The ‘Achilles heel’ of cancer and its implications for the development of novel immunotherapeutic strategies

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Abstract

Over the last century, scientists have embraced the idea of mobilising anti-tumour immune responses in patients with cancer. In the last decade we have seen the rebirth of cancer immunotherapy and its validation in a series of high profile clinical trials following the discovery of several immune-regulatory receptors. Recent studies point towards the tumour mutational load and resulting neoantigen burden as being crucial to tumour cell recognition by the immune system, highlighting a potentially targetable Achilles heel in cancer. In this review we explore the key mechanisms that underpin the recognition of cancerous cells by the immune system and discuss how we may advance immunotherapeutic strategies to target the cancer mutanome in order to stimulate tumour-specific immune responses, ultimately, to improve the clinical outcome for patients with cancer.

Introduction

The concept of cancer immunotherapy originates from observations made by William Coley in the late 19th Century (Figure 1).¹ He documented tumour regression in patients with acute bacterial infections, and proceeded to test bacterial extracts, referred to as ‘Coley’s toxins’, in patients with bone and soft tissue sarcomas. Despite the encouraging results reported by Coley, a lack of understanding with regards to the mechanism of action of these toxins in conjunction with the emergence of radiotherapy
as a treatment modality led to a decline in the use of Coley’s toxin. Subsequent research in cancer immunology however, led to the development and evaluation of several novel immune-therapeutics, namely interleukin-2, interferon gamma (IFNγ), tumour necrosis factor and numerous cancer vaccines.

Whilst several of these therapeutic strategies produced interesting results in patients with solid cancers, the clinical responses were often short-lived and limited to a small fraction of treated patients. The advent of T cell checkpoint molecule inhibitors has revolutionised the therapeutic landscape for patients, enthusing scientists globally to better understand the basic mechanisms that underpin the recognition of cancerous cells by the endogenous immune system. It is evident from published data to date that the tumour mutational load and the consequent generation of neoantigens are one of the important components of an effective anti-tumoural immune response, representing the Achilles heel of cancerous cells. Immunotherapeutic strategies, for example cancer vaccines or adoptive cellular therapy targeted towards tumour neoantigens in combination with checkpoint molecule inhibitors provide a means of delivering a clinically valuable anti-tumoural effect through enhancement and activation of tumour specific immune responses.

**Clinical successes of immune checkpoint therapies**

A significant amount of basic and translational research has been directed towards gaining a better understanding of how the immune response to cancer is regulated. A key inflection point in the history of cancer immunotherapy was the discovery of an immune receptor expressed at high levels by in vitro activated T cells, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4).\(^2\) Whilst its function was elusive at first, a number of experiments demonstrated the role of CTLA-4 as a co-inhibitory receptor, responsible for the downregulation of T cell activity. In the mid 1990s several groups proposed that CTLA-4 would act as an immune checkpoint restricting the activity of tumour reactive T cells.\(^3\)\(^-\)\(^9\) Inhibition of T cell proliferation and IL-2 secretion was mediated following CTLA-4 activation and subsequent data demonstrated the effective rejection of tumours in murine models of cancer using antibodies against CTLA-4.\(^4\)\(^,\)\(^5\) Further evaluation of mechanism of action has suggested that the antibodies can act both by directly blocking inhibitory signals of regulatory T cells and by driving Fcy receptor mediated depletion of tumour infiltrating regulatory T cells expressing higher levels of CTLA-4 than effector T cells.\(^10\)\(^-\)\(^13\)
These key studies gave rise to the concept of immune regulation of T cells involved in anti-tumoural immunity, mediated by CTLA-4, leading to the clinical development of antibodies targeting CTLA-4. Ipilimumab, a fully humanised monoclonal antibody to CTLA-4 was demonstrated to deliver durable responses in patients with advanced melanoma in several clinical trials, leading to its approval by the US Food and Drug Administration (FDA) for the treatment of metastatic melanoma in 2011.

