## EDITORIAL

# Tracking the T-cell repertoire after adoptive therapy

In recent years, there has been increased interest in using immunotherapy to treat cancer. Adoptive T-cell therapy (ACT) involves the isolation and expansion of tumour-specific T cells *in vitro*. Large numbers of tumour-specific T cells are then infused back into cancer patients to provide a substantial 'boost' to anti-cancer immunity. While this personalised therapy has shown promise across various cancer settings, response rates still vary and significant energy is currently being invested into understanding the immunology underpinning these diverse clinical outcomes. Indeed, the burning question at present is how do you identify an effective ACT product from ineffective? Chaouis *et al*.[1] recently probed this question by tracking ACT products using T-cell receptor (TCR) deep sequencing. Using pre- and post-infusion samples from melanoma, breast cancers and Merkel cell carcinoma patients, the authors' tracked clonotypes in the days to months post infusion and compared clonotype parameters with clinical parameters.

TCR deep sequencing is a significant stride forward from previous Sanger sequencing-based methods designed around T receptor beta variable (TRBV) primer-specific polymerase chain reaction (PCR) or unbiased template-switch anchored reverse transcription- PCR (RT-PCR) (reviewed[2]), which can only determine dozens to hundreds of clonotypes per sample. In contrast, TCR deep sequencing allows for thousands to millions of clonotypes to be determined per run; typically profiling every clonotype in an input sample. TCR deep sequencing platforms are based on TRBV primer-specific multiplex PCR on DNA using Illumina sequencing[3] or unbiased template-switch anchored RT-PCR using 454 sequencing[4] or using Illumina sequencing.[5] New technologies include single-cell Drop-seq[6] and single-cell unbiased template-switch anchored RT-PCR[7] that allow determination of TCR α/β (TCRαβ) pairings in single T cells. An additional technique even allows for *de novo* determination of clonotypes using total RNA-Seq data[8] permitting TCR mining across the many publically available RNA-Seq databases.

However given these TCR deep sequencing methodologies are relatively new techniques, Chaouis *et al*[1] first compared sequencing data to conventional quantitative methods such as tetramer staining. After infusion with a MART-1-specific monoclonal ACT product, tetramer frequencies and absolute clonal frequencies correlated well over months of sampling establishing confidence that TCR deep sequencing can be used to quantitatively track ACT-derived T-cells post infusion. Using deep sequencing, the authors showed that tumour-specific T-cell frequencies increased from $<0.001\%$ pre-infusion to a median of 1.5% in the days post infusion. Overall, these frequencies generally waned during the weeks following post infusion. Of note, the clonotype diversity and clonal hierarchy of the ACT products pre-infusion were heterogeneous and could not predict clinical outcome. Additionally, for some patients, the clonal hierarchies in the ACT product did not correlate with the peak clonotype hierarchy's post infusion suggesting considerable restructuring of the T-cell repertoire once the product went *in vivo*. When looking at clonotype persistence after polyclonal ACT infusion, the authors showed that patients with complete remissions maintained high frequency of a single immunodominant clonotype that persisted for many months post infusion.[1] When calculating the 'longevity' of infused clonotypes, they found patients with complete remission had clonotypes that could expand and persist *in vivo* exhibiting half-lives 2- to 13-fold longer than in patients with partial remission, stable disease or progressive disease (Figure 1). Interestingly, across all patients, the majority of clonotypes expanded in T-cell products were not detected in the peripheral blood mononuclear cells (PBMC) before infusion indicating preferential expansion of rare clonotypes ($<0.001\%$) during *in vitro* culture and suggesting some confines of limiting blood sampling even while using a TCR deep sequencing readout.

