Use of different clustering algorithms such as "MacQueen" or "McQuitty" did not substantially alter the classification.[23] Each sample was classified as having severe (≥grade 3) or no/mild (≤grade 2) irAEs (online supplemental file 1).

Prediction of immune-related adverse events

To evaluate data robustness, we subdivided the data set into cohort 1 (N=*54*) and cohort 2 (N=*27*) patient samples. Cohort 1 patient samples were independently clustered into six haplotype groups using k-means clustering of variable gene allele profiles and algorithm "Lloyd", with the elbow method used to identify the optimal number of clusters (online supplemental file 1).[23] Next, the samples in cohort 2 were classified into one of the six cohort 1 haplotype groups using k-nearest neighbor analysis via the knn function in R with k=5. Finally, to visualize results, samples from cohort 2 were projected into the cohort 1 principal component analysis space using the predict function in R and the incidence of irAEs across cohort 2 haplotype groups was noted. Statistical significance for the distribution of severe irAEs across haplotype groups in cohort 2 was calculated via 2×6 Fisher's exact test.

As a second approach we asked whether the TRBV allele profiles of cohort 1 could be used to predict the emergence of severe irAEs in cohort 2 by a k-nearest neighbor classifier.[23] Each sample in cohort 1 was labeled as having severe (1; ≥grade 3) or no/mild (0; ≤grade 2)