The discovery of a second T cell co-inhibitory molecule of the B7 family, programmed death receptor 1 (PD-1)\(^1\) and its ligand programmed death receptor ligand 1 (PD-L1)\(^2\) was later followed by data showing that blockade of the PD-1/PD-L1 axis using monoclonal antibodies could affect similar anti-tumour immune responses in mice with established tumours.\(^3\) The secretion of inflammatory cytokines by infiltrating lymphocytes, for example interferon gamma, leads to the up-regulation of PD-L1 on the tumour cell surface and surrounding tissues, resulting in the inhibition of T cell activity as a result of PD-1/PD-L1 interaction resulting in tumour immune evasion, a process referred to as ‘adaptive immune resistance’.\(^4\)

Numerous trials of anti-PD-1 and anti-PD-L1 therapies have demonstrated impressive clinical responses with survival benefit in a variety of solid\(^5\)-\(^9\) and haematological cancers\(^10\), transforming the outlook for a large number of patients. Currently, nivolumab and pembrolizumab, both monoclonal antibodies to PD-1, are licensed by the FDA for use in advanced melanoma, metastatic non-small cell lung cancer, metastatic squamous cell head and neck cancers and Hodgkin lymphoma (Table 1).

The success of both anti-PD-1 and anti-CTLA-4 therapy has paved the way for the clinical development of a large number of immunomodulatory antibodies, creating much excitement in the field of cancer immunotherapy. However, an important question regarding the precise nature of the antigens that are recognised by heavily regulated lymphocytes remains to be fully answered.
Table 1: Current FDA approved checkpoint molecule inhibitors and clinical trial references.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Clinical Indications</th>
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<tbody>
<tr>
<td>Pembrolizumab (PD-1)</td>
<td>Metastatic melanoma(^{25})</td>
</tr>
<tr>
<td></td>
<td>Metastatic NSCLC(^{29})</td>
</tr>
<tr>
<td></td>
<td>Metastatic squamous cell carcinoma of the head and neck(^{30})</td>
</tr>
<tr>
<td></td>
<td>Hodgkin lymphoma(^{34})</td>
</tr>
<tr>
<td>Atezolizumab (PD-L1)</td>
<td>Locally advanced or metastatic urothelial carcinoma(^{31})</td>
</tr>
<tr>
<td></td>
<td>Metastatic NSCLC(^{26,32})</td>
</tr>
<tr>
<td>Ipilimumab (CTLA-4)</td>
<td>Metastatic melanoma(^{35})</td>
</tr>
<tr>
<td>Ipilimumab and Nivolumab (PD-1 and CTLA-4)</td>
<td>Metastatic melanoma(^{18})</td>
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Characterisation of antigens recognised by tumour infiltrating lymphocytes

Tumour antigens have typically been classified into two main categories based on their distribution within tissues. Tumour-associated antigens (TAAs) include tissue differentiation markers (e.g MART-1, gp100, TRP-1 and TRP-2 proteins) expressed both on normal tissue and tumour cells and cancer-testes antigens (e.g NY-ESO-1 and MAGE-A3) expressed in germ cells and tumour cells (for review please refer to Kawakami et al 2004).\(^{36}\) In contrast, mutations that are found exclusively within cancer cells that are not present in normal tissue give rise to tumour specific antigens often referred to as tumour neoantigens.

In 1991, work carried out Van Der Bruggen et al revealed the presence of a gene encoding the antigen MAGE-1, found within melanoma cells and not in most normal tissues, recognised by cytotoxic lymphocytes in a patient with melanoma.\(^{37}\) This gave rise to the concept of tumour-associated antigens, following which the discovery of several other key self-antigens encoded by gp100, MART-1 and tyrosinase occurred.\(^{38-40}\) The identification of tumour-associated antigens, particularly the finding that these were often shared between patients, drew much attention in the field of
cancer immunology leading to the development of adoptive cellular therapies and cancer vaccines targeted against TAAs.

