REVIEW

T-cell receptor and B-cell receptor repertoire profiling in adaptive immunity

Anastasia Minervina[1], Mikhail Pogorelyy[1,2] & Ilgar Mamedov[1,2,3]

1 Department of Genomics of Adaptive Immunity, M M Shemyakin and Yu A Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russia
2 Institute of Translational Medicine, Pirogov Russian National Research Medical University, Moscow, Russia
3 Laboratory of Molecular Biology, Rogachev Federal Scientific and Clinical Centre of Pediatric Hematology Oncology and Immunology, Moscow, Russia

Correspondence
Ilgar Mamedov, Department of Genomics of Adaptive Immunity, M M Shemyakin and Yu A Ovchinnikov Institute of Bioorganic Chemistry RAS, Miklukho-Maklaya 16/10, Moscow 117997, Russia.
Tel./fax: +74953304288;
e-mail: imamedov78@gmail.com

SUMMARY

B-cell receptors and T-cell receptors are the key molecules responsible for specific antigen recognition in adaptive immunity. The huge diversity of immune receptor repertoires constrained their comprehensive studies in the past. More recently, however, high-throughput sequencing based techniques have revolutionized the field of immune receptor repertoire profiling enabling new insights into the development and function of the adaptive immune system. In this review we describe current methods for immune receptor profiling and software tools used for repertoire reconstruction from raw sequencing data. We also provide examples of how immune repertoire profiling can be used to study adaptive immunity in disease and in the course of organ and bone marrow transplantation.

Transplant International 2019; 32: 1111–1123

Key words
adaptive immunity, repertoire sequencing

Received: 31 March 2019; Revision requested: 9 May 2019; Accepted: 25 June 2019; Published online: 29 July 2019

Introduction

The key molecules in the adaptive immune response are the T-cell receptors (TCRs) and B-cell receptors (BCRs) which are respectively found on the surfaces of T-cells and B-cells. These receptors are heterodimers consisting of α/β or γ/δ chains in the case of TCRs, or heavy and light chains in the case of BCRs. TCRs usually recognize antigenic peptides in complex with major histocompatibility complex (MHC) molecules, whereas BCRs and antibodies bind directly to the antigen surface. The receptor of each mature T or B-cell forms via a specific gene recombination mechanism (V(D)J recombination), in which, one from each of the multiple variable (V), diversity (D) and joining (J) genomically encoded segments is selected and joined together. Recombination occurs in two consecutive stages, wherein D and J segment joining is followed by the addition of a V segment. During this process, exonucleases can remove several nucleotides from the ends of each segment and random nucleotides may then also be added to the segment junctions to form the hypervariable complementarity determining region 3 (CDR3) that is primarily responsible for the antigen recognition. It should be noted that D segment is only present in the TCRβ, TCRδ, and BCR heavy chain; for the other chains only V and J segments are involved in this recombination process. This mechanism enables the generation of a

© 2019 Steunstichting ESOT
doi:10.1111/tri.13475

huge number of different receptor variants, and thus ensures the possibility to recognize virtually any potential alien or self-antigen. The set of T-cell or B-cell receptors in a single individual or in a given sample is called the TCR or BCR repertoire. The formation of each repertoire is the result of multiple processes including: (i) the initial recombination event, which occurs during immune cell maturation, (ii) positive and negative selection of T-cells in the thymus, and (iii) expansion of particular sequences as a result of interaction with the environment, including infectious agents, commensal microbiota and food.

In the past, the study of immune repertoires was greatly limited due to their huge diversity and the skewed distribution of cells with a given receptor. More recently, the development of high-throughput sequencing (HTS) techniques has revolutionized the field of immune repertoire studies. Currently, Illumina is the most widely used platform for repertoire sequencing, as it can achieve sequencing read lengths sufficient to cover the hypervariable CDR3 (for TCRs) and even the entire variable region (for BCRs), generate a high number of reads to reveal a vast majority of variants with a relatively low error rate [1]. Despite the obvious advantages of HTS for immune repertoire studies, there are several obstacles that should be considered. First are the errors that are introduced during amplification, the sequencing process itself, and the reverse transcription (in RNA-based repertoire profiling methods). Correcting these errors is a challenging bioinformatics task, as there are no reference sequences for CDR3s in the genome. The somatic hypermutation of antibodies, which normally occurs outside the CDR3, provides an additional challenge for accurate sequence reconstruction. Second, amplification bias can confound quantitative assessment of particular TCR/BCR containing cell frequency and compromise overall repertoire diversity. Third, it remains a challenge to completely reconstruct receptor structure by the correct pairing of α/β and γ/δ chains for TCRs or heavy/light chains for BCRs in large numbers of cells.

Immunosequencing can provide the information about counts and receptor chain sequences for the TCR/BCR clonotypes present in a repertoire. This information can then be used to: (i) calculate summary statistics of clone size distribution, which in turn gives insight in repertoire diversity and clonality; (ii) track particular lymphocyte clones across time (e.g., before and after treatment) or (iii) space (e.g., between peripheral blood and tumor, or between cell subsets) using nucleotide receptor sequence as an identifier; (iv) examine the sharing of clonal sequences between donors in a cohort of interest, such as patients with a particular disease; (v) examine specific sequence features of antigen receptors such as biased CDR3 length and gene segment usage distribution, or the presence of specific sequence motifs.

Deep profiling of immune repertoires in the course of organ transplantation could be useful for characterizing adaptive immune cells involved in processes related to chronic organ rejection or antiviral immune response under immunosuppressive conditioning. This knowledge can then be used to develop individualized immunosuppressive regimens that target a particular group of recipient cells, or to guide the development of graft tolerance via adoptive transfer of regulatory T-cells (Tregs).

Here, we review existing methods of immune repertoire profiling and describe examples of how repertoire data is being used to gain insights into underlying biology or disease mechanisms. Finally, we review current repertoire studies in organ and hematopoietic stem cell transplantation (HSCT).

HTS-based methods of immune repertoire profiling

There are several strategies for reconstructing TCR or BCR repertoires, and the best choice depends on the question of interest [2–4] (Fig. 1). The first includes repertoire sequencing methods that selectively target TCR/BCR molecules. Targeted repertoire sequencing is cost effective, allowing for the monitoring immune response in large cohorts and at multiple timepoints. These methods can use either DNA [5–7] or RNA [8–13] for library generation and subsequent sequencing, and both have various advantages and limitations as starting material for repertoire sequencing.

RNA-based methods employ 5′ rapid amplification of cDNA ends (RACE)-based template switching [14] or single-stranded RNA ligation [15,16] to add a universal oligo at the 5′ end of the first strand cDNA, and thus minimize quantitative bias via the use of a single primer pair for amplification. RNA-based methods can utilize unique molecular identifiers (UMIs), which comprise a stretch of 8–12 random nucleotides that is used to mark each initial cDNA with a unique ‘molecular barcode’. These represent a powerful tool for TCR/BCR transcript quantification and error correction [17]. DNA-based methods rely on multiplex amplification, which results in quantitation bias that can be partially corrected by the use of a synthetic spike-in template [18]. On the

