T-cell tolerant fraction as a predictor of immune-related adverse events

Jared Ostmeyer,1 Jason Y Park,2 Mitchell S von Itzstein,3 David Hsiehchen,3,4 Farjana Fattah,4 Mary Gwin,3 Rodrigo Catalan,4 Shaheen Khan,2 Prithvi Raj,5 Edward K Wakeland,5 Yang Xie,1,4 David E Gerber6

ABSTRACT

Background Immune checkpoint inhibitor (ICI) therapies may cause unpredictable and potentially severe autoimmune toxicities termed immune-related adverse events (irAEs). Because T cells mediate ICI effects, T cell profiling may provide insight into the risk of irAEs. Here we evaluate a novel metric—the T-cell tolerant fraction—as a predictor of future irAEs.

Methods We examined T-cell receptor beta (TRB) locus sequencing from baseline pretreatment samples from an institutional registry and previously published studies. For each patient, we used TRB sequences to calculate the T-cell tolerant fraction, which was then assessed as a predictor of future irAEs (classified as Common Terminology Criteria for Adverse Event grade 0–1 vs grade ≥2). We then compared the tolerant fraction to TRB clonality and diversity. Finally, the tolerant fraction was assessed on (1) T cells enriched against napsin A, a potential autoantigen of irAEs; (2) thymic versus peripheral blood T cells; and (3) TRBs specific for various infections and autoimmune diseases.

Results A total of 77 patients with cancer (22 from an institutional registry and 55 from published studies) receiving ICI therapy (43 CTLA4, 19 PD1/PDL1, 15 combination CTLA4+PD1/PDL1) were included in the study. The tolerant fraction was significantly lower in cases with clinically significant irAEs (p<0.001) and had an area under the receiver operating curve (AUC) of 0.79. The tolerant fraction was lower for each ICI treatment category, reaching statistical significance for PD1/PDL1 (p=0.21) and combination ICI (p=0.18). The tolerant fraction for T cells enriched against napsin A was lower than other samples. The tolerant fraction was also lower in thymic versus peripheral blood samples, and lower in some (multiple sclerosis) but not other (type 1 diabetes) autoimmune diseases. In our study cohort, TRB clonality had an AUC of 0.62, and TRB diversity had an AUC of 0.60 for predicting irAEs.

Conclusions Among patients receiving ICI, the baseline T-cell tolerant fraction may serve as a predictor of clinically significant irAEs.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Immune-related adverse events (irAEs) occur in a substantial proportion of patients treated with immune checkpoint inhibitors (ICIs). These autoimmune toxicities may affect almost any organ system, and in some cases, may be severe or permanent. Despite a growing body of research in this area, irAEs remain largely unpredictable.

WHAT THIS STUDY ADDS

⇒ To aid in the prediction of irAEs, we introduce a novel parameter for profiling T cell receptor repertoires, termed the ‘tolerant fraction’. Immune tolerance represents a key tenet of normal physiology, as it allows the immune system to distinguish self from non-self. Because irAEs represent ICI-associated autoimmunity, we determined whether T cell tolerance—characterized according to productive or non-productive T-cell receptor β chain sequences—is associated with irAE occurrence. Termed the ‘tolerant fraction’, this novel parameter for profiling T cell receptor repertoires from baseline pre-ICI blood samples was associated with future development of irAE across a range of cancer types treated with a variety of ICI-based therapies.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These findings provide insight into the relationship between immune tolerance and irAE risk. In the future, reliable prediction of irAE could inform selection of patients, ICI regimens, and irAE monitoring approaches.