Several groups including those of Thierry Boon and Hans Schreiber have demonstrated the role of tumour neoantigens in promoting effective anti-tumour immunity. The mutagenisation of a mouse tumour cell line (P815 tumour cell line) led to a highly immunogenic tumour cell variant that was rejected by syngeneic mice due to the expression of novel mutated antigens, resulting in a cytotoxic anti-tumoural immune response.\(^1\) A subsequent study showed the presence of a CD8\(^+\) T lymphocyte response to a peptide arising from a tumour-specific somatic mutation in the coding region of a nucleic acid helicase, p68, in an ultraviolet light-induced murine tumour.\(^2\) In a separate study, CD4\(^+\) T lymphocytes were shown to recognise unique tumour neoantigens expressed exclusively within the tumour cells of an ultraviolet-light induced murine model of cancer.\(^3\)

These studies were bridged to human data by the demonstration of tumour infiltrating lymphocytes capable of recognising a tumour neoantigen encoded by mutated cyclin-dependent kinase 4 (CDK4) in a human melanoma specimen\(^4\), and of others capable of recognising antigen arising from a mutated beta-catenin gene exclusively found within melanoma cells\(^5\). A separate study showed the presence of cytotoxic lymphocytes specific to a neoantigen arising from mutated CASP-8 in a squamous cell carcinoma of the oral cavity.\(^6\) These findings provided initial support that, in humans, tumour specific T cells, which we refer to as neoantigen reactive T cells (NARTs), have the capacity to recognise neoantigens found exclusively within the tumour.

Taken together, data from these studies support the concept of anti-tumoural immune responses against antigens that are expressed as a consequence of the accumulation of mutations within tumours. One can also infer from these studies that the level of tumoural mutational burden may confer a survival advantage through the expression of neoantigens that are recognised by the immune system, driving a specific anti-tumoural immune response. Given that neoantigens are found to occur as a result of mutations that are largely ‘private’ and unique to individual tumours, the therapeutic targeting of neoantigens represented a huge translational challenge during the 1990s, in an era where next generation sequencing was not as economical and readily available as it is today, halting the development of personalised medicine. This led to a shift in the focus of cancer immunology research towards tumour antigens that were
shared between tumours of different patients across varied tumour types, leading to increasing focus on TAAs.

**The renaissance of tumour neoantigens in cancer immunology**

The renaissance of neoantigens has been facilitated by improvements in next generation sequencing techniques and bioinformatics pipelines, including the development of neoantigen peptide prediction algorithms, on a background of disappointing results from immunotherapeutic strategies targeted against TAAs.

In the last fifteen years, a number of studies have pointed towards the importance of neoantigen-directed immune responses. In one study, T cell responses towards neoantigens arising from five tumour-specific mutations were shown to predominate over those against tumour-associated antigens within the same patient.\(^{47}\) A separate study showed the presence of neoantigen reactive CD4\(^+\) cells in the tumours of patients with melanoma.\(^{48}\) Importantly, neoantigen specific T cells have also been detected in the peripheral blood of patients with melanoma.\(^{49}\) Taken together, data from these studies support the role of neoantigens in anti-tumour immunity; however further studies directly comparing the relative contributions of neoantigens versus tumour-associated antigens in the anti-tumoural immune response are necessary.

Further work in murine models supported the development of personalised immunotherapies for patients with cancer. Next generation sequencing and MHC Class I in silico prediction methods were used to identify tumour specific mutations and corresponding predicted peptides in a murine model of MCA-induced fibrosarcoma. A predicted neoantigen arising from mutated spectrin beta-2 was subsequently demonstrated to be a key mediator of the anti-tumoural T cell response. Furthermore, the authors reported that the immunological editing of the cancers in these mice occurred as a result of a selection process dependent on T cells.\(^{50}\) The concept of the immunological editing of cancers relates to earlier work carried out by the same authors\(^ {51}\) and comprises of three key components (for review see Dunn et al 2002).\(^ {52}\) This includes the elimination of tumours through cancer immunosurveillance, maintenance of cancer in an equilibrium state as a result of the endogenous immune response and lastly, tumour escape. Tumours may evade immune-mediated destruction as a result of the clonal evolution of cancerous cells with preferential expansion of tumoural subclones lacking immunogenicity or those with the ability to suppress immune responses leading to tumour progression.
In a separate study, next generation sequencing was employed to identify tumour specific non-synonymous mutations in melanoma cells derived from a B16F10 mouse model. Importantly, in mice harbouring tumours, immunisation with peptides encoded for by non-synonymous mutations predicted to bind to MHC Class I molecules by in silico prediction algorithms lead to tumour control. The importance of MHC Class II presented neoantigens and tumour-reactive CD4+ cells has also been reported. In this study, Kreiter et al proposed a bioinformatics method through which poly-neo-epitope mRNA vaccines could be synthesised based on both MHC Class II predicted binding and expression levels of tumour mutations. They successfully demonstrated that such approaches used to generate vaccines targeted against CD4+ neoantigens resulted in a potent anti-tumour effect in three separate murine models of cancer. Interestingly, through use of the same bioinformatic prediction algorithm, a large number of mutations giving rise to neoantigens predicted to bind to MHC Class II in human cancers were also found.

