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Cancer research in the era of immunogenomics

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ABSTRACT

The most meaningful advancement in cancer treatment in recent years has been the emergence of immunotherapy. Checkpoint inhibitor blockade and adoptive T cell therapy have shown remarkable clinical effects in a wide range of tumour types. Despite these advances, many tumours do not respond to these treatments, which raises the need to further investigate how patients can benefit from immunotherapy. This effort can now take advantage of the recent technological progress in single-cell, high-throughput sequencing and computational efforts. In this review, we will discuss advances in different immunotherapies and the principles of cancer immunogenomics, with an emphasis on the detection of cancer neoantigens with human leucocyte antigen peptidomics, and how these principles can be further used for more efficient clinical output.

INTRODUCTION

Immunotherapy has emerged in the recent decade as a leading therapy against cancer, with therapies such as checkpoint immune blockade now commonly used against many tumours and sometimes given as a first-line therapy.[1] The major immunotherapies commonly administrated target checkpoint molecules on tumour cells that suppress the activation of T cells[2 3] (mainly $CD8^+$ cytotoxic T cells) able to eliminate tumour cells. The checkpoint molecules most commonly targeted are programmed death-1 (PD-1)[4] and cytotoxic T-lymphocyte associated protein 4 (CTLA-4).[5] Unlike targeted therapy against oncogenes (eg, BRAF and MEK), immunotherapy has a lower response rate but a more durable benefit.[6] Immunotherapies have been shown to induce long-lasting disease stabilisation in ~30% of patients,[7 8] and when two immunotherapies are combined, they can improve immune output[9 10] and reach a responsiveness of 60% in the case of patients with cutaneous melanoma.[11]

The majority of patients, however, still do not respond to a single immunotherapy.[12–14] Moreover, as in cancer-targeted therapies, resistance against immunotherapy occurs in many cases.[15] In addition, toxicity and side effects, mainly autoimmune symptoms, might emerge.[16 17] Finally, in some patients with a specific genetic signature, immunotherapy might even worsen disease progression.[18 19] These pitfalls and obstacles are the main challenges in developing better immunotherapies and a deeper understanding of their mechanism of success or failure.

Recent years have seen many new attempts to improve current immunotherapies or to find alternative ones. Novel approaches include the testing of anti-PD-1 or CTLA-4 antibodies in combination with targeted therapy[6] or photodynamic therapy.[20] Many other immune checkpoint molecules expressed by $CD8^+$ T cells, such as TIM-3, LAG-3 and TIGIT, are now being investigated as future therapies.[2 21 22] Other T cell-related molecules, such as CD25, which is expressed on $CD4^+$ T_{regs}[23] or the costimulatory checkpoint molecule OX40,[24] have also been proposed for immunotherapy. In addition, non-T cell-mediated therapies, such as dendritic cell (DC) vaccines,[24 25] local expansion of DCs in the tumour site[26] and natural killer cell therapy,[27] are currently being researched and developed.

However, our understanding of the interactions between tumour and immune cells, and the reasons for the success or failure of a specific immunotherapy within the context of a specific cancer type, is far from complete. The emergence of immunogenomics in the recent decade[28 29] offers modern cancer research the tools to decipher these complicated mechanisms in unprecedented detail and are now advancing the field towards better future clinical benefits.

Applying genomic tools to assess immune biomarkers

Cancer immunogenomics segregates into several branches. In the basic research branch, bulk and single-cell RNA sequencing (scRNA-seq), T cell receptor (TCR) sequencing, mass cytometry and other multidimensional and/or high-throughout methods are used to characterise, phenotype and distinguish both tumour cells and their microenvironment, with a high emphasis on

immune cells, analysed by a myriad of computational tools. In the more clinically oriented branch, whole-exome sequencing, mass spectrometry and various computational approaches are directed towards identifying features of the tumour that can be manipulated therapeutically, such as through vaccination or the identification of T cell clones that can eliminate tumours in a patient-specific manner. These two branches are not dichotomous but rather intertwined and overlap each other in a complimentary manner.