Immune checkpoint inhibitors (ICIs) are now established as standard of care treatments for many cancer types. However, these therapies may elicit unpredictable and potentially severe autoimmune toxicities termed immune-related adverse events (irAEs). As ICI regimens move from advanced disease to early-stage curative settings, concerns over irAEs, which in rare cases may be permanent or fatal, have increased. Furthermore, the growing use of ICI in combinations, now approved for melanoma, kidney cancer, lung cancer, and mesothelioma, pose additional risks for irAE occurrence and severity, especially considering that optimal monitoring for these adverse events remains unclear and their diagnosis may be more challenging than...
diagnosing toxicities from conventional chemotherapy or molecularly targeted therapies.1,2

Approved ICI, including those targeting cytotoxic T lymphocyte antigen 4 (CTLA4), programmed death 1 (PD1) and PD1 ligand (PDL1), and lymphocyte antigen 3 (LAG3), mediate their effects through T cells. T-cell receptor (TCR) repertoire sequencing represents an emerging technology that has been applied to multiple clinical scenarios, including cancer, infection, and ICI efficacy.3 T cell characterization—in particular T-cell receptor β-chain (TRB) sequencing (T-cell receptor beta locus)—has been studied as a means to predict risk of irAEs. Indeed, multiple studies have found that TRB clonality and diversity are associated with irAEs.4–6

Immune tolerance refers to the immune system’s unresponsiveness to substances that would otherwise elicit an immune response. Tolerance arises from prior exposure to an antigen and may be induced centrally (in the thymus) or peripherally (in lymph nodes or other tissues). Immune tolerance represents a key tenet of normal physiology, as it allows the immune system to distinguish self from non-self. Conversely, deficits in tolerance may result in autoimmune disease. Because irAEs represent ICI-associated autoimmunity, here we determine whether T cell tolerance—characterized from TRB sequences which are productive (ie, potentially expressing a TRB protein chain) or non-productive (ie, cannot express a TRB protein chain)—is associated with these toxicities (figure 1A).

**MATERIALS AND METHODS**

**Data collection and sources**

We collected clinical data from patients enrolled in an institutional prospective immunotherapy registry. All participants provided informed consent prior to taking part. As previously described, the registry enrolled patients with cancer planned for but not yet started on ICI. Collected data included demographics, tumor characteristics, treatment information, and irAEs. Due to challenges in determining the occurrence, type, timing, and severity of irAEs,7 8 two oncologists (MvI and DH) experienced in ICI administration and monitoring reviewed each case for toxicities, with discrepancies reviewed and adjudicated by a third experienced clinician (DEG).

---

**Figure 1** The hypothesis and methodology of this study. (A) Patients with a higher percentage of tolerant T cells are anticipated to be at a lower risk of developing an irAE from ICI therapy because their T cells will not recognize self-antigens even after ICI therapy. Conversely, patients with a lower percentage of tolerant T cells are anticipated to be at a higher risk of developing an irAE from ICI therapy. (B) Non-productive and productive TRB sequences are separated from 786 healthy subjects with no known disease. The pool of non-productive TRB sequences cannot express and therefore can be non-tolerant. The pool of productive TRB sequences can express and therefore are tolerant. We characterize each patient with cancer according to the relative overlap of their productive TRB sequences with that of the tolerant pool (from the 786 healthy subjects), which we term the tolerant fraction. The asterisk serves to denote a significant step required to calculate the tolerant fraction—the application of a repair algorithm to modify non-productive TRB sequences for comparison with productive ones (see the Methods section). ICI, immune checkpoint inhibitor; irAE, immune-related adverse event; TRB, T-cell receptor β.
Enrolled patients underwent blood collection at pretreatment baseline immediately prior to ICI therapy initiation. To increase our sample size and potential generalizability of our findings, we also included patients with cancer from three other published cohorts with publicly available data.10–11 Each study had enrolled patients with cancer starting ICI therapy, incorporated irAE-related clinical data, and included blood collection at pretreatment baseline. To evaluate the tolerant fraction in other (non-ICI) clinical settings, we also reviewed published cases that included both thymus and peripheral blood TCR sequencing, as well as TCR sequencing from various disease settings, including infection and autoimmune disease.10–11

Additionally, we included 786 presumed healthy subjects from another study with publicly available data.12 While these subjects underwent blood collection, they did not receive ICI. These subjects were not part of these studies’ cases and controls; rather, data from these subjects were used to define the parameters of T cell tolerance.