Previous pre-clinical data has shown the ability of tumour-reactive CD4+ cells to develop cytotoxic activity leading to tumour rejection in murine B16/BL6 tumour models. Moreover, durable clinical responses to adoptively transferred NY-ESO-1 specific or tumour neoantigen specific CD4+ cells were reported in metastatic melanoma and cholangiocarcinoma respectively. Together, these studies provide support for the role of CD4+ effector cells in the adaptive anti-tumoural immune response. This may be achieved through either direct cytotoxic activity in the context of MHC class II expressing tumours and/or facilitation of CD8+ T cell expansion and effector function to promote immune-mediated tumour cell destruction.

The studies discussed above provide insight into the successful application of next generation sequencing techniques and neoantigen prediction algorithms to identify and characterise TAAs and tumour neoantigens presented by MHC Class I and Class II molecules. Moreover, they highlight the crucial role of CD4+ T cells in generating effective anti-tumoural immune responses and underline the importance of further research in this area, particularly for the development of CD4+ targeted immunotherapeutic strategies for patients with cancer.
The interplay between neoantigen reactive T cells and the response to cancer immunotherapy

Several groups have demonstrated the presence of neoantigen driven T cell responses in human cancers responding to either adoptive cellular therapy and/or immunomodulatory antibodies. The persistence of neoantigen specific T cell clones recognising mutated growth arrested-specific gene 7 (GAS7) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the peripheral blood of a patient with stage IV melanoma reported to have a complete response after adoptive transfer of autologous tumour infiltrating lymphocytes was inferred to be indicative of their role in maintaining a clinical response.58 Moreover, neoantigen reactivity of adoptively transferred T cells was elucidated in three patients with advanced melanoma with objective responses to adoptive cellular therapy59, and neoantigen reactive CD4+ T cells were identified in a patient with metastatic cholangiocarcinoma displaying a response to adoptive cellular therapy.57 Furthermore, neoantigen reactive CD8+ T cell responses were also reported in a patient with advanced melanoma responsive to anti-CTLA-4 therapy.60 These clinical data provide evidence for the role of CD8+ and CD4+ T cells in recognising tumour neoantigens and suggest that immunomodulatory antibodies may act by enhancing the activity of neoantigen reactive T cells to achieve tumour control.

Several studies over the last few years have highlighted the close interplay that exists between the genomic landscape of tumours and the clinical response to checkpoint blockade. Patients with tumours of a relatively high mutational load were found to have a favourable clinical outcome following anti-CTLA-4 therapy.61 Similarly, patients with metastatic melanoma categorised as deriving clinical benefit from anti-CTLA-4 therapy were found to have a significantly higher tumour and mutational load compared to those patients with minimal or no clinical benefit from the drug.62 Moreover, in patients with NSCLC treated with anti-PD-1 therapy, a higher non-synonymous mutational load and neoantigen burden was associated with durable clinical responses and progression free survival. Furthermore, neoantigen-specific CD8+ T cell responses and tumour regression were seen concurrently in a responding patient.63