scRNA-seq[29] is being used more and more frequently to inspect the transcriptome of tumours and their microenvironment.[30] Recent single-cell analyses have characterised both the tumours and participants of the immune system in glioma,[31] melanoma,[32] liver,[33] breast[34] and head and neck[35] cancers. In basic science, this technique is now widely used also to dissect alterations in and modulations of the immune response, such as T cells in melanoma mouse models.[36 37] scRNA-seq can now be complimented by high-dimensional immune profiling on the protein level, using mass cytometry (CyTOF[38]), a technique employed recently, for instance, to profile the human immune response to anti-PD1 treatment[39] and to construct immune atlases of lung adenocarcinoma[40] and clear cell renal cell carcinoma.[41] As in scRNA-seq, CyTOF is now also being applied to profile murine tumour responses in order to gain insights into the human condition.[37 42] Finally, scRNA-seq and CyTOF can be used side-by-side as a means to compare, as was recently done in a mouse model of sarcoma to reveal changes in monocyte/macrophage populations following immunotherapy.[43]

A complementary approach in cancer immunogenomics is the evaluation of the immune status of the tumour, which enables to predict, using computational methods, patient survival following and potential benefit from therapy. Data obtained from The Cancer Genome Atlas, for instance, can be used for this purpose.[44] Several transcriptomics-based algorithms have already been developed to decipher the immune cell composition within tumours.[45] Impressively, some of these algorithms, such as the Cell type Identification By Estimating Relative Subsets Of known RNA Transcripts[46], have been shown to effectively characterise the immune composition of tumours in a quantitative manner comparable to that of immunohistochemistry or flow cytometry.[47 48] Other approaches, such as combining the transcript levels of granzyme A and perforin in order to characterise cytolytic activity,[49] use scRNA-seq for immune profiling[50] or the sequencing of the TCR repertoire as a measure of T cell diversity versus clonality,[51] which can also provide insight into the immune state of a tumour.

Immunogenomics-guided discovery of biomarkers of immunotherapy efficacy

The fact that only a fraction of patients respond to immunotherapy highlights the need for better patient matching and, if possible, early detection, which drives the search for reliable and accessible biomarkers. The issue of the questionable reliability of previously used protein markers, such as PD-L1 expression,[52] in predicting response to immunotherapy[53] may now be resolved by applying more genomic approaches to establish appropriate biomarkers.

The intersection between immunogenomics, cancer genomics and immunotherapy has led to a key question in the field concerning the correlation between mutational load and response to immunotherapy. The current hypothesis in the immunotherapy field is that tumours with an increased mutational load will present more neoantigens and, thus, will be more immunogenic.[54 55] Accordingly, patients with melanoma and lung cancer who respond to checkpoint blockade therapy are often characterised with a high mutational load.[56–58] Another example is colorectal cancer, which is known to be refractory to immunotherapy for most patients,[14] with some clinical benefit demonstrated only in a minority of patients with a high mutational load due to mutations in the mismatch repair genes.[59] Indeed, it was recently reported that a mismatch repair defect can predict a better response to immunotherapies in other cancer types as well.[60 61] However, other reports undermine the correlation between mutational load and response to immunotherapy.[53 62] Specifically, some reports concerning melanoma have shown that neoantigen burden is not correlated with T cell density[63] and that some cancer types, such as clear cell renal cancer carcinoma, do respond to immunotherapy despite having a low mutational burden.[64]

Intratumour heterogeneity (ITH)[65] has been suggested to be another, if not better, predictor of an antitumour immune response, as low tumour heterogeneity was shown to predict response to checkpoint blockade,[51 66] and pan-cancer analyses also support this notion.[67 68] Further support comes from the findings that patients with melanoma who respond to PD-1 blockade exhibit enriched mutations towards BRCA1[53] and that patients who harbour a specific cluster of melanoma germline antigen MAGE-A show resistance to CTLA-4.[69] These observations and their interpretations are currently under heated debate, and their understanding will be instrumental for future patient selection. Finally, other genomic mechanisms may play a role in determining responsiveness to immunotherapy, such as insertion–deletion mutations.[70]

Immunogenomic studies have also proven their use in delineating mechanisms of tumour evasion from elimination by the immune system. One such mechanism is disruption of the antigen-presentation machinery. This can be achieved by acquired mutations of the human leucocyte antigen (HLA) component β2M[71 72] or loss of the HLA alleles.[73] An alternative escape mechanism is disruption of the interferon-γ signalling pathway, which upregulates HLA surface expression on tumour cells and which is manifested by mutations in genes along the pathway, such as the kinases Janus kinase 1 (JAK1) and JAK2 or the downstream transcription factor signal transducer and activator of transcription 1AT1.[74–76] Interferon-γ can activate other tumour escape mechanisms, such

as the upregulation of checkpoint inhibitor molecules on the tumour cell surface, including PDL-1.[77] Tumours can also immunoedit neoantigens and downregulate their expression at the RNA level or delete the mutant alleles on the DNA level.[78 79]