Collectively, this study highlights how TCR deep sequencing could one day provide predictive information on ACT product 'quality' and patient outcome. The authors show that sustained clinical responses result from the *in vitro* expansion of rare clonotypes that go on to dominate the tumour-specific T-cell repertoire *in vivo*. Currently, the origins of these low-frequency clonotypes are not known. These rare T cells could be low-frequency effector cells, naive cells or possibly even memory stem cells,[9,10] which are known to have superior anti-cancer properties.[11] The authors suggested that the culturing method (antigen-pulsed dendritic cells in the presence of interleukin 21 (IL-21)) stimulated naive T cells to expand in the ACT product and subsist *in vivo* in sustained responders. However, this is speculative, and more details are required to understand the origins of these low-frequency clonotypes and how they can be identified and manipulated to improve the impact of ACT products. Given advances in single-cell transcriptomics, which can now provide both the TCRαβ sequence and transcriptional landscape of individual T cells,[7] retrospective analysis of effective clonotypes in ACT could reveal many details about the 'quality' of superior T-cell clonotypes in ACT products. Mechanisms for duplicating this phenotype (cytokine cocktails, CRISPR gene editing and so on) could be developed and applied to GMP manufacture to better 'groom' T-cell products for the *in vivo* environment.
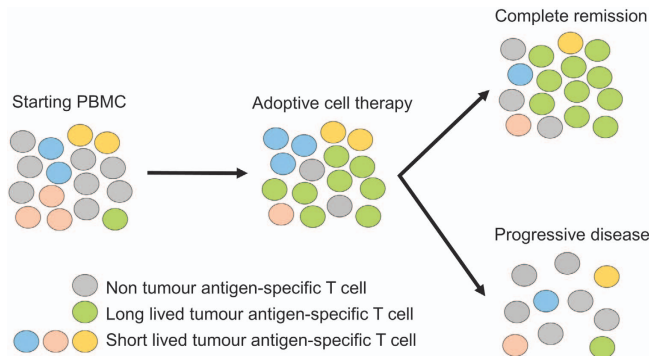
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**Figure 1** Clinical response to adoptive T-cell therapy (ACT) correlates with expanded immunodominant clonotypes that are at very low frequencies *ex vivo*. The figure depicts PBMC that are stimulated with tumour-associated antigens presented by APC to generate an ACT. The polyclonal ACT is infused into patients and a second round of expansion dynamics occurs *in vivo*. Patients with complete remission comprise ACT products with immunodominant clonotypes that have long half-lives post infusion. These clonotypes are at very low frequencies in the starting PBMC material. In contrast, patients with progressive disease comprise ACT products with diverse clonotypes with short half-lives post infusion.

Indeed, persistence is encouraged as TCR deep sequencing can reveal strong correlates of disease. For instance, TCR deep sequencing has been adapted as a tool to assess minimal residual disease in lymphoid malignancies to very high resolution.[12] In immunotherapy, TCR deep sequencing revealed increased TCR clonality within tumour biopsies from advanced melanoma patients correlated with response to monoclonal antibody against PD-1.[13] The same conclusion was drawn from a second group showing increased clonality was predictive of response to PD-1 blockade but not CTLA-4 blockade.[14] Highly expanded clonotypes have also been observed in tumour infiltrating lymphocytes (TIL) in melanoma,[15] where clonal size positively correlated with PD-1 expression. However, some complexity is introduced when taking into account the mutation load of the cancer. A study was recently performed with *de novo* assembly of TCR sequences from RNA-Seq data from 29 cancer types from the The Cancer Genome Atlas (TCGA).[8] They found that TCR diversity of TILs positively correlated with somatic mutation load. However, T-cell clonality does not always associate with positive outcome as shown by large clonal expansions in affected joints in newly diagnosed rheumatoid arthritis patients.[16]

Future studies in ACT immunotherapy should focus on biopsy sampling to: (i) verify that ACT products traffic to the tumour and; (ii) determine precisely which clonotypes are doing the damage at the site of disease. Finally, given that the T-cell repertoire is highly structured[2] increasingly sophisticated measures of TCR parameters (entropy/diversity measurements, TRV/TRJ/CDR3 combinations and biochemistry and so on) could disclose novel correlates of therapeutic response, particularly when paired with T-cell transcriptional profiles[7] in multivariate analysis. Once these novel correlates of disease are established the next challenge will be to develop pipelines that can deliver this information in clinically helpful time frames and at low cost. Overall, bias in the T-cell repertoire has been shown to have important biological implications across a wide range of diseases including malignancy, infectious disease and autoimmunity.[2] It is clear that TCRs 'make a difference'. Likewise, we are in the beginnings of immunomics

and new technologies such as TCR deep sequencing and single-cell transcriptomics will give us new insights into the composition of T-cell repertoires in the blood and tissues in unprecedented detail. Indeed, using these techniques we are now understanding the structure of the T-cell repertoire over the human life course, observing gender differences in T-cell subsets and radical repertoire remodelling with age.[17,18] Immunomics will also allow us to determine how these repertoires are shaped by infection,[19] vaccination and/or distorted by disease. New data gleaned from these and future studies will allow us to understand and one day manipulate the TCR repertoire for positive therapeutic outcomes across many human diseases.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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