**Categorizing irAEs**

We categorized irAEs as clinically significant (Common Terminology Criteria for Adverse Events (CTCAE) grade ≥2) or not clinically significant (CTCAE grade 0–1). We chose this threshold because, in general, grade 2 or greater toxicity implies need for systemic medical intervention, whereas grade 1 toxicity is generally asymptomatic or minimally symptomatic and requires neither ICI modification nor specific systemic treatment.13 Prior studies have employed a similar cut-point.4

**TRB sequencing**

Blood collected from patients in our registry was subjected to TRB sequencing by Adaptive Biotechnologies (Seattle, Washington, USA).14 For this study, we focused on studying the association between T cell tolerance and irAEs from unsorted T cells from peripheral blood samples.

Blood collected from the published cohorts had also been subjected to TRB sequencing by Adaptive Biotechnologies. One of the public cohorts incorporated into the present analysis had sorted T cells into CD4 and CD8 populations, with each subset sequenced separately.4 Because the ratio of TRB sequences from the sorted CD4 and CD8 T cells was approximately 2:1 (the anticipated ratio of CD4 to CD8 T cells in peripheral blood), we merged the TRB sequences from the separately sequenced CD4 and CD8 T cell populations into a single sample. This allowed us to compare the TRB sequences from the sorted T cells to the unsorted T cells from the other cohorts.

**Single-cell sequencing**

Baseline peripheral blood samples from five patients with cancer were subjected to single-cell RNA sequencing using the x10 genomics platform with libraries included to capture TCR sequences, including V(D)J recombination events. After collecting the sequences, we excluded all cells that did not have exactly one TRA and one TRB sequence per cell. We then measured CDR3a and CDR3b lengths.

**Productive and non-productive TRB sequences**

We hypothesized that non-tolerant T cells would be associated with irAE (figure 1A). To study T cell tolerance from TRB DNA sequences, we needed a way to distinguish TRB sequences that are tolerant from TRB sequences that may not be tolerant. In healthy subjects, we considered TRB sequences that are expressed to be tolerant; otherwise, the subject would not be healthy. Productive TRB sequences, which we defined as being in-frame and not containing a stop codon in the rearrangement, are those which can express a TRB chain and are therefore considered tolerant. We also considered TRB sequences that cannot be expressed as not having to be tolerant, as these sequences will not have any effect. Non-productive TRB sequences, which we defined as being either out-of-frame or containing a stop codon in the rearrangement, cannot express a TRB chain and are considered as not being tolerant. Both productive and non-productive TRB sequences in peripheral blood are captured during TRB sequencing.15–19

T cell selection, also known as thymic selection, represents the biological processes ensuring productive TRB sequences are tolerant (online supplemental figure 1). During T cell selection, developing T cells can contain both a productive and non-productive TRB sequence on opposite chromosomes. However, only the productive TRB sequence can express TRB protein chains. Developing T cells expressing TRB chains that are not tolerant are deleted by T cell selection. Therefore, the surviving T cells are those that can express tolerant TRB protein chains. Because the expressed TRB protein chains must be tolerant, the productive TRB sequences expressing the TRB protein chains must also be tolerant. However, the non-productive TRB sequences are under no such constraint, and are carried through T cell selection without regard for whether the sequences are tolerant because the sequences do not express.15–19 After completing T cell selection, the surviving T cells enter peripheral blood, where the TRB sequences can be sequenced.

**Tolerant fraction**

To define parameters for T cell tolerance, we separated non-productive and productive TRB sequences from the 786 presumed healthy subjects (figure 1B).12 The pool of non-productive TRB sequences cannot express and therefore can be non-tolerant, while the pool of productive TRB sequences can express and therefore are tolerant. We characterized each ICI-treated patient in this study according to the relative overlap of their TRB sequences with that of the tolerant and non-tolerant pools (from the 786 presumed healthy subjects). Specifically, we used \( f_{\text{PROD}} \) and \( f_{\text{NON}} \) to denote the fractions of tolerant and non-tolerant pools that overlap (the definition of overlap is provided later) with productive TRB sequences from a
patient with cancer (figure 1B). We calculated the fraction of tolerant T cells for each patient with cancer, termed the tolerant fraction as follows:

\[ TF = \frac{f_{\text{PROD}}}{f_{\text{TOTAL}}} \]

\[ f_{\text{TOTAL}} = f_{\text{PROD}} + f_{\text{NON}} \]

A value of 1 (i.e., 100%) indicates all TRB can be assumed to be tolerant, while a value of 0 (i.e., 0%) indicates none of the TRB can be assumed to be tolerant.