Unbiased genomic screens that use the (clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (CAS9)) whole genome screen hold great potential to uncover new resistance mechanisms, as was shown in tumour mouse models.[80] This method has already yielded considerable findings and the identification of several resistance mechanisms. It was recently shown by a CRISPR screen, for instance, that genes of the SWI/SNF chromatin remodelling complex gain resistance to immunotherapy in murine tumour cells[81] and that knockout of the PBRM1 gene from tumour cells increases cancer sensitivity to immunotherapy, a discovery supported by the finding that human patients with clear cell renal cell carcinoma who carry mutations in PBRM1 respond more favourably to immunotherapy.[64] Another CRISPR screen using human cell lines identified Apelin receptor as a modulator of interferon-γ signalling in tumours via interaction with JAK1.[80] Similarly, the tyrosine phosphatase PTPN2 was recognised as a novel resistance mechanism via a CRISPR screen, with its knockdown enhancing the response to immunotherapy, again via the interferon-γ pathway.[82] The CRISPR-CAS9 technique will enable further identification of genes involved in immunotherapy resistance and will reveal new and, hopefully, stronger biomarkers for better patient matching.

Neoantigen quarry and identification

In addition to the use of immune checkpoint therapy, a more specific and patient-tailored approach, which is possible only due to massively parallel sequencing, is neoantigen identification. Exploring the potential of tumour neoantigens as a therapeutic approach and investigating how neoantigens interact with checkpoint blockade has been a major focus of cancer immunology for the past decade.[1 83 84] Tumour neoantigens are antigens presented exclusively on the tumour cells' human leucocyte antigen (HLA) molecules. They are derived from patient-specific non-synonymous mutations, as well as indels in the cancer cells,[55 70] which are unique from patient to patient. Neoantigen-specific T cells can be found both in the tumour[85] and in the circulation of patients[86 87] and healthy donors.[88] They have been shown to be highly potent in eliminating tumours by both adoptive transfer[89] or using vaccinations that increase their abundance.[90 91] Interestingly, neoantigens are usually considered to activate $CD8^+$ T cells, but neoantigens recognised by $CD4^+$ T cells have also been reported.[92] Furthermore, immunotherapy itself can elicit additional neoantigen-specific T cell responses and can be viewed as an accompanying effect of checkpoint blockade.[93 94]

The potential of neoantigen identification is enormous and better identification and validation of neoantigens is in high demand. Neoantigens can be identified using numerous methods.[95] The initial step involves whole-exome or whole-genome sequencing, to identify patient-specific non-synonymous mutations.[96] The bottleneck continues to be identifying neoantigens from the sequencing data. In recent years, a multitude of computational tools have been generated in order to predict which neoantigens bind the HLAs expressed on the surface of tumour cells with sufficient affinity (reviewed in Hackl *et al*[45]). However, as these technologies are laborious and inaccurate, alternative techniques, such as HLA peptidomics, are now also in use[97 98] (see below). Regardless of the technique used, for each patient the literature describes a very restricted number of validated, rather than predicted, neoantigens (between 0 and 5), which does not correlate with mutational load.[86 87 89 96 99] However, it has been suggested,[95] and recently shown in patients,[100] that it is the quality, or the 'foreignness' of the neoantigen, manifested by its homology to antigens derived from infectious diseases, rather than the actual number of neoantigens, that predicts patient survival. While various algorithms for determining the quality of neoantigens have been developed, a precise understanding of the importance of neoantigen quality is yet to be unravelled.[101 102]

The vast clinical potential of neoantigens, as demonstrated in rodents that neoantigen-based vaccination can induce an antitumour response,[93 103] is now being applied to treat patients in a number of pioneering works. Synthetic RNA-based[91] and peptide-based[104] vaccinations developed via neoantigen querying have been found to be fully immunogenic and to significantly benefit patients with melanoma. These works show that the technology for vaccinating patients with a personalised, antineoantigen construct is possible. Neoantigens can also be used to engineer specific TCRs for T cells taken from patients ex vivo,[105] as adoptive T cell transfer in general, and the technique to transduce T cells with cancer antigen-specific TCR is established for several years.[88 106–109]

The use of HLA peptidomics for neoantigen identification

An effective method for the identification of neoantigens is HLA peptidomics, which involves the co-immunoprecipitation of HLA-I bound peptides and the subsequent analysis of the peptidome using mass spectrometry, following which the peptidome is aligned to whole-exome data from the same samples, thus enabling the detection of bona fide neoantigens that are actually bound to the HLA, rather than predicted in silica. In vitro validation of the reactivity of the found peptides can be done using tumour-infiltrating lymphocytes (TILs) taken from the same patient or effector murine TILs. The method can be used both for quarrying human[110] and murine neoantigens,[111] with recent progress introducing high-throughput screens.[112]