Taken together, data from these studies highlight the intricate relationship that exists between the tumour mutational and neoantigen landscape and the anti-tumoural immune response. Furthermore, they shed light on the mechanistic activity of immunomodulatory antibodies, illustrating how anti-CTLA-4 and anti-PD-1 therapy may be used to counteract the immune regulation of neoantigen reactive T cells. It is
important to note however, that the relationship between tumour mutational load and response to checkpoint blockade is not absolute; metastatic clear cell renal carcinoma has a moderate tumoural mutational load and is associated with durable responses to anti-PD-1 therapy.\textsuperscript{19,64} Moreover, colorectal cancer is not typically considered to be a cancer with a high mutational load\textsuperscript{64} however clinical benefit from anti-PD-1 therapy is reported in a subgroup of these patients.\textsuperscript{65} Patients with DNA mismatch repair deficiency (MMR) were shown to have an increased clinical response to anti-PD-1 therapy likely related to an increased mutational and neoantigen load arising from MMR deficiency in these patients.\textsuperscript{65}

**Intratumoural heterogeneity and anti-tumour immunity**

The concept of genetic intratumoural heterogeneity is well documented in a variety of solid and haematological cancers.\textsuperscript{66-71} Non-synonymous mutations present in every tumour cell give rise to clonal neoantigens that occur early in tumour evolution and are therefore ubiquitously expressed within tumour tissues. In contrast, subclonal or ‘branch’ mutations resulting in the expression of subclonal neoantigens occur later, and so are localised to specific tumour cell subsets.

The impact of intratumoural neoantigen heterogeneity on the anti-tumour immune response has been investigated more recently. A strong relationship between the level of clonal neoantigen burden and overall survival was found in patients with lung adenocarcinoma from analysis of sequencing data within The Cancer Genome Atlas (TCGA) database. More in-depth analysis revealed the upregulation of immune-related genes including CD8, granzyme, IFN\(\gamma\), PD-1, LAG-3, PD-L1 and PD-L2, in patients with a high burden of clonal neoantigens and a relatively low fraction of subclonal neoantigens, indicative of an active anti-tumour immune response within the tumour tissues of these patients. Of note, CD8\(^+\) T cell responses to neoantigens were detected in two patients with early stage NSCLC with a comparable number of predicted neoantigens but markedly different levels of intratumoural neoantigen heterogeneity. Neoantigen reactive T cells identified by MHC-multimer analysis were found to have increased levels of PD-1 and LAG-3 expression on their cell surface indicative of immune regulation of these cells.\textsuperscript{72}

Analysis of genomic sequencing data from anti-PD-1\textsuperscript{63} or anti-CTLA-4\textsuperscript{61} treated cohorts indicated that a high predicted clonal neoantigen burden and low neoantigen heterogeneity in NSCLC and metastatic melanoma was associated with favourable
clinical outcomes. This study highlights fundamental differences in the effectiveness of the anti-tumoural immune response driven by clonal versus subclonal neoantigens. A recent study exploring the tumour neoantigen landscape in matched NSCLC tumour specimens pre and post anti-PD-1 or dual anti-PD-1/anti-CTLA-4 therapy demonstrated genomic and immune-mediated loss of tumour neoantigens in resistant tumours. Moreover, immunotherapy resistance has been shown to correlate with tumour aneuploidy; patients with increased tumoural somatic copy number alterations were found to have reduced survival following anti-CTLA-4 therapy compared to those with reduced tumour aneuploidy. A lack of cytotoxic immune infiltration in tumours with high levels of tumour aneuploidy may contribute to the reduced survival observed in these anti-CTLA-4 treated patients.

The studies discussed above provide insight into firstly, the importance of clonal neoantigens in predicting response to checkpoint blockade and secondly, the potential impact of checkpoint blockade and/or tumour aneuploidy in the immunological editing of neoantigens that may in fact lead to tumour immune evasion. Therapeutically targeting a wide repertoire of clonal neoantigens may theoretically provide an effective method of targeting the cancer mutanome although this is yet to be proven (Figure 2).