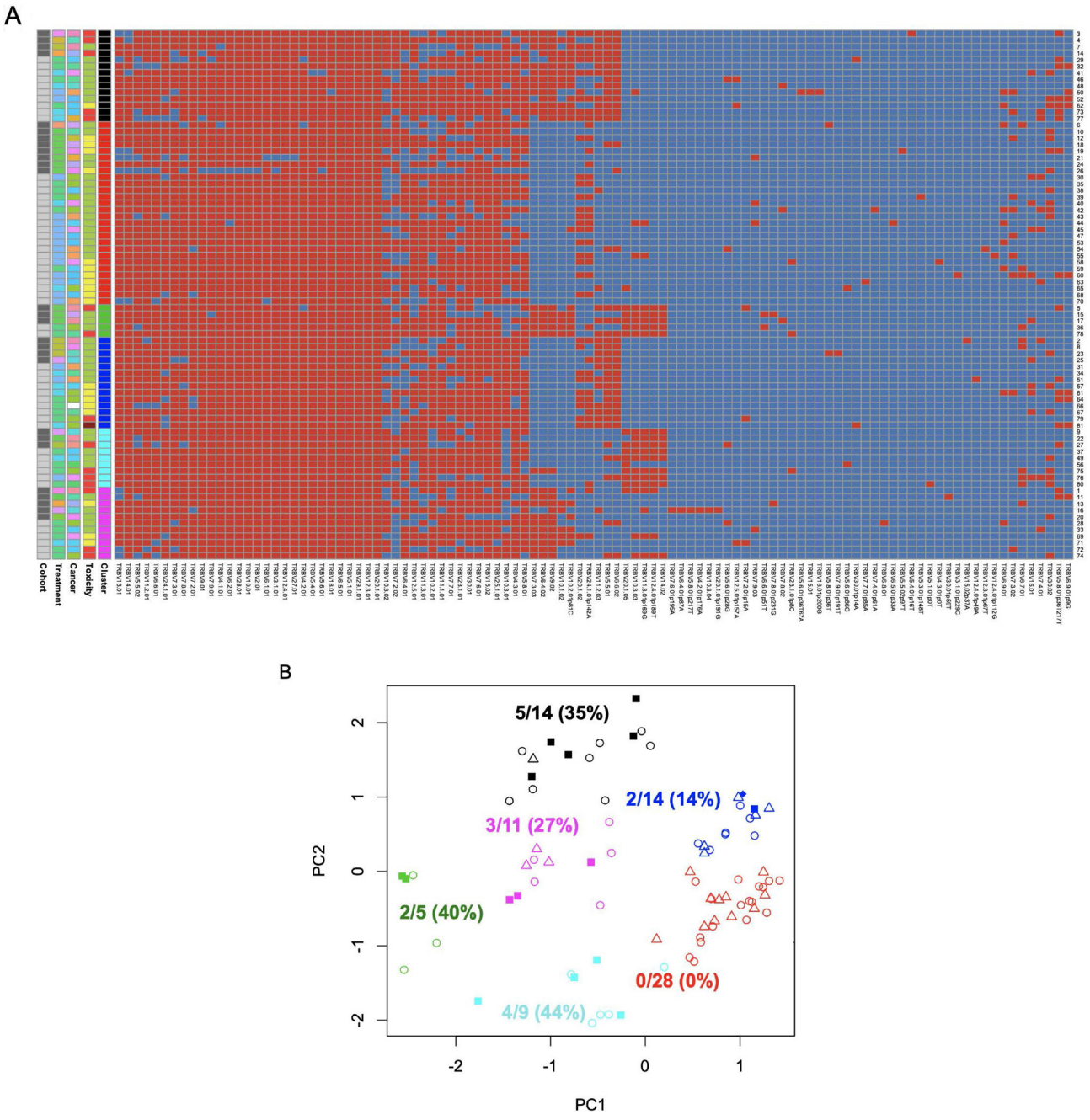

Figure 1 (A) Heatmap of TRBV allele profiles for 81 patients with advanced cancer treated with ICIs. TCRB repertoires were used to construct variable gene allele profiles for each individual. The sets of alleles detected for each individual are displayed in heatmap form, where each row represents a different individual and each column a different variable gene allele. Red tiles indicate that an allele was detected in an individual while blue tiles indicate absence of allele. Columns are arranged via hierarchical clustering, while rows are arranged according to haplotype group classification produced by k-means clustering. IMGT allele names are displayed along the X-axis; alleles having lowercase "p" in name correspond to putative novel alleles absent from the IMGT database as identified by Ion Reporter (methods). To the left, cluster column indicates the haplotype group classification (black: haplotype 1, red: haplotype 2, green: haplotype 3, blue: haplotype 4, turquoise: haplotype 5, and dark pink: haplotype 6). Toxicity column indicates grade of immune-related adverse events (green: grade 0 or 1; yellow: grade 2; red: grade 3; maroon: grade 4). Cancer and treatment columns highlight the different immunotherapy treatments and cancer types in the data set, with each color representing a different cancer or treatment. The cohort column indicates the origin of the sample (gray: cohort 1, Roswell Park Comprehensive Cancer Center; dark gray: cohort 2, The University of Texas MD Anderson Cancer Center). (B) Principal component analysis of allele profiles, highlighting immune-related adverse events. Samples are displayed according to the two largest principal components derived from analysis of the TRBV allele profile matrix. Samples are colored according to the haplotype group (cluster column in figure 1A) label identified via k-means clustering, while symbol shape indicates the grade of the immune-related adverse events. Grade 0 and 1 immune-related adverse events are plotted with the same symbol. Figure 1A adapted from Reference #22 with permission from the Journal of Immunotherapy and Precision Oncology (Innovations Journals).

irAEs. TRBV allele profiles were used to train a k-nearest neighbor classifier (via scikit-learn KNeighborsClassifier function in Python with n_neighbors=5, weights="distance", algorithm="brute", p=*1*).[23] ROC (receiver-operator characteristic) and AUC (area under the curve) values were calculated via the scikit-learn roc and auc functions.[23]

RESULTS

TCRB receptor repertoires were used to construct variable gene allele profiles for each sample, which we then applied to subdivide the data into six major types of allele profiles, which we termed haplotype groups.[24] The allele profiles of each sample, sorted by haplotype group, are presented in figure 1A, along with key annotations. We next categorized each sample as having either no/mild irAEs (≤grade 2) or severe irAEs (≥grade 3) under the general principle that irAEs of grade 2 or lower (except myocarditis, neurologic, and hematologic irAEs) do not increase morbidity and are generally manageable without significant modification to the therapeutic regimen, while grade 3 or higher irAEs may significantly increase morbidity and require termination of therapy.[4 25]

Strikingly, we observed that the incidence of severe irAEs varied markedly across the six haplotype groups: members of one haplotype group (group 2), accounting for 33% of samples, appeared to be protected against severe irAEs (0% frequency; figure 1B haplotype 2, red color), while 14–44% of patients in the other haplotype groups had severe irAEs (p=4.4E−4, Fisher's exact test). T-cell repertoire richness and evenness (ie, normalized Shannon entropy) did not differ markedly across haplotype groups (online supplemental file 1), nor did they differ across samples of different irAE grades (online supplemental file 1).