The TRB sequences are subjected to in-silico processing and filtering steps before being used in the calculation of the tolerant fraction. These steps are summarized in online supplemental figure 2 and described in the preceding sections.

**Translating TRB DNA sequences to protein sequences**

T cell tolerance is based on the expressed TRB proteins, not the nucleotide sequences of the genes, motivating us to compare productive to non-productive TRB sequences as protein sequences. Therefore, all TRB sequences were translated in silico to predicted protein sequences. While productive TRB sequences can be translated to protein sequences, non-productive TRB sequences cannot. We previously developed an algorithm to computationally repair non-productive TRB sequences, thereby allowing repaired TRB sequences to be translated to productive protein sequences. To maximally preserve the original biological sequences, which contain complex and intricate biases from V(D)J recombination, our algorithm repairs each non-productive TRB sequence using the fewest alterations required to obtain a productive copy.

This algorithm, based on the type of repair required, handles each non-productive TRB sequence as one of three cases:

1. For TRB sequences that are non-productive because the open reading frame of the J segment is one position ahead of the open reading frame of the V segment, our algorithm removes any single nucleotide at a somatic junction to bring the segments into the same open reading frame (online supplemental figure 3B).
2. For TRB sequences that are non-productive because the open reading frame of the J segment is two positions ahead of the open reading frame of the V segment, our algorithm removes any two nucleotides at the somatic junctions to bring the segments into the same open reading frame.
3. For TRB sequences that are non-productive because of a stop codon in a somatic junction, we mutate any nucleotide in the somatic junction encoding the stop codon to attempt to convert it to an amino acid residue (online supplemental figure 3C).

Additional details about the repairing process can be found in supplementary materials of our previous publication.

There are multiple ways to repair a non-productive TRB sequence (online supplemental figure 3B,C). Each way of repairing the TRB sequence can result in a different protein sequence. This study uses all protein sequences obtained from repairing the same non-productive TRB sequence as part of the calculation for the tolerant fraction.

**Discarding TRB with ‘short’ CDR3 sequences**

A tolerant T-cell receptor (TCR) is one that does not recognize self-antigens. Therefore, we focused on aspects of the TCR that influence antigen recognition. A T cell receptor (TCR) is a heterodimer of a TRA and TRB chain that each contribute a complimentary determining region 3 (CDR3) for antigen recognition. We hypothesized that the TCR chain with the longer CDR3 will contribute more to antigen recognition than will the TCR chain with the shorter CDR3 (online supplemental figure 4A–C). Because we only had TRB sequences for this study, we discarded TRB sequences with a ‘short’ CDR3 based on the assumption that a shorter CDR3 may not contribute as much to antigen recognition and therefore may not be as relevant to T cell tolerance (online supplemental figure 5A). To investigate our hypothesis, we examined the correlation between CDR3 lengths for individual cells. We also generated a rendering using Visual Molecular Dynamics from the 3-dimensional x-ray crystallographic structure using Protein Data Bank ID 4jrx to demonstrate TRB chain CDR3 interactions with antigen. We considered multiple CDR3 length cut-offs to determine the threshold providing the best discrimination.

**Trimming CDR3 sequences**

Although the CDR3 of each TRB is involved in antigen recognition, not all amino acid residues within the CDR3 can make direct contact with antigens. Published analyses of 3D X-ray crystallographic structures of TRB in contact with antigen have found that the first and last three CDR3 amino acid residues do not directly contact the antigen. Based on these studies, to determine the tolerant fraction, we removed the first and last three amino acid residues from each CDR3, as these residues would not be expected to contribute to tolerance (online supplemental figure 5B).