**T cell receptor (TCR) repertoire and clonality in cancer**

The repertoire of antigen specific T cells is generated in the thymus during the process of T cell differentiation as a result of somatic recombination of both alpha and beta chains of the T cell receptor, followed by central deletional tolerance of the most highly self-reactive T cells. The somatic rearrangement of either V and J segments or V, D, J segments of alpha and beta chains respectively, gives rise to the highly variable complementarity determining region 3 (CDR3) of the TCR, key in determining the antigen specificity of individual T cell clones.

The level of mutational burden and genomic heterogeneity demonstrated in a variety of solid cancers could be reflected in the clonality and repertoire of tumour-reactive lymphocytes found within the tumour microenvironment, but data is currently limited. Tumours with a high mutational load and resulting neoantigen burden may give rise to a more diverse intra-tumoural T cell repertoire due to the large number of antigens presented to the immune system. In line with this, an association between T cell diversity and mutational load has previously been reported through the analysis of reconstructed CDR3 regions from RNAseq data of samples within the TCGA database.
Previous studies examining the effects of anti-CTLA-4 therapy on the TCR repertoire have shown peripheral blood TCR repertoire diversification following therapy\textsuperscript{76} and improved overall survival in patients who maintained highly abundant TCR clones present in the blood prior to commencement of anti-CTLA-4 therapy\textsuperscript{77}. Anti-CTLA-4 therapy has also been described to significantly increase the number of newly detected CD8\textsuperscript{+} melanoma specific T cell clones.\textsuperscript{78}

The response to anti-PD-1 therapy in metastatic melanoma has previously been associated with a more clonal and less diverse intratumoural TCR repertoire at baseline in melanoma specimens. Furthermore, in responding patients, a significant increase in the number of expanded TCR clones following anti-PD-1 therapy was reported, indicative of an enhanced oligoclonal T cell response within tumours of patients with metastatic melanoma.\textsuperscript{79}

Heterogeneity in the repertoire of T cells infiltrating different regions of clear cell renal carcinomas has been demonstrated using multi-region TCR sequencing\textsuperscript{80}, and spatial heterogeneity of TILs infiltrating oesophageal cancers is also documented.\textsuperscript{81} Interestingly, in the latter study, deeper analysis limited to the 100 most abundant TCR clones revealed a high degree of overlapping TCRs between tumour regions within each individual patient.\textsuperscript{81} Theoretically these TCR clones, present across multiple regions of the tumour may expand in response to common antigens found in all tumour regions, although the specificity of tumoural clones seen across multiple regions of a tumour remains to be elucidated.

**Therapeutic approaches to target the cancer mutanome and future perspectives**

Vaccination strategies, adoptive cellular therapies or the adoptive transfer of engineered T cells targeting tumour neoantigens, in conjunction with checkpoint molecule antibodies, represent some of the key avenues for targeting the cancer
mutanome that are currently being explored in a number of clinical trials. Clinical trials of personalised neoantigen vaccines +/- checkpoint blockade are recruiting in a variety of solid cancers (Table 2). Early phase trials of neoantigen based adoptive cellular therapies and trials of engineered T cells harbouring TCRs against neoantigens are eagerly awaited.

<table>
<thead>
<tr>
<th>Clinical trials of neoantigen targeted immunotherapies</th>
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<tr>
<td><strong>Trial name</strong></td>
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<tr>
<td>A Personalised Cancer Vaccine (NEO-PV-01) with Nivolumab for Patients With Melanoma, Lung Cancer or Bladder Cancer</td>
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<tr>
<td>A Phase I Study With a Personalised NeoAntigen Cancer Vaccine in Melanoma</td>
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<tr>
<td>A Phase I Personalised NeoAntigen Cancer Vaccine With Radiotherapy for Patients With MGMT Unmethylated, Newly Diagnosed Glioblastoma</td>
</tr>
<tr>
<td>Neoeptope-based Personalised Vaccine Approach in Patients With Newly Diagnosed Glioblastoma</td>
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**Table 2: Clinical trials of neoantigen targeted immunotherapy currently open to recruitment.**