© 2019 Steunstichting ESOT

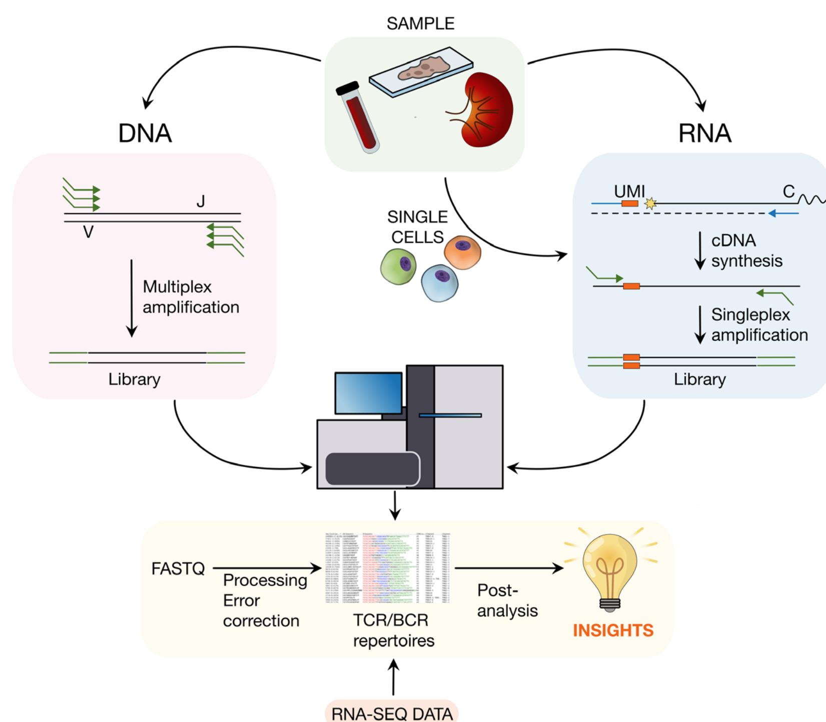

Figure 1 Methods of immune repertoire profiling. Immune repertoires can be derived from DNA or RNA extracted from a sample of interest. Unique molecular identifiers are used to mark each T-cell receptor/B-cell receptor molecule with a unique barcode that can be used to correct sequencing errors and amplification bias. Immune receptor sequences can also be extracted from bulk RNA-Seq data.

plus side, DNA-based approaches are resistant to expression level variations, since each cell contains only a single DNA copy of the gene encoding each chain of the TCR/BCR. This is particularly important for the analysis of B-cells, as BCR transcription levels can vary by as much as 1000-fold, making RNA-based methods impractical without additional manipulations. For example, precise FACS sorting of certain populations can be used to filter out subpopulations with high BCR expression (e.g., plasma cells), and performing experiments with multiple biological replicates makes it possible to estimate reproducibility and quantify the noise introduced by variable expression levels. As an alternative to multiplex or 5′ RACE-based amplification one can employ the hybridization-based enrichment of receptor RNA or DNA molecules with RNA baits [19,20]. These hybridize to TCR or BCR molecules, and thus can be used to capture and directly sequence them after few amplification cycles. BCR repertoire sequencing can also be coupled with proteomics to reconstruct the antibody repertoire at the protein level [21]. Alternatively, TCR or BCR repertoires can be extracted from bulk RNA sequencing data obtained from any immune cell-containing tissue [22,23]. Obviously, only a small portion of such sequencing reads will correspond to TCRs or BCRs, and a very high sequencing depth is therefore required to derive informative repertoires [24].

Another powerful group of methods for studying repertoires are the single cell-based approaches [3,25]. These include methods that specifically target both chains of immune receptor molecules [26–30], as well as standard single-cell transcriptomics analyses from which the sequences of immune receptors are extracted [31–35]. In the first case, additional information on cell phenotype can be obtained either through separation by FACS sorting with specific surface markers or by targeted amplification of functional genes characteristic of different cell subsets [28]. The single-cell-based approaches have obvious advantages over other methods as they are able to reconstruct complete receptor structures by pairing both chains in a given T or B-cell, and also link them to a particular immune cell's phenotype.

Many computational approaches have been developed in the last decade to process and analyze HTS immune repertoire data [36], including software tools that can be used to extract TCR or BCR repertoires from raw sequencing reads (e.g., MiXCR, Vidjil, IMSEQ, IgReC [37–40]). This typically includes creating a list of clonotypes with determined CDR3, V, D, and J segments and their borders, as well as each clonotype's count and basic error correction, based on the collapsing of similar sequences. Such an error-correction approach works quite well for TCR repertoires but is less applicable to the analysis of BCRs, which have hypermutations not

© 2019 Steunstichting ESOT

only in the randomly-generated CDR3 but also in genomically encoded segments. The most robust approach to correct errors introduced during PCR or HTS is based on the use of UMIs [37–42], which also allows for more precise measurement of repertoire diversity. In addition to the above-described clonotype-building programs, many tools for post-analysis of repertoires have been developed (e.g., tcR, IgAT, IMEX, VDJtools [43–46]). These tools are used to assess the characteristics of different individual repertoires, including repertoire diversity and the usage of gene segments, in order to build evolutionary trees of hypermutated antibodies, and to compare individual repertoires to each other. Few programs have been developed that can predict TCR antigen specificity [47–49] based on their sequence. Databases containing TCR sequences with known antigen specificities [50,51] are also used to characterize repertoires.

Researchers can use the information obtained from repertoire sequencing in a variety of different ways (see Fig. 2), which are explored in great detail in the sections below.

Clone size distribution statistics

The potential diversity of a given TCR/BCR repertoire is many orders of magnitude larger (e.g., 10^{14} for TCRβ [52]) than the diversity that is actually realized in any organism. The latter is estimated to be at least 10^{8} clonotypes for TCRβ, which is still larger than the diversity observed in the largest repertoire sequencing experiments [53], while a typical repertoire sequencing experiment samples less than one million cells. Inferring diversity from small samples is challenging, and many different metrics have been proposed for this task (Shannon entropy [54], RECON [55], DivE [56], Renyi entropy (https://arxiv.org/abs/1604.00487), and its transformation, Hill number-based diversity estimators [57], Chao2 estimator [58]). V(D)J recombination and thymic selection result in a very diverse repertoire of naive T-cells with more or less even distribution of clone sizes. Clones can subsequently expand upon contact with their cognate antigen, which skews the observed clone size distribution such that a relatively small number of expanded clones occupies disproportionate space in the repertoire. There are a variety of clonality statistics that can reflect these biases, such as the Gini-Simpson index [59]. In many cases, the clonality statistics are just the inverse of the diversity statistics, such that higher clonality typically means lower diversity. The number of ‘expanded clonotypes’ is often used as another way to describe narrowed repertoires. While there is no generally accepted definition, ‘expanded clonotype’ usually refers to a particular T-cell clone that either occupies a substantial fraction of the sequenced repertoire (e.g., >1%) or is repeatedly found in many

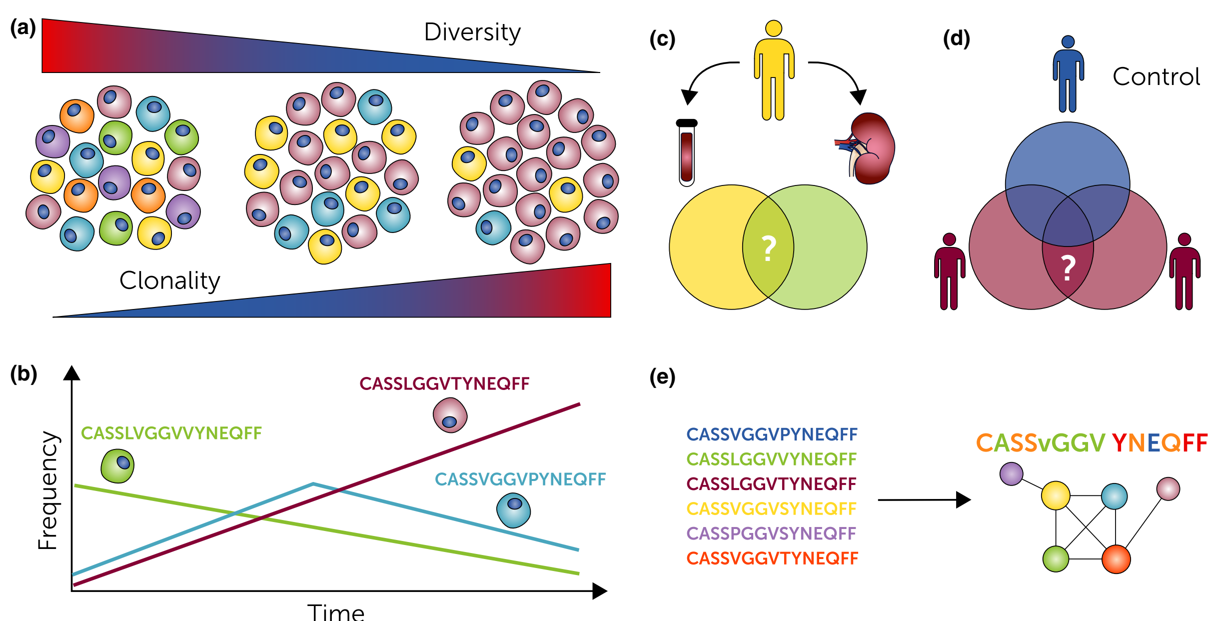

Figure 2 Insights from immune repertoire analysis. Analysis of repertoire data can offer a variety of valuable immunological information, including: (a) clone size distribution statistics such as diversity and clonality, (b) tracking of clones in time, (c) physical/phenotypic space, (d) sharing between individuals and (e) clonal sequence features (motifs, VJ-usage biases etc.).