**Definition of overlap**

In this study, we calculated the relative overlap between TRB sequences from each ICI-treated patient with cancer and TRB sequences from either the tolerant or non-tolerant pools. We defined the overlap between two sets of TRB sequences as the region where TRB sequences from each set have identical trimmed CDR3 protein sequences (online supplemental figure 5C). The relative overlap was then calculated by taking the number of TRB sequences in the overlapping region and dividing by the total number of TRB sequences from one of the sets. Dividing by the total number of TRB sequences from one of the sets prevented the size (i.e., number of TRB sequences) of that set from influencing the calculation. For this step, we divided by the total number of TRB sequences from the tolerant or non-tolerant pools, whichever set was being considered.
When calculating relative overlap, we did not incorporate the template count of TRB sequences but did include duplicate TRB sequences in the tolerant and non-tolerant pools resulting from the aggregation of the 786 healthy subjects. It is also worth noting the template count of TRB sequences from the ICI-treated patients with cancer (figure 1B) did not alter the calculation of relative overlap, which was verified by running the calculation with and without the template count of the TRB sequences from patients with cancer.

Statistical analysis
Our null hypothesis was that the tolerant fraction of patients with a grade 0–1 irAE was not higher than the tolerant fraction of patients with a grade ≥2irAE. The alternative hypothesis was that it was higher. Because we were not testing the converse association, we used a one-sided test of our null hypothesis. Specifically, p values were calculated using a one-sided Mann-Whitney U test assuming the null hypothesis. Because we considered multiple cut-offs for the CDR3 sequence lengths, we adjusted p values using a Bonferroni correction. P values for TRB clonality and diversity were also calculated using a one-sided Mann-Whitney U test, with the alternative hypothesis inverted for TRB diversity.

T cell enrichment against an irAE antigen
To assess the tolerant fraction of the specific T cells that contribute to irAEs, we sought a dataset that was enriched for T cells involved in irAEs responding to a self-antigen. A recent study identified napsin A as a T cell antigen responsible in part for irAEs in patients with lung cancer. Starting with peripheral blood from four patients with lung cancer, this previous study enriched for T cells with cancer, this previous study enriched for T cells with napsin A by coculturing T cells with napsin A expressing cells. Similar to napsin A, T cells were then subjected to TRB sequencing by Adaptive Biotechnologies, and the results were downloaded to calculate the tolerant fraction of these TRB sequences.

TRB sequences from thymus and peripheral blood samples
To evaluate the tolerant fraction further, we examined publicly available TRB sequences from matched thymus and peripheral blood samples from pediatric patients undergoing corrective cardiac surgery and then made publicly available. The samples were subjected to TRB sequencing by Adaptive Biotechnologies, and the results were downloaded to calculate the tolerant fraction of these TRB sequences. Because the thymus is enriched with developing T cells not yet removed by T cell selection, we hypothesized that thymus samples would have a lower tolerant fraction than peripheral samples.

Disease-specific TRB sequences
We also examined TRB sequences from various clinical disease scenarios, including infection (influenza, coronavirus), and autoimmune diseases (type 1 diabetes mellitus (T1DM) and multiple sclerosis (MS)). For these analyses, TRB sequences did not come from individual patients, but were instead curated from pooled published literature available through the Immune Epitope Database. In general, each study from the pool of publications contributes a small number of TRB sequences, with TRB sequences being sourced from tissue samples and peripheral blood using a diverse array of experimental methodologies. For each clinical disease condition, we aggregated TRB sequences, which allowed calculation of the tolerant fraction. The complete list of antigens associated with each of these TRB sequences is listed in online supplemental materials.

RESULTS
A total of 77 patients (including 22 from our institutional registry and 55 from three published studies) were included in our primary analysis (online supplemental tables 1 and 2). ICI types included anti-CTLA4 (n=43), anti-PD1/PDL1 (n=19), and combination anti-CTLA4 plus anti-PD1/PDL1 (n=15) as follows: ipilimumab (n=22), tremelimumab (n=21), atezolizumab (n=2), avelumab (n=1), durvalumab (n=2), nivolumab (n=9), pembrolizumab (n=5), and ipilimumab plus nivolumab (n=15). Cancer types included the following: melanoma (n=45), prostate cancer (n=10), non-small cell lung cancer (n=4), small cell lung cancer (n=1), mesothelioma (n=1), bladder cancer (n=1), and renal cell carcinoma (n=1). Overall, 53 patients (69%) experienced a grade≥2irAE.

