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High-Throughput immunogenetics for precision medicine in cancer

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ABSTRACT

Cancer is characterized by an extremely complex biological background, which hinders personalized therapeutic interventions. Precision medicine promises to overcome this obstacle through integrating information from different 'subsystems', including the host, the external environment, the tumor itself and the tumor microenvironment. Immunogenetics is an essential tool that allows dissecting both lymphoid cancer ontogeny at both a cell-intrinsic and a cell-extrinsic level, i.e. through characterizing micro-environmental interactions, with a view to precision medicine. This is particularly thanks to the introduction of powerful, high-throughput approaches i.e. next generation sequencing, which allow the comprehensive characterization of immune repertoires. Indeed, NGS immunogenetic analysis (Immune-seq) has emerged as key to both understanding cancer pathogenesis and improving the accuracy of clinical decision making in oncology. Immune-seq has applications in lymphoid malignancies, assisting in the diagnosis e.g. through differentiating from reactive conditions, as well as in disease monitoring through accurate assessment of minimal residual disease. Moreover, Immune-seq facilitates the study of T cell receptor clonal dynamics in critical clinical contexts, including transplantation as well as innovative immunotherapy for solid cancers. The clinical utility of Immune-seq represents the focus of the present contribution, where we highlight what can be achieved but also what must be addressed in order to maximally realize the promise of Immune-seq in precision medicine in cancer.

1. Introduction

The remarkable clinical heterogeneity observed in cancer, both within a given entity but also between different entities, stems from the underlying biological heterogeneity which impacts on the disease course and the response to treatment. [1,2] *A propos* the latter, the "one-size--fits-all" approach in cancer treatment, whereby patients with the same type of cancer are treated uniformly, leads to markedly diverse outcomes, even when the malignant clones share a particular molecular profile [3]. This is hardly surprising, given that the natural history of cancer is shaped not only by cell-intrinsic aberrations but also by their complex interplay with lifestyle and host-related factors, including the tumor microenvironment (TME), hence, by inference, with the host immune system [4,5]. Understanding the bidirectional interactions between the malignant cells and the host immune system is therefore of essence in realizing precision medicine in cancer.

Studies from the early 1990′s have demonstrated that tumor regression can be achieved through stimulation of immune responses [6]. This evidence has spurred, amongst others, the interest in cancer immunology, a fast-evolving field that focuses on the interactions between the malignant cells and the diverse cell populations of the immune system. Immunogenetics and, more recently, immunogenomics are pillars of cancer immunology since they offer the possibility to dissect the repertoires of adaptive immune responses at the molecular level. Such studies revolve around the characterization of antigen receptors of B and T cells, namely, the B cell receptor immunoglobulin

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(BcR IG) and the T cell receptor (TR), respectively. The underlying rationale is that relevant information may uncover unique signatures linked with cancer ontogeny, diagnosis and response to treatment [7–10]. This translates into novel 'tools' for refined understanding of disease pathophysiology but also more accurate diagnosis, prognosis and prediction, and even therapeutic decision making.

In recent years, the introduction of next-generation sequencing (NGS)-based methodologies in the field of immunogenomics (defined here as Immune-seq) enabled a far more comprehensive study of BcR IG/TR gene repertoires than ever before, especially as it concerns clonal diversity and intraclonal dynamics [11–13]. Immune-seq has found two main applications in malignancies of lymphoid cells: (i) clonality assessment for the differential diagnosis from reactive lymphoproliferations that are typically polyclonal, in contrast to the former that usually display a 'classic' monoclonal profile; and (ii) monitoring of response to treatment through the sensitive detection of minimal residual disease (MRD) [14]. Moreover, Immune-seq is increasingly being utilized in the study of other cancers, both hematopoietic and solid, where the aim is to characterize lymphoid clones with potential anti-tumor activity in order to further guide therapeutic immunomodulatory interventions [15]. Indicatively, Immune-seq constitutes the basis for the identification of immunogenic tumor-specific antigens required for novel T-cell based immunotherapy designs that are currently being applied in cancer patients [11].

2. *In vitro* and *in silico* considerations for Immune-seq

Cells of the adaptive immune system recognize antigens through a remarkably diverse repertoire consolidated by the concerted action of mainly two mechanisms operating during their differentiation: (i) DNA recombination of IG/TR variable (V), diversity (D) and joining (J) genes; and (ii) further antigen-dependent maturation, which is critically dependent on selection processes [16–18]. Immune-seq aspires to capturing in molecular detail the specifics of both mechanisms, which inevitably poses several challenges.