© 2019 Steunstichting ESOT

sampling replicates [60]. These 'expanded clonotypes' should not be mistaken with clonotypes that expanded between timepoints in the longitudinal studies (see section below).

The estimation of various clonality and diversity metrics is a part of most repertoire sequencing studies. Summary statistics reduce the complexity of repertoire data to a single number per sample, thus enabling the comparison of experimental groups with routine statistical tests in order to identify meaningful correlates of disease, health, or therapeutic response. In autoimmune disease diminished diversity and increased clonality at an active disease site may be a sign of an ongoing autoreactive clonotype expansion. For example, patients with systemic lupus erythematosus exhibit less diverse TCR repertoires in peripheral blood than healthy controls, and their clone size distribution is skewed towards more expanded clonotypes [61]. TCR clonal expansion in the ileum mucosa of Crohn's disease patients has been shown to significantly correlate with smoking status at the time of the assay, and more importantly, has also been found to be predictive of disease relapse such that patients with postoperative recurrence have a higher proportion of high-frequency clones in their TCR repertoire [62]. Matos *et al.* [63] showed the presence of large oligoclonal subpopulations of α/β T-cells in clinically resolved skin lesions from patients with psoriasis. A large fraction of these T-cells was able to produce IL-17A, which is believed to be an important driver of psoriasis pathogenesis. Interpretation of repertoire sequencing results is particularly complicated in modern cancer research focused on immunotherapy. Different repertoire summary statistics could be especially helpful in this case, offering potential markers of immunotherapy efficacy. For example, higher intra-tumoral TCR clonality has been observed in responders to anti-PD1 antibody treatment at both pre-treatment and during-treatment timepoints, whereas no such effect was observed for CTLA4 blockade therapy [64–67]. In contrast, higher TCR repertoire diversity in the peripheral blood after CTLA4 blockade was correlated with drug-related toxicities in prostate cancer [68,69] and metastatic melanoma [70].

Even though biased clone size distribution may correlate with a condition of interest, the underlying mechanisms must be proven and investigated in detail using methods we describe further below. When using repertoire summary statistics, one should also be aware that differences in both sampling and sequencing depth may confound diversity or clonality estimates, making it important to properly normalize the data [60].

Clonal tracking in time

Due to the high diversity of immune repertoires, nucleotide sequences of antigen receptor chains can be used as unique identifiers for lymphocyte clones. Comparison of the frequencies of clonotypes in a repertoire before and after an immune challenge by an antigen can directly show the expansion of responding clonotypes.

Clonotype tracking has been used to investigate TCR repertoire alterations after yellow fever (YF) immunization – a safe model of acute viral infection in humans [71]. In the recent study, each donor responded to the vaccine with ~1000 different clonotypes corresponding to both cytotoxic and helper T-cells [72], with dynamics similar to those observed in previous studies using MHC-multimer and T-cell activation marker staining [73,74]. The specificity of a substantial fraction of the HTS-identified YF-responding clonotypes was confirmed by independent assays [72]. In another study, Qi *et al.* [75] tracked the expansion of varicella-zoster virus (VZV)-specific CD4+ T-cells after immunization with a live vaccine, and showed both reactivation of previously existing memory cells and recruitment of VZV-specific naive T-cells.

In the case of B-cells, it is generally more interesting to track the total amount of a particular serum antibody produced by a given clone rather than the frequency of the cells with such BCR in the peripheral blood, which may be low and is generally irreproducible between timepoints, except for the most abundant clones [76]. This can be achieved by combining repertoire sequencing with proteomics, where mass-spectrometry is used to determine the serum concentration of antibodies produced by B-cells. Lee *et al.* [21] used such an approach to show that ~60% of serum antibodies identified after seasonal influenza vaccination were present in serum prior to immunization.

Clonal tracking has also been used to measure the effects of therapeutic interventions. Using TCRβ sequencing, Chapuis *et al.* [77] were able to track tumor-specific, adoptively-transferred cytotoxic T-cells in peripheral blood of melanoma patients. The frequency of these T-cell clonotypes peaked at day 7, and then declined for most clonotypes to baseline levels 30 days after the transfer. Interestingly, in patients with a complete response, a single clonotype dominated after the transfer, and was detectable in blood up to 300 days post-transfer. The authors speculated that these expanded clones are responsible for tumor control. In other work, de Jong *et al.* [78] and coauthors performed a longitudinal study of the effects of a pan-JAK

© 2019 Steunstichting ESOT

inhibitor drug on T-cells in patients with alopecia areata, an autoimmune hair loss disorder. They found that the scalp repertoire of patients was enriched with abundant CD8+ T-cell clones, and the concentration of these clones decreased significantly in response to therapy. In some cases, the abundance of clones in a peripheral repertoire may be independent of disease symptom progression. For example, in patients with systemic lupus erythematosus, Thapa *et al.* [61] found no significant alterations between TCRβ repertoires at timepoints of disease quiescence and at a flare.

Sequencing based clonal tracking in time is a very useful tool for revealing clonotypes responding to different challenges without any prior knowledge of antigens causing clonal expansions. However, the pre-challenge timepoint is needed for clonal expansion identification. In YF-vaccination [72] study contraction dynamics from the peak of the response to the later timepoint was used to overcome this challenge. In general, strong clonal expansion in tissue of interest is needed to identify disease-associated clonotypes using this approach.

Clonal tracking in space

One can also use nucleotide sequences of antigen receptors to track clones between body compartments, or between cell subsets of different phenotypes. In this way, researchers can identify clones significantly enriched in a subpopulation of interest, such as T-cells infiltrating a tumor or a transplanted organ compared to the background (e.g., peripheral blood), or to estimate an overlap of TCR/BCR repertoires between different organs. It was recently shown that both PD1+CD8+ [79] and CD4+ Treg clonotypes [80] from peripheral blood match corresponding tumor-resident clones and appear to be tumor-reactive. Similarly, Wong *et al.* [81] found that the repertoire of urine-derived lymphocytes accurately represents the tumor microenvironment repertoire in patients with muscle invasive bladder cancer. These studies thus provide a method to sample intra-tumoral repertoires non-invasively.

The overlap between tissues is likely to be specific to memory cells but practically absent for naive T-cells. Thome *et al.* [82] demonstrated this by sequencing repertoires of effector memory and naive T-cells from inguinal, lung-draining lymph nodes and spleens of deceased donors. Meng *et al.* [83] sequenced BCR repertoires in eight tissue samples from six donors. Strikingly, they found two non-overlapping B-cell networks inside the body: one spanning blood-rich tissues (i.e., blood, bone marrow, mesenteric lymph node, and lung) and another spanning the gastrointestinal (GI) tract (i.e., jejunum, ileum, and colon). The construction the B-cell lineage trees revealed differences between these two networks. In blood-rich tissues, each branch of the tree was tissue-specific, whereas in the GI tract, each branch contained clones from different parts of the intestine, suggesting the proliferation and intensive migration of somatically hypermutated clonotypes inside the GI tract. For all donors, the most highly hypermutated B-cells were found in the jejunum, suggesting intense interactions with the microbiome and environmental antigens at this site.