We recently successfully used HLA peptidomics to identify neoantigens from different melanoma metastasis and cell lines, accompanied by in-depth characterisation

of the T cell landscape.[98] Using this technique, we are able to show that despite the low number of neoantigens detected, these handful of neoantigens are in fact highly robust—with two neoantigens sufficing to eliminate 90% of human melanomas when co-cultured with patient-matched TILs. As these analyses were done on late-stage tumours, it may be the case that more neoantigens were presented at earlier stages of the disease and then immunoedited or that indeed each tumour harbours only a very limited number of neoantigens or that the detection level of HLA peptidomics is rather limited. Either way, identification of a few targetable antigens can have great clinical significance. Thus, HLA peptidomics can be sufficient to discover immunodominant neoantigens, and a future pipeline for the detection of neoantigens for clinical use can be envisioned.

A possible future application for the HLA peptidomics technique is to identify neoantigens derived from recurring mutations, which are very frequent in cancer.[113] A main characteristic of melanoma, for instance, is recurrent mutations in BRAF, NRAS and NF1, though, to date none of these genes was ever reported to harbour a neoantigen, as the vast majority of neoantigens are derived from passenger, rather than driver, mutations. However, the KRAS G12D mutation was shown to generate a reactive neoantigen in a patient with metastatic colorectal cancer,[105] and recurrent hotspot p53 mutations were shown to generate an immunogenic T cell response in ovarian cancer.[114] Interestingly, it was reported that patients with desmpolatic melanoma, a rare form of melanoma characterised by a high number of mutations in NF1, respond very well (70%) to anti-PD1 therapy, compared with ~30% in cutaneous melanoma.[115] These observations are of vast importance since, unlike most neoantigens, which are private and patient specific, these neoantigens might be shared between different patients and may be used in the future as an off-the-shelf product. However, the detection of these recurrent neoantigens might be extremely challenging, since there seems to be a strong evolutionary pressure to prevent these neoantigens from being presented. Despite this, future technology with higher resolution might facilitate the detection of neoantigens derived from recurrent mutations.

Future directions

As discussed above, immunotherapy strategies that enhance the antitumour T cell response, such as checkpoint inhibitors and adoptive T cell therapy, exhibit remarkable clinical effects in a wide range of tumour types. However, many tumours do not respond to checkpoint inhibitors and the determinants of treatment efficacy remain largely unknown. Neoantigens that arise as a consequence of somatic mutations within the tumour represent an attractive means to promote immune recognition in cancer.[93] Indeed, high mutational and neoantigen load in tumours have been associated with an enhanced response to immune checkpoint blockade therapy.[57 58 116 117] The use of neoantigen-formulated vaccines has further emphasised the power latent in neoantigen-targeted immunotherapy.[90 118] Cutaneous melanoma, which is among the most highly mutated malignancies,[119] has the highest objective response rates to checkpoint blockade (~60% on combined CTLA-4 and PD-1 blockade).[11] Yet, the reasons for the lack of response in a substantial number of patients remain obscure and call for investigation of mechanisms beyond mutational load. Indeed, ITH may influence immune surveillance[51 66] and pan-cancer analyses show better survival for tumours with low ITH.[67 68] Clearly, by using the various unbiased and comprehensive tools described in this review, additional biomarkers that will predict response to immunotherapy will arise, allowing for superior patient matching for future immunotherapies.

Importantly, the assumption behind immune checkpoint inhibitor therapy is that an antitumoral immune potential waits to be unleashed against tumour-presented antigens that drive the effector T cell response.[93 120] The limitations in this therapeutic modality can be addressed by combining immune checkpoint inhibitors with the priming of patient T cells towards tumour neoantigens. Yet, one needs to ensure that targeted neoantigens are pronouncedly presented to the immune system and prompt strong T cell responses. Various vaccine systems have been suggested and tested for neoantigen delivery.[121 122] However, the most suitable vaccine format, including how to choose which neoantigens to target and how many neoantigens need to be included in the vaccine regimen, is yet to be determined.

Finally, the intense efforts to identify novel strategies to overcome therapeutic resistance to immunotherapy through a deep understanding of the contribution of the tumour genetic composition, the immune microenvironment and neoantigen presentation are expected to generate new insights with significant clinical importance in the immediate future.

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