To evaluate the robustness of this finding, we examined whether cohort 1 samples could be used to predict severe irAEs in cohort 2. We repeated principal component analysis and k-means clustering with cohort 1 samples and then used the resultant haplotype labels to assign cohort 2 samples to haplotype groups via k-nearest neighbor analysis. We again observed a non-random distribution of irAEs across haplotype groups (figure 2A, p=0.03, Fisher's exact test), with no samples classified as haplotype group 2 having severe irAEs. As a complementary approach, we asked whether a k-nearest neighbor classifier trained on cohort 1 allele profiles and irAE labels (0: irAE ≤grade 2; 1: irAE ≥grade 3) could predict the presence or absence of severe irAEs in cohort 2 patient samples. The classifier was able to predict irAEs in cohort 2, as demonstrated by the analysis of the receiver-operator characteristic curve (area under the curve of 0.90, figure 2B).

Finally, to provide insight into the basis for the differential distribution of irAEs across haplotype groups, we asked whether there were allele profile features that distinguished haplotype group 2 from other haplotype groups.[24] As shown in our previous work,[22] haplotype group 2 members had fewer unique alleles and uncommon alleles (defined as those present in <50% of the sample set) than members of other haplotype groups. This indicated that haplotype group 2 members tended to be homozygous for an allele haplotype common in this cohort, while members of other groups had higher *TRBV* allele heterozygosity and carried TRBV haplotypes that were uncommon (online supplemental file 1; P=*1.7E−4* and *3.6E−13* for group 2 number of unique alleles and uncommon alleles, respectively, compared with other groups, Student's t-test). Furthermore, there was a notable positive correlation between the mean number of uncommon alleles per haplotype group and the incidence of severe irAEs (online supplemental file 1, Spearman correlation=0.83).

DISCUSSION

ICIs are an effective class of immunotherapeutic agents used for the treatment of several cancers, though ICI-associated irAEs remain a key challenge. The findings from our study support the notion that genetic variation within the TCRB locus contributes to irAEs following treatment with ICIs. Our sample set included individuals treated for a variety of cancers with both mono and combination checkpoint blockade agents, suggesting that the predictive value of *TRBV* polymorphism is not restricted to a single cancer type or immunotherapy regimen. Presumably, VDJ recombination, the somatic recombination between variable (V), diversity (D), and joining (J) immunoglobulin gene segments, in carriers of autoreactive *TRBV* alleles frequently yields autoantigen recognizing TCRs, some of which are not eliminated via thymic negative selection. Current models of T-cell maturation suggest that T-cells with autoreactive TCRs are either eliminated outright by thymic negative selection or persist as deactivated T-cells in the periphery.[26] Hypothetically, the latter population may be re-activated by ICIs to mediate irAEs. A second and compatible possibility is that ICIs reduce the efficacy of thymic negative selection,[27] leading to the generation of disproportionately large numbers of autoreactive T-cells in carriers of autoreactive *TRBV* alleles.

This study was restricted to white individuals receiving treatment with ICIs. Future studies should address whether TRBV allele haplotypes also predict irAEs following the administration of other immunotherapeutic agents, and whether protective haplotypes are present in other population groups. At a higher level, the observation that protected haplotype group 2 members tend to be homozygous for the most common alleles in whites suggests that the population frequency of *TRBV* alleles may be determined by both autoimmunity-mediated negative selection and positive selection owing to beneficial disease antigen recognition, similar to proposals regarding the existence of balanced functional polymorphisms within the HLA locus.[28]