One of the major challenges concerns the unbiased and comprehensive characterization of the clonal diversity within a sample. The most widely used approaches for the detection of clonally rearranged BcR IG/TR genes are based on the use of (i) multiplex PCR assays with multiple primers, or (ii) rapid amplification of 5′ complementary DNA ends (5′RACE) on RNA samples based on the template-switch effect [19,20]. Both approaches require additional steps of PCR amplification for library preparation, thus raising concerns about the potential introduction of biases due to putative variations in the amplification efficiency of the primers, amplification stochasticity, template-switching and polymerase errors. Alternatively, one could use a hybridization capture or pull-down methodology for the enrichment of BcR IG/TR loci DNA using small probes, potentially leading to the reduction of sequencing biases of all target regions [21,22]. In principle, this approach would allow studying in parallel (in a single assay) chromosomal translocations involving the BcR IG/TR gene loci, which are quite common in hematological malignancies [23].

Letting the *in vitro* ('wet') issues aside, another seminal challenge concerns the *in silico* interpretation of the findings. Indicatively, when it comes to assessing the clonality status of a given sample, the unprecedented depth of resolution provided by Immune-seq enables the detection of clonotypes (i.e. unique BcR IG/TR gene rearrangements) at levels that can be low yet above the polyclonal 'background'. Are these minor clonotypes relevant? Answering this question will require a refinement of current definitions for what constitutes clonality and whether/how it relates with malignancy, that will likely be 'context-dependent' i.e. vary depending on the biological or the clinical setting.

Adding to this complex landscape and considering that the BcR IG and TR are heterodimer receptors of heavy/light chains and alpha/beta or gamma/delta chains, respectively, it should be carefully decided when the analysis of both chains is required, as paired analysis currently requires special approaches and is quite costly, rendering it accessible to specialized research labs only [24,25]. Nevertheless, single-cell sequencing approaches are rapidly evolving, offering an eventual viable alternative to the classic laborious protocols: admittedly, however, they will also remain a reserve for the few, at least in the short/mid-term [26].

Obviously, much effort is needed to refine the design of the experimental procedures and transfer them in the clinical routine. Collaborative actions are focusing on the design, standardization and validation of protocols targeting BcR IG/TR genes for both research and diagnostic purposes. Indicatively, this is our objective within the EuroClonality-NGS Working Group (https://euroclonalityngs.org/usr/pub/pub.php), a European network of laboratories experienced in Immune-seq, supplemented by laboratories with expertise in diagnosis and disease monitoring (including clonality assessment and MRD studies) and immunoinformatics and bioinformatics.

Another major challenge in Immune-seq arises from the enormous computational load of analyzing hundreds of thousands or even millions of BcR IG/TR gene rearrangement sequences in order to accurately describe the clonal architecture, complexity, diversity as well as, in the case of BcR IG, the somatic hypermutation (SHM) status. The scientific community has risen to this challenge through developing purpose-built softwares and pipelines that can be either customized for specific clinical and biological contexts or widely flexible in order to facilitate a wider application. Furthermore, the complex nature of Immune-seq has led to differentiating the analytical procedure into two parts: (i) 'basic' bioinformatics analysis, which includes the processing and filtering of the raw sequencing data, sequence annotation or V(D)J gene assignment and clonotype computation; and, (ii) 'advanced' or metadata analysis, which extends from the assessment of the clonal architecture to the identification of specific BcR IG/TR gene combinations, the detailed description of complementarity determining region 3 (CDR3) length and physicochemical properties, the mapping of clonal dynamics overtime and, most importantly, the comparative analyses between different samples/individuals/diseases.

Other important analytical challenges concern the validation of the NGS data quality and, for IG gene analysis, the distinction between true SHM and sequencing/PCR-induced artifacts; the development of intuitive visualizations; as well as the design of a user-friendly working environment, especially in a clinical context. Ideally, Immune-seq analytical pipelines should also be enriched with basic as well as advanced statistical tools in order to provide end-to-end solutions. Finally, time and data management should be performed according to current needs. Given all the aforementioned aspects of Immune-seq, the standardization of the entire NGS workflow is critical: concepts and standards are needed more than ever for NGS IG/TR data, including consistent definition and annotation rules in order to ensure accuracy, consistency and coherence of immunogenetics data in research and clinical applications. Currently, several Immune-seq analytical pipelines are publicly available that perform both basic as well as metadata analysis: notable examples concern IMGT/HighV-quest [27], MiXCR [28], the Vidjil platform [29], ARRestST|Interrogate [30], and TRIP [31], amongst others.