Clonal sharing

Although TCR/BCR nucleotide sequences can be used as unique clone identifiers, the occurrence of highly similar or identical receptor amino acid sequences between individuals is not impossible. Indeed, thousands of such ‘convergent’ immune receptor sequences can be found in bulk receptor repertoires from different individuals [53,57,84]. Clonotypes present in more than a single donor are termed ‘public’, whereas clonotypes specific to an individual are considered ‘private’. The V(D)J recombination mechanism generates some antigen receptor sequences much more frequently than others. There are efficient algorithms that can calculate the probability of generation for a given antigen receptor sequence [52,85,86], and these could theoretically estimate the number of donors sharing such sequence [87]. Thus, there is no sharp border between public and private clonotypes, but rather a continuous spectrum of publicness defined by the probability of an antigen receptor being produced by the recombination machinery. Such purely stochastic sharing provides little information for biomedical research, but TCR/BCR sharing is also defined by selection, both in the thymus and in the periphery in response to pathogens. The latter leads to clonal expansion and loss of the naive lymphocyte phenotype, and thus makes antigen-experienced clones easier to sample in a repertoire sequencing experiment. This means that patients sharing a disease may also share clonotypes recognizing disease-specific antigens that would not be detected in repertoires of healthy people.

A great example of this was provided by Emerson *et al.* [88]. Their study featured the largest cohort of donors subjected to repertoire sequencing to date: 666 individuals with a known cytomegalovirus (CMV) infection status. CMV-associated clonotypes were identified as predominantly shared between CMV-positive patients

© 2019 Steunstichting ESOT

using Fisher's exact test. The resulting collection of CMV-associated clonotypes was afterwards used to create an accurate classifier of donor CMV status, which was also tested on an independent cohort [48]. In another study, Faham *et al.* [89] used a similar approach to identify a set of TCR amino acid sequences overrepresented in a cohort of ankylosing spondylitis patients. At the same time, Komech *et al.* [90] identified the similar motif in an independent cohort, using a different statistical approach based on the estimation of TCR recombination probability (described in detail in [91]). Sharing of putatively pathogenic clonotypes was also described in psoriasis [63]. Latorre *et al.* [92] showed sharing of hypocretin-specific T-cell clones between patients suffering from narcolepsy. In some patients, self-reactive clonotypes were among the most frequent in peripheral blood, suggesting intense clonal expansion. These findings have solidified evidence for narcolepsy being an autoimmune disorder. Public BCR sequences have also been found in response to several infections, including dengue [93], HIV [94], influenza [95], and malaria [96]. It has been speculated that stimulation of such clones could represent a goal for novel vaccines, as they co-occur in many people.

However, there are pitfalls to strategies focused on shared clonotypes. First, for TCRs, sharing depends on the recognition of the same epitope, where the implication is that the antigen is presented by the same or highly similar MHC-alleles. A reanalysis of the above-mentioned data from Emerson *et al.* [88] by Dewitt *et al.* [97] identified clusters of TCRs that co-occur with certain MHC-alleles, many of which contained previously identified TCRs specific to common pathogens. Second, for some epitopes there might be few or no public sequences [98]. In the majority of cases, antigen-specific clones are unique to a single donor, with little sharing observed even between genetically identical twins [72,75].

Overall TCR/BCR sequence sharing in large cohorts of patients may provide a diagnostic signature, and may in some cases identify T-cell and B-cell clonotypes participating in the immune response to disease.

Clonal sequence features

B cell receptor and TCR specificities of are encoded in their sequences, although there is currently no algorithm that can reliably predict which epitope a TCR or BCR recognizes based on its sequence. However, it is still possible to identify specific TCR/BCR sequence features that are crucial for the recognition of antigens associated with the disease of interest. TCRs recognizing the same epitope often have highly similar sequences, and several sequence similarity measures have been recently proposed to cluster TCRs recognizing them [47–49]. Shared TCR motifs arise by the same mechanism as described for public clonotype generation in the previous section: independent convergent recombination of the same sequence feature in many precursor cells, followed by clonal selection for the cognate antigen in the periphery.

In some cases, CDR1 and CDR2 sequences encoded by germline V segments make important contacts with the antigen, leading to strong biases in V segment usage among antigen-specific clonotypes. For example, TCRs recognizing the $NS4b_{214–222}$ peptide from YF virus frequently incorporate the alpha chain V segment TRAV12, which was shown to make a contact with the peptide [99]. There are many other such examples that have been observed in antigen-specific TCR repertoires [100,101]. Similar V segment usage biases are also often found in repertoires of B-cell subpopulations. Blum *et al.* [102] identified preferential usage of certain IGHV segments in the plasmablast repertoires of Lyme disease patients. Tucci *et al.* [103] reported disrupted B-cell repertoires in a cohort of chronic HCV patients due to preferential usage of several IGHV segments in IGM memory B-cells. While CDR3 may be conserved within a subset of antigen-specific sequences, even withinTCRs recognizing one epitope, there are frequently many distinct motifs, each successfully solving the task of antigen recognition [47,104].

Sometimes sequences analysis can reveal very unexpected sequence features. Tan *et al.* [105] isolated B-cells producing broadly reactive antibodies to Plasmodium-infected erythrocytes. BCR sequencing revealed an unusually large (~100 nt) insertion between the V and DJ segments of the antibody heavy chains, which appeared to be a collagen-binding domain from the LAIR1 gene. Such antibodies were found in 5–10% of donors from malaria-endemic regions but were abcent in European donors. Strikingly, in all observed cases, the LAIR1-containing antibodies were derived from a single clone, suggesting that this insertion happens rarely but is subsequently favored by selection in response to malaria antigens. Such genome segment insertions represent a novel and poorly understood mechanism of antibody diversification. Such insertions are not specific to LAIR1; in European cohorts, 1 in every 1000 B-cells has such an insertion from almost any part of the genome [96]. Antigen specificity and function of these chimeric BCRs remain to be determined.

© 2019 Steunstichting ESOT

Immune repertoire profiling in organ and stem cells transplantation

Only a few studies have reported immune repertoire profiling in the context of organ transplantation. Yang *et al.* [106] have reported TCRβ repertoire profiling of six patients in the course of liver transplantation. The authors found some shared clonotypes that were characteristic for transplant recipients and absent in the blood of healthy controls. Common sequences were also observed at certain timepoints pre- and post-transplantation. These findings remain to be validated by further analysis, including calculation of recombination probability, and searching for similar sequences in databases of TCRs with known specificity or MHC association. Other studies [107,108] have compared bulk TCRβ repertoires before and after kidney transplantation. The authors found that repertoire diversity is decreased after transplantation, suggesting clonal expansion of donor T-cells. In two other studies on kidney transplantation [109,110], the authors first identified a TCRβ repertoire of donor-reactive cells using repertoire sequencing of mixed lymphocyte reaction (MLR)-expanded T-cells [111] for each donor-recipient pair. These pools of donor-reactive repertoires were tracked after combined kidney and bone marrow transplantation (CKBMT) in patients that developed allograft tolerance and in non-tolerant patients. The authors showed that in the blood of patients who developed graft tolerance the number of donor-reactive T-cells was lower than in non-tolerant patients [109]. In the other study [110], the number of donor-specific Treg cells was positively correlated with immunotolerance in CKBMT patients.

B cell receptor repertoire reconstruction after CKBMT in four patients [112] showed that the diversity of B-cell receptors in graft-tolerant subjects recovered to pre-transplant levels after 1 year post-transplantation.

Hematopoietic stem cell transplantation is used for the treatment of various diseases, including autoimmune disorders, cancer, and primary immunodeficiencies [113–116]. The success of HSCT-based therapy depends on immune repertoire reconstruction and its ability to eliminate malignant cells, control infections after conditioning, and suppress autoimmune reactions. Many studies on immune repertoire profiling in the course of HSCT have revealed details about repertoire reconstitution after autologous and allogeneic stem cell transplantation under various accompanying therapy and graft manipulations. The curative effect of autologous HSCT in autoimmune diseases is believed to be a result of repertoire resetting that establishes a new balance between autoreactive effector cells and suppressive Treg cells. Indeed, recent TCRβ repertoire studies show that in patients with multiple sclerosis, CD4+ cells can be completely renewed after HSCT, whereas CD8 cells retain some signatures of the pre-transplantation repertoire [117]. Other work [118] reports increased Treg diversity in patients in remission for autoimmune juvenile idiopathic arthritis or juvenile dermatomyositis after transplantation.