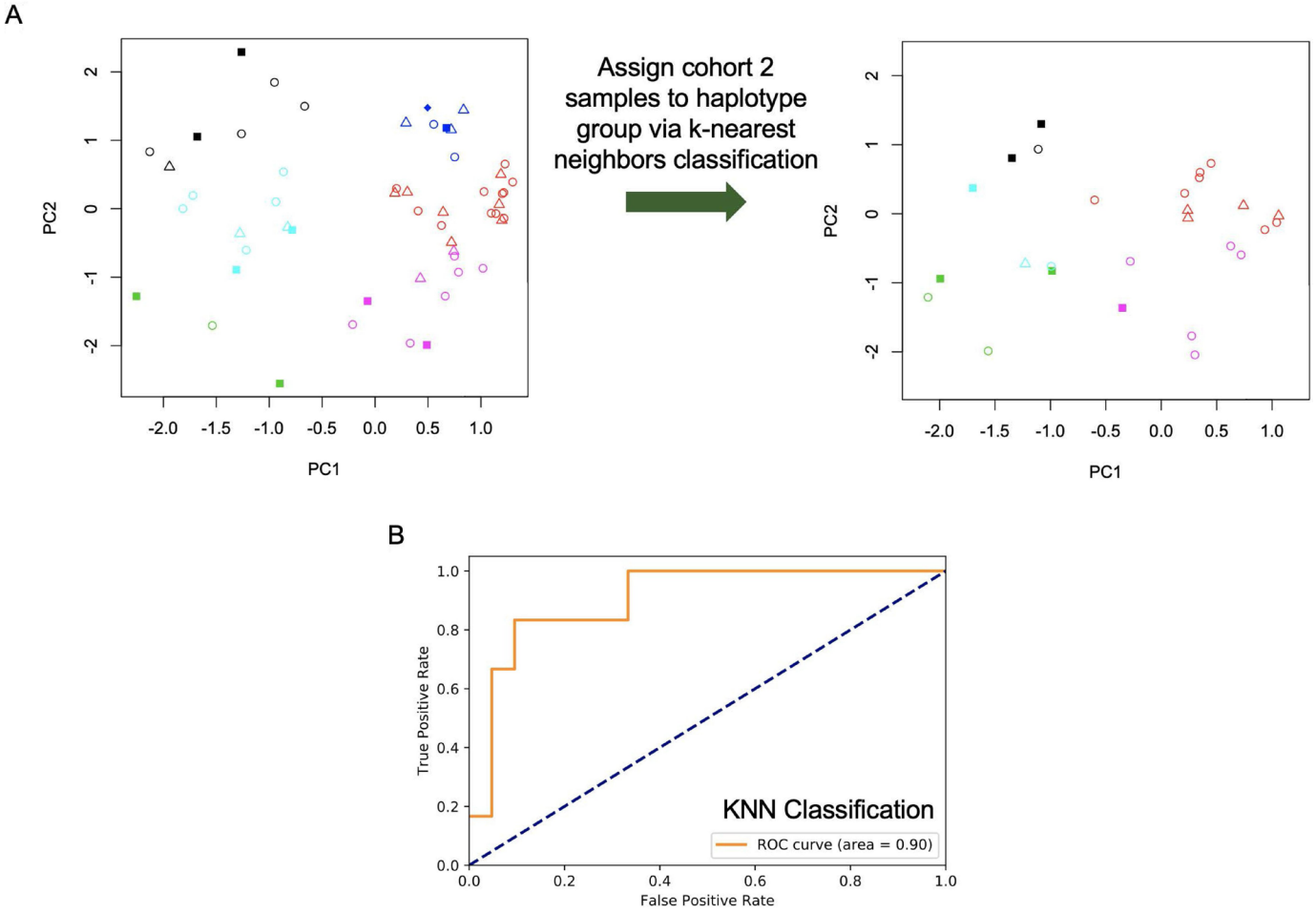

Figure 2 (A) Classification of cohort 2 samples using cohort 1 allele profiles. K-means clustering was used to subdivide cohort 1 samples into one of six haplotype groups. Cohort 1 labels were used to classify cohort 2 samples into cohort 1 groups. Cohort 1 samples are arranged according to the two largest principal components, while cohort 2 samples are projected into cohort 1 principal component space. The different haplotype groups are indicated as follows: black: haplotype 1, red: haplotype 2, green: haplotype 3, blue: haplotype 4, turquoise: haplotype 5, and dark pink: haplotype 6. Grade 0 and 1 immune-related adverse events are plotted with the same symbol. (B) Receiver operator curve (ROC) for k-nearest neighbor (KNN) classifier trained on cohort 1 then tested on cohort 2 samples.

Identification of patients at risk for severe irAEs is an important initial step towards a personalized approach to effectively manage irAEs.[8] This simple and clinically applicable test could allow patients and physicians to understand the patients' risk of severe irAEs, potentially in combination with demographic factors such as age,[29] sex,[30–32] race and ethnicity,[33–35] Eastern Cooperative Oncology Group (ECOG) performance status,[36] and, socioeconomic factors, which have elsewhere been investigated as contributors to autoimmune disease.[30] Inclusion of such immunogenomic and socioeconomic attributes in a risk assessment model would facilitate risk stratification of patients. Such an approach would ultimately enable development of personalized ICI therapeutic regimens, irAE-monitoring care plans and/or proactive risk mitigation plans prior to initiating ICI treatment.