3. Cell-intrinsic markers of lymphoid cancers

3.1. Establishing the clonality of B and T cell lymphoproliferations

Lymphoma diagnostics is grounded on the integration of clinical, cytological, histopathological, immunophenotypic and molecular data [32]. This approach allows establishing a diagnosis in the great majority of cases. However, a sizeable minority, ~10−15%, of cases pose a diagnostic challenge necessitating additional analyses for reaching definitive conclusions, including clonality assessment [33,34].

Since B and T cell malignancies arise from the clonal expansion of a single cell, BcR IG and TR genes constitute a unique marker shared by all

the malignant cells within an individual patient. Hence, BcR IG/TR gene rearrangements can be used as clonal markers ('molecular fingerprint') for the individual tumor. In fact, BcR IG/TR clonality assessment has proven a valuable aid in the differential diagnosis of malignant (i.e. clonal) from non-malignant/reactive (i.e. poly/oligo-clonal) lymphoproliferations. This is particularly relevant when the latter share overlapping features with the former; examples abound for both B and T cell entities e.g. persistent polyclonal B lymphocytosis versus splenic small B cell lymphoma [35], marginal zone lymphoma versus marginal zone hyperplasia [36,37], T-large granular (T-LGL) leukemia versus reactive T-LGL lymphoproliferations [38,39], to name but a few. Traditional methodologies, such as heteroduplex GeneScan analysis/spectratyping, have been widely used to that purpose; however, their analytical capacity is limited [33,40]. Moreover, false negative results due to extensive SHM (in the case of B cell entities) preventing primer annealing or suboptimal DNA samples only complicate the analysis. Finally, oligoclonality at the initial diagnosis admixed normal/reactive T and B cells generating strong polyclonal background and clonal evolution of IG/TR gene rearrangements between diagnosis and relapse might be other confounding factors [41–43].

The advent of NGS-based methodologies has brought an entirely new dimension to clonality assessment. In contrast to previous technologies, such as GeneScan analysis/spectratyping, NGS-based clonality assessment is performed through BcR IG/TR clonotype computation, which in turn, takes into account the composition of each individual sequence. Thus, clonality assessment using NGS-based IG/TR gene rearrangement sequences could be largely used for the in-depth qualitative and quantitative characterization of B and T cell populations, in both health and disease [44,45]. In addition, this approach may provide important information in regard to: (i) the clonal relationship of BcR IG/TR gene rearrangements from different anatomical locations or over time [46]; and, (ii) the assessment of intraclonal diversification in mature B cell malignancies due to ongoing SHM [47,48]. Of note, Immune-seq is also quite efficient for the assessment of clonality in samples with suboptimal DNA quality, such as formalin-fixed, paraffin-embedded tissues.

However, despite the significant advantages of NGS-based IG/TR clonality assessment over traditional approaches, several issues remain unresolved. In particular, Immune-seq analysis still mostly relies on an initial multiplex PCR step, which is hampered by the same limitations as all other PCR-based IG/TR studies, i.e. polymorphisms or SHM that could prevent primer annealing. Additionally, the diverse amplification efficiency leading to unequal amplification of different rearrangements in multiplex PCR potentially prevents precise quantification. Thus, NGS-based clonality assessment requires multiple complementary BcR IG/TR targets to ensure a high detection rate, accomplished by multiplexing of targets and samples for the sequencing step following initial separate PCRs [45]. An alternative approach utilizes a hybridization capture or pull-down methodology mentioned previously [22,49]. Finally, perhaps most importantly, the depth of resolution afforded by NGS can lead to diagnostic conundrums, seriously challenging the (simplistic) distinction between monoclonal/malignant versus poly-oligoclonal/reactive [14]. For instance, persistent clonal expansions can be detected in refractory celiac disease for many years without any evidence of enteropathy-associated T cell lymphoma; [50] on the other hand, cases of symptomatic T-LGL leukemia can display an oligoclonal profile [51]. Altogether, it becomes increasingly evident that a reappraisal is warranted regarding the current definitions as to what constitutes clonality in a clinically meaningful context and what is the cut-off, if any, to accurately distinguish reactive from malignant lymphoproliferations. The major applications of Immune-seq in clonality assessment are depicted graphically in Fig. 1.