For cancer patients undergoing HSCT, allogeneic transplanted cells are intended to kill malignant cells that escape patient adaptive immunity (GVT, Graft-versus-tumor). In addition, undifferentiated cells from the graft provide faster immune repertoire reconstruction, which is needed to control opportunistic infections. However, mature donor cells can also attack the patient's organs due to antigen or HLA mismatches, leading to graft-versus-host disease (GVHD), a common cause of HSCT-associated mortality. Thus, grafts are commonly depleted of mature T-cells by *ex vivo* antibody (anti-TCRα/β) capture or by chemical agents such as anti-thymocyte globulin or cyclophosphamide [119]. TCRβ repertoire evaluation has revealed lower diversity of both CD4+ and CD8+ subsets in recipients of T-cell-depleted grafts after 6 months compared to cord blood graft recipients [120]. In the early stages of immune repertoire reconstitution (1–4 months post-transplantation), T-cell clones originate from rapidly increasing non-depleted T-cells of the donor, which are eventually replaced by a new, diverse repertoire, with naive cells coming from the thymus after approximately 1 or 2 years [121]. The former population of early T-cells may originate from donor naive T-cells [122] and differentiate into a special type of memory stem T-cells; alternatively, they come mainly from the blood/marrow memory CD8+ compartment [123]. Further systematic investigations of various HSCT protocols are needed to fully unravel the source of early repertoire reconstitution. Oligoclonal T effector memory cell expansions correlate with CMV activation [124,125], and these CMV-specific clonal expansions contract the underlying naive CD8+ repertoire. A study based on TCRα repertoire sequencing [126] indicated that, unlike for the TCRβ repertoire, the vast majority (up to 80%) of early post-transplant cells at 2 and 6 months share their CDR3 sequences with donor memory T-cells. Surprisingly, 41–61% of naive T-cell TCR sequences post-transplantation are found among memory TCRs of the donor graft. These results could be potentially confounded by sharing of convergently recombined public TCRα chains with a high probability of generation.

© 2019 Steunstichting ESOT

Several studies indicate that both donor TCR repertoire diversity [127–129] and post-transplant recipient repertoire diversity correlate with better prognosis, including lower risk of GVHD or relapse risk and CMV re-activation. Accordingly, Gkazi et al. [127] proposed to use accurate UMI-based TCRβ repertoire diversity measures for monitoring of immune repertoire reconstitution in the clinic.

Concluding remarks and future perspectives

The specific molecular mechanisms underlying chronic rejection or immune tolerance after organ transplantation are still largely unknown. The vast majority of currently published studies are based on small cohorts, lack detailed phenotypic or functional characterization of reactive cells and are focused on bulk repertoires of peripheral blood. As a consequence these studies are mainly descriptive and lack sufficient biological insights. Moving forward, intensive study of immune repertoires pre- and post-transplantation, identification and phenotypic characterization of specific responding clones will help to unravel these mechanisms and enable targeted personalized manipulation of the adaptive immune response.

At present, single cell-based approaches are the most powerful way to study immune repertoires, as these can link the complete receptor sequence to functional traits of the cell. This knowledge can then be complemented with direct determination of antigen specificity based on libraries of antigen-loaded MHC-multimers [130]. Although existing commercial solutions are still very expensive, extensive efforts of many research groups and biotech companies will decrease prices in the near future. The accumulation of single-cell TCR/BCR sequencing data will also require further development of computational tools for both primary (e.g., receptor sequence reconstruction, chain pairing, and gene expression levels) and post (e.g., functional characterization of cells, comparison of datasets, and identification of disease/immune response-associated sequences) data analysis.

Another critical issue in immune repertoire studies is the ability to predict receptor specificity based on its sequence. Despite considerable progress in this field, the determinants of antigenic specificity for the vast majority of receptors sequenced to date are totally unknown. Only a small subset of receptors are known to be associated with particular conditions such as ankylosing spondylitis, colon cancer and melanoma, or pathogens such as CMV, influenza, YF vaccine. And a tiny portion of this subset could be linked to the particular MHC-peptide pair or epitope, but this number (and thus diagnostic power of repertoire sequencing) grows steadily. With further advances in the field, a clinician should ultimately be able to get a comprehensive view of adaptive immune status from a single repertoire sequencing survey of a patient, which provides insights into predisposition to autoimmune disease, resistance against certain infections, immune response to cancer, treatment efficiency, etc. Deeper knowledge of immune receptor specificity will also lead to the development of specific therapies that could change the landscape of immune repertoire depleting the undesired immune cells and expanding the advantageous ones.

Funding

This work was supported by Russian Foundation for Basic Research grants (RFBR 19-54-12011 and RFBR 18-29-09132-mk).

Conflicts of interest

The authors have declared no conflicts of interest.

REFERENCES

1. Bolotin DA, Mamedov IZ, Britanova OV, *et al.* Next generation sequencing for TCR repertoire profiling: platform-specific features and correction algorithms. *Eur J Immunol* 2012; **42**: 3073.
2. Boyd SD, Crowe JE Jr. Deep sequencing and human antibody repertoire analysis. *Curr Opin Immunol* 2016; **40**: 103.
3. Friedensohn S, Khan TA, Reddy ST. Advanced methodologies in high-throughput sequencing of immune repertoires. *Trends Biotechnol* 2017; **35**: 203.
4. Rosati E, Dowds CM, Liaskou E, Henriksen EKK, Karlsen TH, Franke A. Overview of methodologies for T-cell receptor repertoire analysis. *BMC Biotechnol* 2017; **17**: 61.
5. Kitaura K, Shini T, Matsutani T, Suzuki R. A new high-throughput sequencing method for determining diversity and similarity of T cell receptor (TCR) alpha and beta repertoires and identifying potential new invariant TCR alpha chains. *BMC Immunol* 2016; **17**: 38.
6. Lin SG, Ba Z, Du Z, Zhang Y, Hu J, Alt FW. Highly sensitive and unbiased