To assess our hypothesis that longer TCR CDR3 are most involved in antigen interactions, and therefore, most relevant to autoimmune phenomena including irAE, we examined various characteristics of TCR length (online supplemental figures 4, 5). We found no significant correlation between CDR3a and CDR3b lengths within individual cancers (Pearson correlation coefficient r=0.0062) (online supplemental figure 4B). Online supplemental figure 4C demonstrates a representative three-dimensional X-ray crystallographic structure in which the longer TCR chain achieves greater antigen contact (≈55% of amino acid residues) than does the shorter TCR chain (≈25% of residues). We then considered TCR chain length cutoffs of 2, 9, 10, 11, 12, 13, 14, 15, and 16 amino acid residues (online supplemental figure 4D), observing a clear association between higher TCR chain length cut-off and performance of the tolerant fraction to predict irAE. A length of 15 amino acid residues provided the best discrimination and was therefore selected as a cut-off.

To evaluate our hypothesis that baseline T cell tolerance is associated with lower risk of autoimmune toxicity, we measured the TRB tolerant fraction in patients with and without clinically significant irAEs (figure 2A). As hypothesized, tolerant fraction values are higher in patients with grade 0–1 irAEs compared with patients with grade ≥2 irAEs (p<0.001). To distinguish the two outcomes (grade ≥2 vs grade 0–1 irAE), a threshold can be applied to the
tolerant fraction. As shown in figure 2B, for all possible cut-offs for tolerant fraction values, the true positive rate was almost always greater than the false positive rate. The area under the curve (AUC) of the receiver operator characteristics (ROC) was 0.79. Among patients with grade 0–1 irAEs, 18 of 24 had a tolerant fraction ≥85.2% (75% sensitivity). Among patients with grade ≥2 irAEs, 39 of 53 had a tolerant fraction <85.2% (74% specificity).

To validate the tolerant fraction on an external dataset, we sought a dataset of T cells enriched against an antigen associated with irAEs on which we could assess our metric. Napsin A is an antigen expressed in over 80% of lung adenocarcinoma cases,29 T cells enriched against napsin A have been associated with lung inflammation driving pulmonary irAEs, and a dataset of TRB sequenced from T cells enriched against napsin A was recently published.28 Tolerant fraction for three samples enriched against napsin A were 75.0%, 68.8%, and 77.6% (figure 2C), falling into the range (<82.5%) associated with irAEs. The tolerant fraction could not be calculated for a fourth sample that did not have overlap with the TRB sequences from the 786 healthy subjects.

We also evaluated the predictive performance of the tolerant fraction for specific ICI treatment types (CTLA4, PD1/PDL1, combination CTLA4+PD1/PDL1) (figure 3). For all three categories, the tolerant fraction was lower in cases grade ≥2 irAE. This difference reached statistical significance for CTLA4 (n=43; p<0.001) and showed non-significant trends for PD1/PDL1 (n=19; p=0.21) and combination CTLA4+PD1/PDL1 (n=15; p=0.18).

To evaluate the TRB tolerant fraction in various clinical disease states, we examined publicly available individual patient and pooled TRB sequences (figure 4). In an immunologically healthy population of pediatric patients...
undergoing surgery for congenital cardiac defects, matched thymus and peripheral samples demonstrated substantially lower TRB tolerant fractions for thymic TRB sequences (figure 4A). TRBs specific for infection (influenza and coronavirus) demonstrated a tolerant fraction in the 85%–86% range, while TRBs specific for T1DM had a higher tolerant fraction (≈87%), and TRBs specific for MS had a lower tolerant fraction (≈84%) (figure 4B).

Finally, to place our findings in context of other predictive parameters, we also evaluated TRB diversity and clonality in our 77 cases of ICI-treated patients with cancer (figure 5). For TRB diversity, the ROC AUC was 0.60 (p=0.07) (figure 5A,B). For TRB clonality, the ROC AUC was 0.62 (p=0.05) (figure 5C,D).