3.2. MRD detection in lymphoid malignancies

Recent studies have provided conclusive evidence that NGS-based MRD assessment using IG/TR gene rearrangements as the molecular target can be applied to malignancies of lymphoid cells, both immature (i.e. acute lymphoblastic leukemia) and mature (i.e. lymphomas and multiple myeloma) (Fig. 2), and is potentially even more sensitive than established methodologies (multi-color flow cytometry, allele-specific RQ-PCR) [52–55]. Against that, however, robust and accurate identification of the correct index clone is not trivial, most often due to the caveats mentioned above (particularly, misannealing of the primers or differential primer annealing efficiency) but also due to low cell infiltration in the analyzed sample [56,57]. Another critical issue that requires refinement concerns the quantification of the residual clonal cells and is especially critical in patients with B cell lymphomas receiving anti-B cell treatment (e.g. anti-CD20 antibodies) where the normal, polyclonal B cell compartment is completely depleted. In such cases, Immune-seq may lead to a considerable overestimation of MRD, requiring the use of appropriate internal controls. (14)

Unsurprisingly, Immune-seq has already been applied extensively in ALL. The sensitivity of the methodology can reach 10^{-6}, which corresponds to a single malignant cell in a million normal B cells [54,56,58].

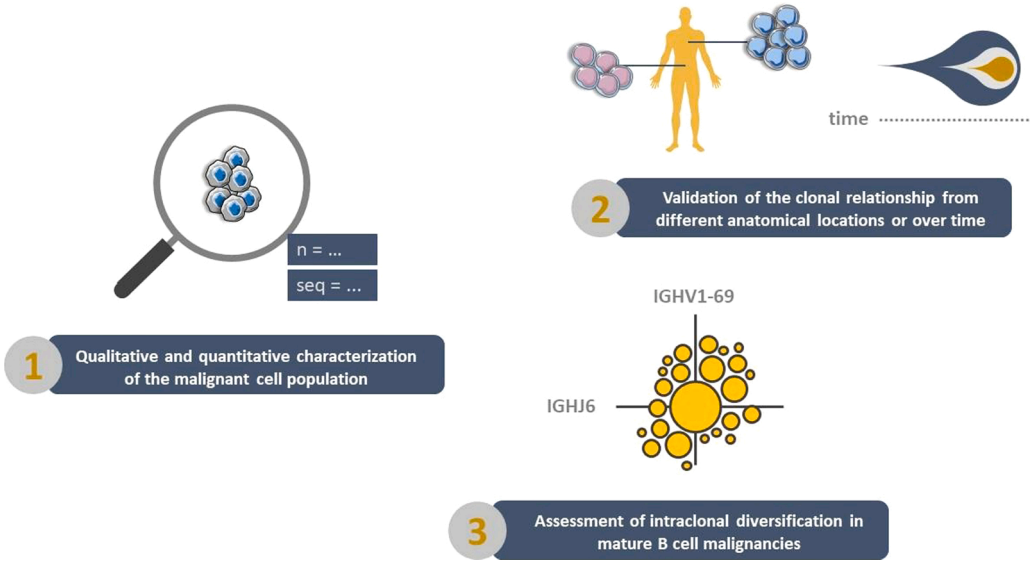

Fig. 1. Applications of Immune-seq for clonality assessment and disease monitoring after therapy (i.e. detection of minimal residual disease, MRD) in patients with lymphoid malignancies.