© 2019 Steunstichting ESOT

approach for elucidating antibody repertoires. *Proc Natl Acad Sci USA* 2016; **113**: 7846.
7. Robins HS, Campregher PV, Srivastava SK, *et al.* Comprehensive assessment of T-cell receptor beta-chain diversity in alphabeta T cells. *Blood* 2009; **114**: 4099.
8. Cole C, Volden R, Dharmadhikari S, Scelfo-Dalbey C, Vollmers C. Highly accurate sequencing of full-length immune repertoire amplicons using Tn5-enabled and molecular identifier-guided amplicon assembly. *J Immunol* 2016; **196**: 2902.
9. Freeman JD, Warren RL, Webb JR, Nelson BH, Holt RA. Profiling the T-cell receptor beta-chain repertoire by massively parallel sequencing. *Genome Res* 2009; **19**: 1817.
10. Mamedov IZ, Britanova OV, Zvyagin IV, *et al.* Preparing unbiased T-cell receptor and antibody cDNA libraries for the deep next generation sequencing profiling. *Front Immunol* 2013; **4**: 456.
11. Pogorelyy MV, Elhanati Y, Marcou Q, *et al.* Persisting fetal clonotypes influence the structure and overlap of adult human T cell receptor repertoires. *PLoS Comput Biol* 2017; **13**: e1005572.
12. Quigley MF, Almeida JR, Price DA, Douek DC. Unbiased molecular analysis of T cell receptor expression using template-switch anchored RT-PCR. *Curr Protoc Immunol* 2011; 1. Chapter 10: Unit10 33.
13. Turchaninova MA, Davydov A, Britanova OV, *et al.* High-quality full-length immunoglobulin profiling with unique molecular barcoding. *Nat Protoc* 2016; **11**: 1599.
14. Matz M, Shagin D, Bogdanova E, *et al.* Amplification of cDNA ends based on template-switching effect and step-out PCR. *Nucleic Acids Res* 1999; **27**: 1558.
15. Best K, Oakes T, Heather JM, Shawe-Taylor J, Chain B. Computational analysis of stochastic heterogeneity in PCR amplification efficiency revealed by single molecule barcoding. *Sci Rep* 2015; **5**: 14629.
16. Oakes T, Heather JM, Best K, *et al.* Quantitative characterization of the T cell receptor repertoire of naive and memory subsets using an integrated experimental and computational pipeline which is robust, economical, and versatile. *Front Immunol* 2017; **8**: 1267.
17. Kivioja T, Vaharautio A, Karlsson K, *et al.* Counting absolute numbers of molecules using unique molecular identifiers. *Nat Methods* 2011; **9**: 72.
18. Carlson CS, Emerson RO, Sherwood AM, *et al.* Using synthetic templates to design an unbiased multiplex PCR assay. *Nat Commun* 2013; **4**: 2680.
19. Linnemann C, Heemskerk B, Kvistborg P, *et al.* High-throughput identification of antigen-specific TCRs by TCR gene capture. *Nat Med* 2013; **19**: 1534.
20. Ruggiero E, Nicolay JP, Fronza R, *et al.* High-resolution analysis of the human T-cell receptor repertoire. *Nat Commun* 2015; **6**: 8081.
21. Lee J, Boutz DR, Chromikova V, *et al.* Molecular-level analysis of the serum antibody repertoire in young adults before and after seasonal influenza vaccination. *Nat Med* 2016; **22**: 1456.
22. Bolotin DA, Poslavsky S, Davydov AN, *et al.* Antigen receptor repertoire profiling from RNA-seq data. *Nat Biotechnol* 2017; **35**: 908.
23. Schrama D, Ritter C, Becker JC. T cell receptor repertoire usage in cancer as a surrogate marker for immune responses. *Semin Immunopathol* 2017; **39**: 255.
24. Brown SD, Raeburn LA, Holt RA. Profiling tissue-resident T cell repertoires by RNA sequencing. *Genome Med* 2015; **7**: 125.
25. De Simone M, Rossetti G, Pagani M. Single cell T cell receptor sequencing: techniques and future challenges. *Front Immunol* 2018; **9**: 1638.
26. Busse CE, Czogiel I, Braun P, Arndt PF, Wardemann H. Single-cell based high-throughput sequencing of full-length immunoglobulin heavy and light chain genes. *Eur J Immunol* 2014; **44**: 597.
27. DeKosky BJ, Ippolito GC, Deschner RP, *et al.* High-throughput sequencing of the paired human immunoglobulin heavy and light chain repertoire. *Nat Biotechnol* 2013; **31**: 166.
28. Han A, Glanville J, Hansmann L, Davis MM. Linking T-cell receptor sequence to functional phenotype at the single-cell level. *Nat Biotechnol* 2014; **32**: 684.
29. Murugan R, Imkeller K, Busse CE, Wardemann H. Direct high-throughput amplification and sequencing of immunoglobulin genes from single human B cells. *Eur J Immunol* 2015; **45**: 2698.
30. Turchaninova MA, Britanova OV, Bolotin DA, *et al.* Pairing of T-cell receptor chains via emulsion PCR. *Eur J Immunol* 2013; **43**: 2507.
31. Afik S, Yates KB, Bi K, *et al.* Targeted reconstruction of T cell receptor sequence from single cell RNA-seq links CDR3 length to T cell differentiation state. *Nucleic Acids Res* 2017; **45**: e148.
32. Eltahla AA, Rizzetto S, Pirozyan MR, *et al.* Linking the T cell receptor to the single cell transcriptome in antigen-specific human T cells. *Immunol Cell Biol* 2016; **94**: 604.
33. Redmond D, Poran A, Elemento O. Single-cell TCRseq: paired recovery of entire T-cell alpha and beta chain transcripts in T-cell receptors from single-cell RNAseq. *Genome Med* 2016; **8**: 80.
34. Rizzetto S, Koppstein DNP, Samir J, *et al.* B-cell receptor reconstruction from single-cell RNA-seq with VDJPuzzle. *Bioinformatics* 2018; **34**: 2846.
35. Stubbington MJT, Lonnberg T, Proserpio V, *et al.* T cell fate and clonality inference from single-cell transcriptomes. *Nat Methods* 2016; **13**: 329.
36. Miho E, Yermanos A, Weber CR, Berger CT, Reddy ST, Greiff V. Computational strategies for dissecting the high-dimensional complexity of adaptive immune repertoires. *Front Immunol* 2018; **9**: 224.
37. Bolotin DA, Poslavsky S, Mitrophanov I, *et al.* MiXCR: software for comprehensive adaptive immunity profiling. *Nat Methods* 2015; **12**: 380.
38. Duez M, Giraud M, Herbert R, Rocher T, Salson M, Thonier F. Vidjil: a web platform for analysis of high-throughput repertoire sequencing. *PLoS One* 2016; **11**: e0166126.
39. Kuchenbecker L, Nienen M, Hecht J, *et al.* IMSEQ–a fast and error aware approach to immunogenetic sequence analysis. *Bioinformatics* 2015; **31**: 2963.
40. Shlemov A, Bankevich S, Bzikadze A, Turchaninova MA, Safonova Y, Pevzner PA. Reconstructing antibody repertoires from error-prone immunosequencing reads. *J Immunol* 2017; **199**: 3369.
41. Parekh S, Ziegenhain C, Vieth B, Enard W, Hellmann I. zUMIs – a fast and flexible pipeline to process RNA sequencing data with UMIs. *Gigascience* 2018; **7**: 1.
42. Shugay M, Britanova OV, Merzlyak EM, *et al.* Towards error-free profiling of immune repertoires. *Nat Methods* 2014; **11**: 653.
43. Nazarov VI, Pogorelyy MV, Komech EA, *et al.* tcR: an R package for T cell receptor repertoire advanced data analysis. *BMC Bioinformatics* 2015; **16**: 175.