DISCUSSION

irAEs remain a major concern in immuno-oncology. These autoimmune toxicities may affect almost any organ system. In rare cases, they may be permanent or even fatal. The lack of understanding of these clinical phenomena is apparent through the relatively blunt approach to patient selection and monitoring. Apart from the observation that patients with pre-existing autoimmune disease may face heightened risk of autoimmune disease flare and irAEs, and patients with organ transplant may face risk of organ rejection, there are no clear methods to identify high-risk populations.30 Similarly, recommendations for irAEs monitoring range from following only thyroid, liver, and renal function to extensive panels including these parameters as well as assessment of cardiac, pulmonary, pituitary, adrenal, and pancreatic function.31–35

In response to a clear clinical need, characterization of patient T cell function through such parameters as TRB diversity and clonality has emerged as a potential approach to irAE prediction. Premised on the tenet that tolerance suppresses anti-self immunity, in this study we proposed a new T cell metric—the tolerant fraction—as a potential means to identify patients at heightened risk for future irAEs. With this approach, we identified a specific tolerant fraction threshold able to distinguish between low-risk and high-risk of future irAE in a cohort comprizing both published and unpublished TRB and clinical data from cases with diverse cancer types treated with various types of immunotherapy. Consistent with the overall concept of tolerance, patients with more tolerant T cells, manifest as a higher tolerant fraction, were less likely to develop clinically significant irAEs. When examined by type of ICI treatment, CTLA4, PD1/PDL1, and combination therapy groups all had lower tolerant fractions among irAE cases. This difference reached statistical significance for CTLA4 but not for the other treatment categories. The lack of statistical significance in the PD1/PDL1 and combination ICI groups could reflect small sample sizes, as each of these groups was less than half the size of the CTLA4 group.

With an ROC AUC approximately 0.8, the tolerant fraction had superior performance to both TRB diversity and clonality (ROC AUC approximately 0.6) for irAE prediction in this study. We also noted a clear association
between low tolerance and presence of T cell populations enriched against irAE-associated antigens. The relatively low performance of TRB diversity and clonality in this study compared with prior reports could be due to a number of factors: the inclusion of multiple types of ICI therapies; the aggregation of samples from multiple studies; and the exclusive use of baseline, pretreatment samples.

The association between immune tolerance and irAEs is consistent with our earlier observations linking immune dysregulation with immunotherapy toxicity. Specifically, a signature featuring low baseline but marked increases in IFNγ-inducible cytokines/chemokines involved in T cell recruitment and activation was associated with heightened risk of future irAEs.8 Clinical correlates of this relationship include the subset of primary immunodeficiencies termed diseases of immune dysregulation, in which patients with impaired immunity develop autoimmune and inflammatory disorders.36 Similar observations have been made in cases of acquired immunodeficiency such as HIV.37

Our analyses of the tolerant fraction in other populations provide further insights into this novel parameter. As we hypothesized, the tolerant fraction from thymus-derived TRB sequences was lower than that of peripheral blood TRB sequences. Presumably, this observation reflects the fact that the thymus is enriched with developing T cells, including non-tolerant T cells not yet removed by T cell selection. Our observations from other disease states are more nuanced. Patients with infections had TRB tolerant fractions similar to those from patients with congenital cardiac defects. However, TRB sequences from patients with autoimmune diseases varied widely. As anticipated, patients with MS had lower tolerant fractions; however, patients with T1DM had high tolerant fractions. A previous study has observed that thymic selection does not remove T cells specific for T1DM, even in healthy individuals, offering a potential explanation for the high tolerant fraction value for this disease.38 These data suggest that the TRB tolerant fraction might be similar for patients with and without T1DM, as both populations will contain T1DM-specific TRB sequences.

Potential clinical applications of the tolerant fraction require consideration of certain factors. First, we recognize the relatively narrow range between the extremes of tolerant fraction values in our cohort, an observation that could also be applied to T cell clonality and diversity parameters. A potential explanation for this observation is that only a small fraction of pretreatment T cells are mechanistically associated with subsequent irAEs, consistent with observations