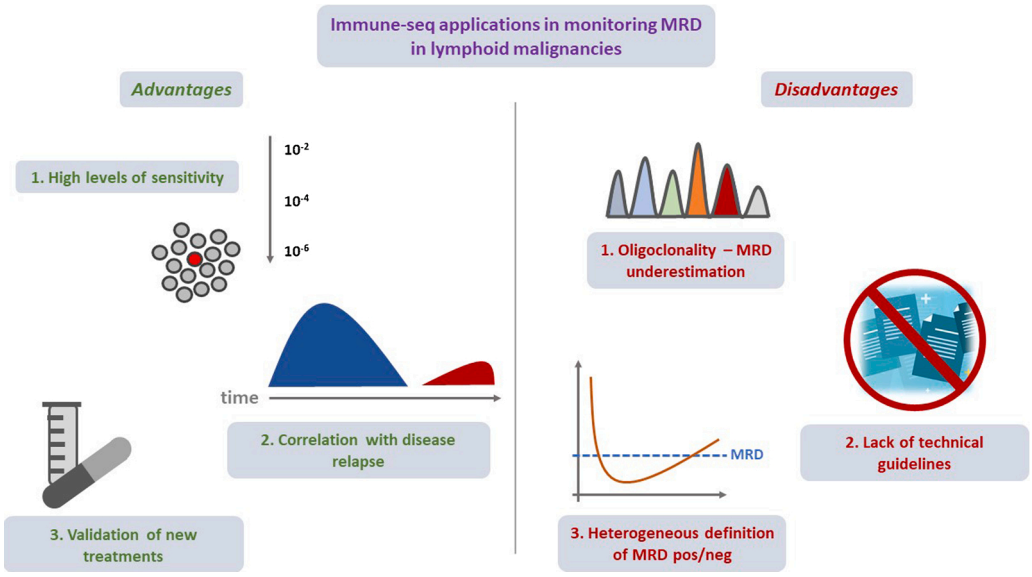

Fig. 2. Advantages and disadvantages of Immune-seq applications in monitoring MRD in lymphoid malignancies.

The strong correlation between MRD levels and risk of relapse and the prognostic significance of MRD assessment during and after therapy has already been acknowledged in both pediatric and adult ALL [59]. The major challenge for MRD assessment in ALL stems from ongoing IG/TR gene rearrangements leading to the frequent occurrence of oligoclonality (e.g. IGH D–J to IGH V-D–J changes or IGHV substitutions of complete IGH V-D–J rearrangements) [60]. Using an Immune-seq approach, oligoclonality was identified in the vast majority of childhood B cell precursor ALL cases by comparing the IGH D–J stem of the index sequence with the IGH D–J stem of different complete IGH gene rearrangements [46]. Of interest, about 10 % of cases showed >1000 related sequences that could greatly affect the outcome of MRD assessment. Whether such potentially related sequences should also be tracked in follow-up samples has not yet been prospectively analyzed.

Immune-seq has been applied for MRD detection also in chronic lymphocytic leukemia (CLL) with quite promising results [53,61–66]. Indeed, accumulating evidence supports its potential clinical value in view of highly effective approved treatments with the BCL2 inhibitor venetoclax [67–70] as well as combinations of venetoclax with Bruton's tyrosine kinase inhibitors (ibrutinib, acalabrutinib) +/- anti-CD20 antibodies, currently in clinical trials [71]. That notwithstanding, challenges remain, particularly for CLL, but also in other mature B cell lymphomas, where ongoing SHM may underlie intraclonal diversity, potentially leading to a decrease of the amplification efficacy of the index BcR IG rearrangement and, thereby, to a low or even false-negative MRD result [72]. This can be compensated, at least in part, by relying on additional index sequences from other BcR IG targets (e.g. IG light chain gene rearrangements). Turning to multiple myeloma (MM), recent studies have demonstrated that Immune-seq can be used for the evaluation of MRD status as a prognostic marker in MM [57,73,74], that could be used for the validation of new treatment approaches, leading to the refinement of the criteria for MRD assessment by the International Myeloma Working Group [75]. These developments indicate that Immune-seq will be a valid alternative to multiparametric flow cytometry, the current 'gold standard' for MRD detection in MM [75,76,77]. Furthermore, they are both timely and pertinent given the recent approval of MRD as an intermediate endpoint for drug licensing in randomized studies by regulatory authorities in Europe.

It is worth mentioning that technical guidelines are currently lacking for Immune-seq based MRD computation, while the definition of MRD positivity/negativity is very heterogeneous in the literature, hindering comparisons between studies. Considering the potential higher sensitivity of Immune-seq, minimal technical requirements have therefore to be defined, including the theoretical sensitivity for a single sample analyzed for MRD but also the clinical (i.e. prognostic) relevance of low MRD levels [60].