© 2019 Steunstichting ESOT

44. Rogosch T, Kerzel S, Hoi KH, *et al*. Immunoglobulin analysis tool: a novel tool for the analysis of human and mouse heavy and light chain transcripts. *Front Immunol* 2012; **3**: 176.
45. Schaller S, Weinberger J, Jimenez-Heredia R, *et al*. ImmunExplorer (IMEX): a software framework for diversity and clonality analyses of immunoglobulins and T cell receptors on the basis of IMGT/HighV-QUEST preprocessed NGS data. *BMC Bioinformatics* 2015; **16**: 252.
46. Shugay M, Bagaev DV, Turchaninova MA, *et al*. VDJtools: unifying post-analysis of T cell receptor repertoires. *PLoS Comput Biol* 2015; **11**: e1004503.
47. Dash P, Fiore-Gartland AJ, Hertz T, *et al*. Quantifiable predictive features define epitope-specific T cell receptor repertoires. *Nature* 2017; **547**: 89.
48. De Neuter N, Bittremieux W, Beirnaert C, *et al*. On the feasibility of mining CD8 + T cell receptor patterns underlying immunogenic peptide recognition. *Immunogenetics* 2018; **70**: 159.
49. Glanville J, Huang H, Nau A, *et al*. Identifying specificity groups in the T cell receptor repertoire. *Nature* 2017; **547**: 94.
50. Shugay M, Bagaev DV, Zvyagin IV, *et al*. VDJdb: a curated database of T-cell receptor sequences with known antigen specificity. *Nucleic Acids Res* 2018; **46**: D419.
51. Tickotsky N, Sagiv T, Prilusky J, Shifrut E, Friedman N. McPAS-TCR: a manually curated catalogue of pathology-associated T cell receptor sequences. *Bioinformatics* 2017; **33**: 2924.
52. Murugan A, Mora T, Walczak AM, Callan CG Jr. Statistical inference of the generation probability of T-cell receptors from sequence repertoires. *Proc Natl Acad Sci USA* 2012; **109**: 16161.
53. Briney B, Inderbitzin A, Joyce C, Burton DR. Commonality despite exceptional diversity in the baseline human antibody repertoire. *Nature* 2019; **566**: 393.
54. Chaara W, Gonzalez-Tort A, Florez LM, Klatzmann D, Mariotti-Ferrandiz E, Six A. RepSeq data representativeness and robustness assessment by shannon entropy. *Front Immunol* 2018; **9**: 1038.
55. Kaplinsky J, Arnaout R. Robust estimates of overall immune-repertoire diversity from high-throughput measurements on samples. *Nat Commun* 2016; **7**: 11881.
56. Laydon DJ, Melamed A, Sim A, *et al*. Quantification of HTLV-1 clonality and TCR diversity. *PLoS Comput Biol* 2014; **10**: e1003646.
57. Greiff V, Weber CR, Palme J, *et al*. Learning the high-dimensional immunogenomic features that predict public and private antibody repertoires. *J Immunol* 2017; **199**: 2985.
58. Lee SM, Chao A. Estimating population size via sample coverage for closed capture-recapture models. *Biometrics* 1994; **50**: 88.
59. Shifrut E, Baruch K, Gal H, *et al*. CD4(+) T cell-receptor repertoire diversity is compromised in the spleen but not in the bone marrow of aged mice due to private and sporadic clonal expansions. *Front Immunol* 2013; **4**: 379.
60. Rosenfeld AM, Meng W, Chen DY, *et al*. Computational evaluation of B-cell clone sizes in bulk populations. *Front Immunol* 2018; **9**: 1472.
61. Thapa DR, Tonikian R, Sun C, *et al*. Longitudinal analysis of peripheral blood T cell receptor diversity in patients with systemic lupus erythematosus by next-generation sequencing. *Arthritis Res Ther* 2015; **17**: 132.
62. Allez M, Auzolle C, Ngollo M, *et al*. T cell clonal expansions in ileal Crohn's disease are associated with smoking behaviour and postoperative recurrence. *Gut* 2019; 1.
63. Matos TR, O'Malley JT, Lowry EL, *et al*. Clinically resolved psoriatic lesions contain psoriasis-specific IL-17-producing alphabeta T cell clones. *J Clin Invest* 2017; **127**: 4031.
64. Amaria RN, Reddy SM, Tawbi HA, *et al*. Publisher correction: neoadjuvant immune checkpoint blockade in high-risk resectable melanoma. *Nat Med* 2018; **24**: 1942.
65. Forde PM, Chaft JE, Smith KN, *et al*. Neoadjuvant PD-1 blockade in resectable lung cancer. *N Engl J Med* 2018; **378**: 1976.
66. Roh W, Chen PL, Reuben A, *et al*. Integrated molecular analysis of tumor biopsies on sequential CTLA-4 and PD-1 blockade reveals markers of response and resistance. *Sci Transl Med* 2017; **9**: eaah3560.
67. Tumeh PC, Harview CL, Yearley JH, *et al*. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014; **515**: 568.
68. Oh DY, Cham J, Zhang L, *et al*. Immune toxicities elicted by CTLA-4 blockade in cancer patients are associated with early diversification of the T-cell repertoire. *Cancer Res* 2017; **77**: 1322.
69. Subudhi SK, Aparicio A, Gao J, *et al*. Clonal expansion of CD8 T cells in the systemic circulation precedes development of ipilimumab-induced toxicities. *Proc Natl Acad Sci USA* 2016; **113**: 11919.
70. Robert L, Tsoi J, Wang X, *et al*. CTLA4 blockade broadens the peripheral T-cell receptor repertoire. *Clin Cancer Res* 2014; **20**: 2424.
71. DeWitt WS, Emerson RO, Lindau P, *et al*. Dynamics of the cytotoxic T cell response to a model of acute viral infection. *J Virol* 2015; **89**: 4517.
72. Pogorelyy MV, Minervina AA, Touzel MP, *et al*. Precise tracking of vaccine-responding T cell clones reveals convergent and personalized response in identical twins. *Proc Natl Acad Sci USA* 2018; **115**: 12704.
73. Akondy RS, Monson ND, Miller JD, *et al*. The yellow fever virus vaccine induces a broad and polyfunctional human memory CD8 + T cell response. *J Immunol* 2009; **183**: 7919.
74. Miller JD, van der Most RG, Akondy RS, *et al*. Human effector and memory CD8 + T cell responses to smallpox and yellow fever vaccines. *Immunity* 2008; **28**: 710.
75. Qi Q, Cavanagh MM, Le Saux S, *et al*. Diversification of the antigen-specific T cell receptor repertoire after varicella zoster vaccination. *Sci Transl Med* 2016; **8**: 332ra46.
76. Galson JD, Truck J, Fowler A, *et al*. In-depth assessment of within-individual and inter-individual variation in the B cell receptor repertoire. *Front Immunol* 2015; **6**: 531.
77. Chapuis AG, Desmarais C, Emerson R, *et al*. Tracking the fate and origin of clinically relevant adoptively transferred CD8(+) T cells in vivo. *Sci Immunol* 2018; **2**: eaal2568.
78. de Jong A, Jabbari A, Dai Z, *et al*. High-throughput T cell receptor sequencing identifies clonally expanded CD8 + T cell populations in alopecia areata. *JCI Insight* 2018; **3**: e121949.
79. Gros A, Parkhurst MR, Tran E, *et al*. Prospective identification of neoantigen-specific lymphocytes in the peripheral blood of melanoma patients. *Nat Med* 2016; **22**: 433.
80. Ahmadzadeh M, Pasetto A, Jia L, *et al*. Tumor-infiltrating human CD4(+) regulatory T cells display a distinct TCR repertoire and exhibit tumor and neoantigen reactivity. *Sci Immunol* 2019; **4**: eaao4310.