Establishing predictive biomarkers for ICI toxicity could significantly alter the way in which care is provided in both the metastatic setting or adjuvant setting. For example, ICI candidates could be stratified into high-risk and low-risk groups based on their underlying risk for severe irAEs, then managed according to their risk profile. In the metastatic setting, this approach would enable patients and their treating physicians to formulate personalized irAE-monitoring care plans to mitigate irAEs. Patients at a high-risk of severe irAEs could be monitored aggressively, with proactive supportive care consultations, intensified interventions such as administration of systemic corticosteroids, early introduction of other immunosuppressants like infliximab, mycophenolate mofetil, or intravenous immune globulin in refractory cases, and early assessments such as endoscopic evaluation or bronchoscopy[25] when symptoms warrant during periods of therapy when the patient is predicted to have an increased risk of irAEs. Likewise, physicians could tailor the therapy to match the patient's risk profile, for example, by increasing the dosing interval or temporarily withholding immunotherapy, such that the immune system remains active without crossing the toxicity threshold. Importantly, such a personalized irAE monitoring plan will either prevent

or identify irAEs at an early stage, at which point they are potentially reversible with immunosuppressive therapy. For patients with the highest risk, it may be preferable to choose alternate treatment options beyond ICI. By contrast, patients with the lowest risk could potentially receive more aggressive dosing or a different sequencing of therapies. In the adjuvant setting, this approach could guide selection of therapeutic options depending on the patient's risk profile. For example, patients with high risk for severe irAEs can opt for alternative anticancer therapy over immunotherapy that could have serious or fatal consequences. Thus, by predicting the risk of severe irAEs by long amplicon TCRB repertoire sequencing, we may be able to maximize the flexibility of therapeutic options, and ultimately maximize durable clinical benefit while minimizing negative impacts on quality of life.

The lack of robust markers for predicting irAEs has served to disincentivize use of efficacious ICI modalities having significant irAE-mediated toxicity. For example, a recent phase 3 trial evaluating the efficacy of the CTLA-4 pathway inhibitor ipilimumab versus placebo for melanoma demonstrated a median RFS of 26.1 months versus 17.1 months for ipilimumab and placebo, respectively (p=0.0013), with a 5-year overall survival rate of 65.4% versus 54.4% for ipilimumab and placebo, respectively (p=0.001).[37] However, 41.6% of patients in the ipilumumab arm experienced severe irAEs (including five deaths attributed to ipilimumab) compared with 2.7% in the placebo group. A similar phase 3 study evaluated the PD-1 pathway blockade agent pembrolizumab (N=*514*) versus placebo (N=*505*) as adjuvant therapy in patients with melanoma.[38] The 1-year RFS rate with pembrolizumab was 75.4% versus 61% with placebo (p<*0.001*). However, 7.1% of patients treated with pembrolizumab had severe irAEs (including one death attributed to pembrolizumab) versus 0.6% with placebo. The significantly higher incidence of irAEs associated with ipilimumab and lack of a predictive safety biomarker has led to the de facto discontinuation of this therapeutic agent, despite its proven antitumor efficacy and potential synergy with treatment modalities targeting other immune checkpoint pathways; with an informative safety biomarker this would not be the case.

Finally, beyond cancer immunotherapy, one potential implication of these findings is that the *TRBV* polymorphism may serve as a predictive biomarker for chronic autoimmune disease.[23] This may be particularly true for diseases with a strong HLA component and missing heritability[12] (ie, rheumatoid arthritis and type 1 diabetes), given that the polymorphism detailed here affects portions of the TCRB chain that directly interact with HLA. Testing this hypothesis will require the analysis of *TRBV* allele profiles in groups of individuals diagnosed with autoimmune disease and matched disease-free controls.

In summary, the ability to identify individuals at risk for severe irAEs using long-amplicon TCRB repertoire sequencing has the potential to improve patient outcomes by reducing irAE morbidity and enabling personalized immunotherapy regimens that leverage a more diverse armamentarium of immune checkpoint modulators. Further investigations are needed to expand, validate, refine, and assess the utility of this approach for potential future use in a clinical setting.

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