4. Reactive T cell populations as cell-extrinsic markers in cancer

4.1. T cell receptor gene repertoire analysis for monitoring immune reconstitution after transplantation

Clonotype dynamics and detailed profiling of the repertoire is required when monitoring the re-establishment of immune repertoires after ablation, particularly in the context of allogeneic hematopoietic stem cell transplantation (allo-HSCT) and, most importantly, during surveillance for graft-versus-host disease (GvHD). Widely-used approaches for this type of analysis are based on TR spectratyping, however these are inherently hampered by limited resolution [78,79]. Immune-seq holds great potential for overcoming this limitation, enabling the systematic, longitudinal and comprehensive characterization and tracking of clones even at very low frequencies, allowing for timely clinical interventions. This is especially relevant considering that early recovery of repertoire diversity has emerged as a benchmark for decreased risk for GvHD and relapse [80,81]. Moreover, in patients undergoing allo-HSCT for hematological malignancies, detailed characterization of the TR repertoire could serve both for monitoring graft clones with possible anti-leukemia effects as well as cytotoxic clones that eventually trigger GvHD [82,83].

One of the major complications of solid organ transplantation is graft rejection, which is mediated by alloreactive T cells triggered by HLA incompatibility [84]. The development of *in vitro* assays for accurately assessing a specific T cell responses against an organ allograft would be a valuable clinical tool for this context, however still represents a not fully accomplished goal [85]. Most of the currently used methods for alloreactivity testing in these cases are based on qualitative/functional assays or indirect methods of determination, like ELISPOT for interferon-γ (IFN-γ)-producing T cells, lacking high resolution and specificity [85–87]. These issues are overcome by NGS methodologies enabling the sequencing of thousands of different TRs and, thus, offering the possibility for longitudinal monitoring of alloreactive T cell clones even at low frequencies. Such clones could be utilized as markers for tailored immunosuppressive therapy, further contributing to the fine balance that should be retained between rejection and too strong

immunosuppression [88]. These applications are illustrated in Fig. 3.

4.2. T cell receptor gene repertoire analysis in cancer immunotherapy

The rapid progress in cancer immunology should translate into refined understanding of the molecular mechanisms underlying cancer development and progression, as well as the response to treatment. Adaptive immune responses within the tumor microenvironment appear to be major determinants of the clinical course in each cancer patient, as highlighted by the prominent breakthrough in cancer immunotherapies achieved in the last few years [89]. Immunotherapeutic protocols include a variety of treatment manipulations, including immune checkpoint inhibitors, adoptive cell therapies, and therapeutic cancer vaccines that aim to boost anti-tumor responses in solid tumors and hematological malignancies [90].

T cells are inherently capable to attack and eliminate cancer cells through neo-antigen recognition thanks to the highly diverse pool of the available TR specificities [91]. Immune-seq offers the opportunity to characterize in-depth the repertoire of T cells across a range of tumor types and, thus, obtain insight into both antigen selection mechanisms but also the type of the implicated antigens (albeit only indirectly) [92]. Of note, due to the complex micro-environmental influences, the TR repertoire may differ significantly between different anatomical sites: consequently, diverse TR profiles have been described when comparing tumor infiltrating lymphocytes (TILs) versus peripheral blood T cells in the same patient [93]. Although specific anti-tumor T cells have been identified in both tumor sites and in the periphery of cancer patients, and the TIL density has been associated with good prognosis in many cancer types, complete tumor eradication cannot be achieved [91]. Several defects progressively acquired during the disease course can lead to defective immune responses, including low proliferative capacity and reduced cytotoxic functions of T cells, likely in a context of exhaustion, which is characterized by overexpression of multiple inhibitory molecules [94].

Immunotherapy aims to ameliorate these defects, taking the brakes off immunosuppression, and has already demonstrated promising results in many cases. However, only a small fraction of cancer patients experience long-term benefits by immunotherapy, while resistance to therapy and low response rate are major setbacks in these approaches [20]. In this frame, monitoring of TR dynamics during immunotherapy by Immune-seq could guide immunomodulatory and T-cell based interventions [95]. Moreover, data from large-scale Immune-seq studies could serve as a valuable input for personalized designs in each individual malignancy [20].

5. Immune-seq in the context of immune-based therapies

5.1. T cell receptor immunoprofiling in patients treated with immune checkpoint inhibitors

One of the main mechanisms of immune silencing during cancer revolves around the increased expression of co-inhibitory receptors (CTLA-4, PD-1, TIM-3, TIGIT, and LAG-3) on the surface of T cells [96–98]. Under normal conditions, these receptors are strictly controlling T cell responses and autoreactivity, but in cancer their overexpression has been associated with dampening of anti-tumor responses [99]. Blockade treatment of co-inhibitory receptors, also referred as immune checkpoint inhibitor (ICI) therapy, can reverse immunosuppression with impressive results in melanoma and non-small cell lung carcinoma (NSCLC), amongst others [100–102]. However, not all patients benefit from this treatment. Arguably, the unique properties of the TME that shape immune responses play a pivotal role in determining the success of ICI therapy, hence highlighting the need for new predictive biomarkers to foresee the outcome.