© 2019 Steunstichting ESOT

81. Wong YNS, Joshi K, Khetrapal P, *et al*. Urine-derived lymphocytes as a non-invasive measure of the bladder tumor immune microenvironment. *J Exp Med* 2018; **215**: 2748.
82. Thome JJ, Grinshpun B, Kumar BV, *et al*. Longterm maintenance of human naive T cells through in situ homeostasis in lymphoid tissue sites. *Sci Immunol* 2016; **1**: eaah6506.
83. Meng W, Zhang B, Schwartz GW, *et al*. An atlas of B-cell clonal distribution in the human body. *Nat Biotechnol* 2016; **35**: 879.
84. Venturi V, Quigley MF, Greenaway HY, *et al*. A mechanism for TCR sharing between T cell subsets and individuals revealed by pyrosequencing. *J Immunol* 2011; **186**: 4285.
85. Marcou Q, Mora T, Walczak AM. High-throughput immune repertoire analysis with IGoR. *Nat Commun* 2018; **9**: 561.
86. Sethna Z, Elhanati Y, Callan CG Jr, Walczak AM, Mora T. OLGA: fast computation of generation probabilities of B- and T-cell receptor amino acid sequences and motifs. *Bioinformatics* 2019; btz035.
87. Elhanati Y, Sethna Z, Callan CG Jr, Mora T, Walczak AM. Predicting the spectrum of TCR repertoire sharing with a data-driven model of recombination. *Immunol Rev* 2018; **284**: 167.
88. Emerson RO, DeWitt WS, Vignali M, *et al*. Immunosequencing identifies signatures of cytomegalovirus exposure history and HLA-mediated effects on the T cell repertoire. *Nat Genet* 2017; **49**: 659.
89. Faham M, Carlton V, Moorhead M, *et al*. Discovery of T cell receptor beta motifs specific to HLA-B27-positive ankylosing spondylitis by deep repertoire sequence analysis. *Arthritis Rheumatol* 2017; **69**: 774.
90. Komech EA, Pogorelyy MV, Egorov ES, *et al*. CD8 + T cells with characteristic T cell receptor beta motif are detected in blood and expanded in synovial fluid of ankylosing spondylitis patients. *Rheumatology (Oxford)* 2018; **57**: 1097.
91. Pogorelyy MV, Minervina AA, Chudakov DM, *et al*. Method for identification of condition-associated public antigen receptor sequences. *Elife* 2018; **7**: e33050.
92. Latorre D, Kallweit U, Armentani E, *et al*. T cells in patients with narcolepsy target self-antigens of hypocretin neurons. *Nature* 2018; **562**: 63.
93. Parameswaran P, Liu Y, Roskin KM, *et al*. Convergent antibody signatures in human dengue. *Cell Host Microbe* 2013; **13**: 691.
94. Setliff I, McDonnell WJ, Raju N, *et al*. Multi-donor longitudinal antibody repertoire sequencing reveals the existence of public antibody clonotypes in HIV-1 infection. *Cell Host Microbe* 2018; **23**: 845 e6.
95. Jackson KJ, Liu Y, Roskin KM, *et al*. Human responses to influenza vaccination show seroconversion signatures and convergent antibody rearrangements. *Cell Host Microbe* 2014; **16**: 105.
96. Pieper K, Tan J, Piccoli L, *et al*. Public antibodies to malaria antigens generated by two LAIR1 insertion modalities. *Nature* 2017; **548**: 597.
97. DeWitt WS 3rd, Smith A, Schoch G, Hansen JA, Matsen F, Bradley P. Human T cell receptor occurrence patterns encode immune history, genetic background, and receptor specificity. *Elife* 2018; **7**: e38358.
98. Eugster A, Lindner A, Catani M, *et al*. High diversity in the TCR repertoire of GAD65 autoantigen-specific human CD4 + T cells. *J Immunol* 2015; **194**: 2531.
99. Bovay A, Zoete V, Dolton G, *et al*. T cell receptor alpha variable 12-2 bias in the immunodominant response to Yellow fever virus. *Eur J Immunol* 2018; **48**: 258.
100. Miles JJ, Thammanichanond D, Moneer S, *et al*. Antigen-driven patterns of TCR bias are shared across diverse outcomes of human hepatitis C virus infection. *J Immunol* 2011; **186**: 901.
101. Turner SJ, Doherty PC, McCluskey J, Rossjohn J. Structural determinants of T-cell receptor bias in immunity. *Nat Rev Immunol* 2006; **6**: 883.
102. Blum LK, Adamska JZ, Martin DS, *et al*. Robust B cell responses predict rapid resolution of lyme disease. *Front Immunol* 2018; **9**: 1634.
103. Tucci FA, Kitanovski S, Johansson P, *et al*. Biased IGH VDJ gene repertoire and clonal expansions in B cells of chronically hepatitis C virus-infected individuals. *Blood* 2018; **131**: 546.
104. Chen G, Yang X, Ko A, *et al*. Sequence and structural analyses reveal distinct and highly diverse human CD8(+) TCR repertoires to immunodominant viral antigens. *Cell Rep* 2017; **19**: 569.
105. Tan J, Pieper K, Piccoli L, *et al*. A LAIR1 insertion generates broadly reactive antibodies against malaria variant antigens. *Nature* 2016; **529**: 105.
106. Yang G, Ou M, Chen H, *et al*. Characteristic analysis of TCR beta-chain CDR3 repertoire for pre- and post-liver transplantation. *Oncotarget* 2018; **9**: 34506.
107. Alachkar H, Mutonga M, Kato T, *et al*. Quantitative characterization of T-cell repertoire and biomarkers in kidney transplant rejection. *BMC Nephrol* 2016; **17**: 181.
108. Lai L, Wang L, Chen H, *et al*. T cell repertoire following kidney transplantation revealed by high-throughput sequencing. *Transpl Immunol* 2016; **39**: 34.
109. Morris H, DeWolf S, Robins H, *et al*. Tracking donor-reactive T cells: evidence for clonal deletion in tolerant kidney transplant patients. *Sci Transl Med* 2015; **7**: 272ra10.
110. Savage TM, Shonts BA, Obradovic A, *et al*. Early expansion of donor-specific Tregs in tolerant kidney transplant recipients. *JCI Insight* 2018; **3**: e124086.
111. Emerson RO, Mathew JM, Konieczna IM, Robins HS, Leventhal JR. Defining the alloreactive T cell repertoire using high-throughput sequencing of mixed lymphocyte reaction culture. *PLoS One* 2014; **9**: e111943.
112. Gao B, Gu Y, Rong C, *et al*. Dynamics of B cell recovery in kidney/bone marrow transplant recipients. *Transplantation* 2017; **101**: 2722.
113. Chabannon C, Kuball J, Bondanza A, *et al*. Hematopoietic stem cell transplantation in its 60s: a platform for cellular therapies. *Sci Transl Med* 2018; **10**: eaap9630.
114. Kang E, Gennery A. Hematopoietic stem cell transplantation for primary immunodeficiencies. *Hematol Oncol Clin North Am* 2014; **28**: 1157.
115. Singh AK, McGuirk JP. Allogeneic stem cell transplantation: a historical and scientific overview. *Cancer Res* 2016; **76**: 6445.
116. Swart JF, Delemarre EM, van Wijk F, *et al*. Haematopoietic stem cell transplantation for autoimmune diseases. *Nat Rev Rheumatol* 2017; **13**: 244.
117. Muraro PA, Robins H, Malhotra S, *et al*. T cell repertoire following autologous stem cell transplantation for multiple sclerosis. *J Clin Invest* 2014; **124**: 1168.
118. Delemarre EM, van den Broek T, Mijnheer G, *et al*. Autologous stem cell transplantation aids autoimmune patients by functional renewal and TCR diversification of regulatory T cells. *Blood* 2016; **127**: 91.

© 2019 Steunstichting ESOT

119. Saad A, Lamb LS. Ex vivo T-cell depletion in allogeneic hematopoietic stem cell transplant: past, present and future. *Bone Marrow Transplant* 2017; **52**: 1241.
120. van Heijst JW, Ceberio I, Lipuma LB, *et al*. Quantitative assessment of T cell repertoire recovery after hematopoietic stem cell transplantation. *Nat Med* 2013; **19**: 372.
121. Zvyagin IV, Mamedov IZ, Tatarinova OV, *et al*. Tracking T-cell immune reconstitution after TCRalphabeta/CD19-depleted hematopoietic cells transplantation in children. *Leukemia* 2017; **31**: 1145.
122. Cieri N, Oliveira G, Greco R, *et al*. Generation of human memory stem T cells after haploidentical T-replete hematopoietic stem cell transplantation. *Blood* 2015; **125**: 2865.
123. Kanakry CG, Coffey DG, Towlerton AM, *et al*. Origin and evolution of the T cell repertoire after posttransplantation cyclophosphamide. *JCI Insight* 2016; **1**: e86252.
124. Link CS, Eugster A, Heidenreich F, *et al*. Abundant cytomegalovirus (CMV) reactive clonotypes in the CD8 (+) T cell receptor alpha repertoire following allogeneic transplantation. *Clin Exp Immunol* 2016; **184**: 389.
125. Suessmuth Y, Mukherjee R, Watkins B, *et al*. CMV reactivation drives posttransplant T-cell reconstitution and results in defects in the underlying TCRbeta repertoire. *Blood* 2015; **125**: 3835.
126. Link-Rachner CS, Eugster A, Rucker-Braun E, *et al*. T-cell receptor-alpha repertoire of CD8 + T cells following allogeneic stem cell transplantation using next-generation sequencing. *Haematologica* 2019; **104**: 622.
127. Gkazi AS, Margetts BK, Attenborough T, *et al*. Clinical T cell receptor repertoire deep sequencing and analysis: an application to monitor immune reconstitution following cord blood transplantation. *Front Immunol* 2018; **9**: 2547.
128. Ritter J, Seitz V, Balzer H, *et al*. Donor CD4 T cell diversity determines virus reactivation in patients after HLA-matched allogeneic stem cell transplantation. *Am J Transplant* 2015; **15**: 2170.
129. Yew PY, Alachkar H, Yamaguchi R, *et al*. Quantitative characterization of T-cell repertoire in allogeneic hematopoietic stem cell transplant recipients. *Bone Marrow Transplant* 2015; **50**: 1227.
130. Zhang SQ, Ma KY, Schonnesen AA, *et al*. High-throughput determination of the antigen specificities of T cell receptors in single cells. *Nat Biotechnol* 2018; **36**: 1156.

© 2019 Steunstichting ESOT