---

**Figure 5** TRB diversity and clonality as predictors of irAE. (A) The 0%, 25%, 50%, 75%, and 100% quartiles shown using box-and-whisker plots of TRB diversity for patients with grade ≥2 irAEs (red) vs grade 0–1 irAEs (blue). Dots represent outliers (exceeding 1.5 the IQR). See methods section for how to interpret the p value. (B) ROC plot demonstrating the true and false positive rates resulting from different thresholds of TRB diversity for distinguishing the two outcomes (grade ≥2 vs grade 0–1 irAE). The area under the curve (AUC) is 0.60. (C) The 0%, 25%, 50%, 75%, and 100% quartiles shown using box-and-whisker plots of TRB clonality for patients with grade ≥2 irAEs (red) vs grade 0–1 irAEs (blue). Dots represent outliers (exceeding 1.5 the IQR). See the Methods section for how to interpret the p value. (D) ROC plot demonstrating the true and false positive rates resulting from different thresholds of TRB clonality for distinguishing the two outcomes (grade ≥2 vs grade 0–1 irAEs). The AUC is 0.62. irAE, immune-related adverse event; ROC, receiver operator characteristics; TRB, T-cell receptor β.
that only a few hundred T cell clones are found to clonally expand during an irAE.  

Strengths of the current study include the total number of patients, which exceeds those in a number of previously published studies of TRB-based irAE correlative biomarkers.  

Our study also included diverse cancer and ICI treatment types. At the same time, we recognize the inherent complexity and imperfect nature of negative selection. The large numbers of TRB sequences included in our analyses may help overcome the potential noise introduced by autoreactive clones leaking out from the thymus and escaping peripheral tolerance mechanisms. Additionally, from a clinical perspective, the T-cell tolerant fraction has the favorable characteristic of being a pretreatment baseline parameter. In contrast, assessment of T cell clonal expansion or diversification requires assessment of immune cell populations over time. Indeed, the availability of pretreatment guidance could be particularly helpful for those areas of greatest current need: selection of patients and monitoring. Limitations of the study include relatively small sample sizes remaining after categorizing cases according to ICI type. Further testing of the tolerant fraction, particularly among individuals treated with PD1/PDL1 or combination CTLA4+PD1/PDL1, is needed.

In conclusion, we have identified a novel parameter, the T-cell tolerant fraction, which is associated with future development of clinically significant irAEs. In this study, the tolerant fraction has better predictive ability than pretreatment TRB clonality or diversity. Furthermore, in contrast to dynamic T cell clonal expansion or diversification, the tolerant fraction may be determined prior to IC1 initiation, thereby potentially informing up-front selection of patients, treatments, and monitoring.

**Present affiliations** The present affiliation of Jared Ostmeyer is: Amgen Inc, Dallas, Texas, USA.

**Contributors** JO and DEG designed the overall study, provided insights on experimental design and interpretation of results, and wrote the manuscript. JO performed primary analyses, JYP, MvI, DH, FF, SK, PR, EKW and YX provided insights on experimental design and interpretation of results, MvI, DH, MG and RC collected and reviewed clinical data. FF oversaw experiment performance.

**Funding** Funded in part by the National Institute of Allergy and Infectious Disease (U10AI156189-01; to DEG, EKW, YX), an American Cancer Society-Melanoma Research Alliance Team Award (MRAT-18-114-01-LIB; to DEG), a V Foundation Robin Roberts Cancer Survivorship Award (DT2019-007- to DEG), the University of Texas Lung Cancer Specialized Program of Research Excellence (SPORE) (P50CA070907-21), a Mary Kay Ash International Fellowship (to RC), the University of Texas Stimulating Access to Research in Residency (UT-SARR, R38HL150214 to MG), and the Harold C. Simmons Comprehensive Cancer Center Data Sciences Shared Resource (P130 CA 142543-03).

**Disclaimer** The funders were not involved in study design, conduct, or reporting.

**Competing interests** JO and DEG report a US patent application, SK, FF, JYP, YX, EKW and DEG report a US patent application (62/854,025).

**Patient consent for publication** Consent obtained directly from patient(s).

**Ethics approval** This study involves human participants and was approved by UT Southwestern IRBSTU 082015-053. Participants gave informed consent to participate in the study before taking part.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data are available on reasonable request.

**Supplemental material** This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

**Open access** This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See http://creativecommons.org/licenses/by-nc/4.0/

**ORCID iD**

David E Gerber http://orcid.org/0000-0002-7812-6741

**REFERENCES**


Open access