TR repertoire profiling in TILs pre- and post-treatment has emerged as a reliable biomarker for immune response to ICI treatment, while also offering valuable information regarding the mechanism of action of immunotherapy [103–105]. Studies examining the relation of TR clonality and diversity with the response to treatment in melanoma demonstrated that increased TR clonality was associated with response to PD-1 blockade, while elevated pre-treatment TR diversity had a positive impact on survival with anti-CTLA-4 monotherapy [106–108]. Similar findings also emerged from Immune-seq studies in pancreatic cancer, where certain TR profiles were predictive for response to anti–CTLA-4 and anti–PD-1 treatment, also offering the rationale for sequential administration of these ICIs [109].

5.2. Adoptive cell transfer

Adoptive cell transfer (ACT) in cancer is based on the selective expansion and administration of immune cells with anti-tumor properties, in a highly personalized manner [110]. The first successful application of this treatment was implemented in melanoma patients [111]. Data from Immune-seq studies have been used in order to identify public TR beta chain motifs responsible for the recognition of the dominant melanoma epitope in Melan-A, the most commonly recognized antigen by peripheral T cells and TILs in HLA-A2+ melanoma patients, with obvious therapeutic implications [112]. Following these studies, administration of tumor-reactive T cells that target melanoma-specific antigens led to complete cancer regression [113]. ACT has also been implemented in epithelial cancers as well, either using *ex vivo* expanded host cells or genetically engineered T cells with chimeric antigen

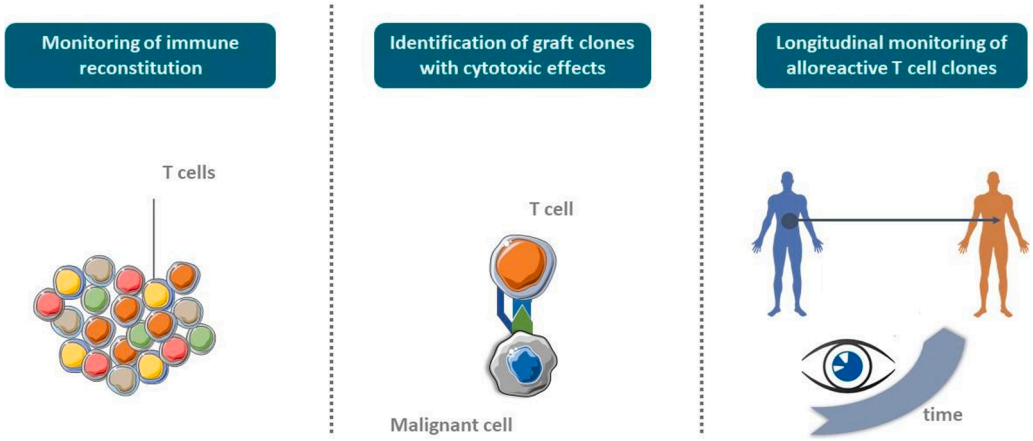

Fig. 3. T cell receptor gene repertoire analysis using Immune-seq for monitoring immune reconstitution and graft versus host disease after transplantation.

receptors (CARs) [114]. CAR-T cell therapy has been widely used also in hematological malignancies, albeit with variable efficacy, depending on the underlying neoplasia subtype [115].

Validated biomarkers are essential for predicting the response to ACT as well. In this context, extensive TR profiling, as well as functional characterization of the T cell pool with respect to candidates for ACT is essential, in order to ensure the best chances for tumor eradication while at the same time avoiding toxicities in the normal tissues. In general, improved TR diversity has been observed after ACT in patients with both leukemia and solid tumors [116].

Adaptive immune responses are also mediated by B cells, whose role in the TME is largely overlooked. Studies support that tumor infiltration by B cells associates with favorable prognosis in many cancer types, such as ovarian and breast cancer, likely due to their function as antigen-presenting cells, but also due to inducing T cell functions and producing specific-antibodies [20,117,118]. Finally, additional evidence supports that anti-melanoma B and T cell responses were possible under the presence of functional lymphoid compartments, suggesting the possibility of novel ACT protocols based on both T and B cells [119]. Taken together, Immune-seq for patients undergoing ACT could conceivably extend to include not only the TR but also the BcR IG gene repertoire.

5.3. Vaccine therapy

Cancer therapeutic vaccines represent an alternative emerging treatment in cancer that is based on the invigoration of immune responses through reshaping the immune repertoire and promoting clonal expansion of T and B cells as a response to tumor-specific antigens [120,121]. TR immunoprofiling in vaccine therapy is useful both in vaccine design and in monitoring of the efficacy of the vaccine [122]. Deep TR sequencing is required in order to identify clones that may respond to neo-antigens based on the assumption that TRs recognizing the same antigen will be sharing conserved sequences [122]. Although indirect, this immunogenetic information can assist neo-antigen discovery, eventually facilitating vaccine production [123]. As in the case of ACT, combined TR and BcR IG immunoprofiling has a definite position in this context as well, allowing to identify tumor-reactive T cells and antibodies that target specific tumor-derived epitopes rather than public antigens. Additionally, tracking these clones after vaccination and quantifying the degree of clonal expansion by Immune-seq offers the possibility for real-time monitoring of vaccine efficacy [20]. (Fig. 4)

6. Concluding remarks

Immune-seq has already proven highly relevant to the study of cancer in line with the concepts of precision medicine. This notion is exemplified by the seminal contribution of Immune-seq to both the diagnosis and monitoring of lymphoid malignancies as well as the study of both hematological and solid cancers, through the identification and characterization of lymphoid clones with putative anti-tumor capacity in the context of immunotherapy. Given the high level of complexity of the adaptive immune system, continuous refinement of NGS methodologies and *in silico* (immunoinformatics) analysis is warranted, along with robust validation of Immune-seq-based biomarkers. Only thus will the promise of immunogenomics be realized with obvious implications for implementing precision medicine in the routine clinical setting of cancer.

Author contributions

All authors contributed to the article and approved the submitted version. E.V. and A.A. wrote the manuscript. F.D., A.W.L. and A.C. edited the text and gave final approval.

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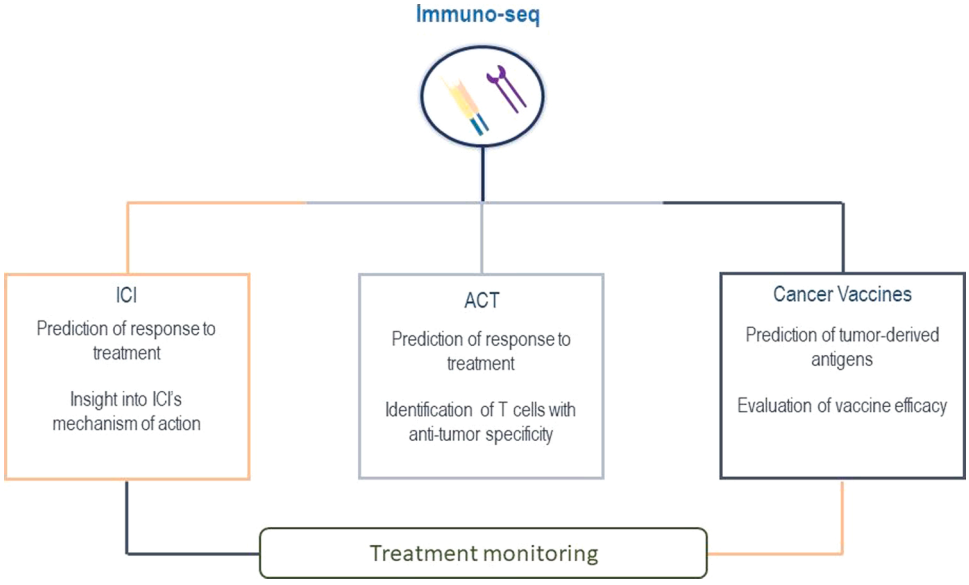

Fig. 4. Immune-seq as a tool for guiding cancer immunotherapy: High-throughput immunogenetic analysis of adaptive immune receptors has been highlighted as a novel predictive marker of response to Immune Checkpoint Inhibitors (ICI) and Adoptive Cell Transfer (ACT) treatments; Immuno-seq serves as a valuable tool for the prediction of tumor-derived antigens leading to more efficient vaccine designs.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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