As we move closer to achieving our goal of delivering personalised medicine for many of our patients, we must be thorough in our approach. It is clear, that such bespoke approaches to target cancers will involve sequencing and identification of tumour-specific mutations, in silico prediction of resulting neoantigen sequences according to individual patient HLA types and predicted strength of neoantigen peptide binding to MHC to guide appropriate selection of neoantigen peptide sequences. Moreover, neoantigen targeted adoptive cellular therapies will rely upon information generated from in vitro assays testing the reactivity of tumour infiltrating lymphocytes stimulated with synthesised peptides. It is critical that we recognise that despite the advances in next generation sequencing and bioinformatics methods, these techniques are themselves imperfect; the limitations of neoantigen prediction algorithms and verification of whether tumour cells actually express such predicted neoantigens poses a significant challenge. The therapeutic targeting of neoantigens may minimise the risks of toxicities in cancer patients undergoing checkpoint blockade, since these are often related to immune responses to tumour associated self antigens which may also
be expressed in some normal tissues. Nevertheless, the possibility that T cell receptors may show some degree of cross-reactivity with unrelated self-antigens may still exist.

In the context of neoantigen-targeted vaccine therapy, the expansion of regulatory T cells following vaccination is a likely possibility, thus combination of vaccines with therapeutic agents that either deplete regulatory T cells or limit the action of inhibitory cytokines e.g. indoleamine 2,3-dioxygenase (IDO), transforming growth factor beta (TGF-β) or interleukin 10 (IL-10) may be required to achieve successful clinical outcomes. The effectiveness of clonal neoantigen targeted adoptive cellular therapies will rely on infiltration of transferred T cells into the tumour microenvironment and the use of appropriate combinational strategies to overcome the immune regulation of these cells. The possibility exists that tumour immune escape may occur as a result of tumour resistance to IFN-γ signalling, as described previously in the context of acquired resistance to anti-PD-1 therapy. Successful therapeutic strategies of how best to overcome tumour resistance in this context remain to be elucidated. Despite these challenges, however, there is hope for optimism that we may finally have found an exploitable Achilles heel in our battle against cancer.

Acknowledgements

K.J. is funded by the Sam Keen Foundation and UCL/Royal Marsden Hospital NIHR Biomedical Research Centres. S.A.Q. is a CRUK Senior Cancer Research Fellow and is funded by a CRUK Biotherapeutic Program Grant and a CRUK Senior Cancer Research Fellowship. K.S.P. receives funding from the NIHR BTRU for Stem Cells and Immunotherapies, of which he is the Scientific Director.

References


Key milestones in cancer immunotherapy

1. **The birth of cancer immunotherapy**
   - 1893
   - William Coley described tumour regression in patients with bone and soft tissue sarcomas harbouring acute bacterial infections.¹

2. **Discovery of CTLA-4**
   - 1987
   - CTLA-4 gene cloned and CTLA-4 reported as a new member of the immunoglobulin superfamily.²

3. **Discovery of tumour neoantigens**
   - 1988
   - Report of tumour neoantigens in a mutagenised mouse tumour cell line, capable of generating a cytotoxic anti-tumoural immune response.⁴¹

4. **Discovery of tumour associated antigens**
   - 1991
   - Discovery of the first tumour associated antigen encoded by the MAGE-1 gene.³⁷

5. **Anti-tumour effect of PD-1/PD-L1 axis inhibition**
   - 1992
   - First demonstration of tumour growth suppression in PD-L1+ murine models of cancer through therapeutic blockade of the PD-1/PD-L1 axis using anti-PD-L1.¹⁶

6. **Anti-tumour effect of anti-CTLA-4**
   - 1996
   - The regression of tumours in murine models of cancer using antibodies to CTLA-4 first described.⁵

7. **Genetic determinants of response to checkpoint blockade**
   - 2002
   - Several studies demonstrate the importance of tumour mutational load and neoantigen burden in response to checkpoint blockade.⁶¹-⁶³, ²²

8. **FDA approval of ipilimumab, nivolumab and pembrolizumab**
   - 2011-17

9. **FDA approval of**
   - 2014-16
Immunotherapeutic targeting of cancer’s Achilles heel

Tumour cells (patterned circles) comprising of two types of neoantigens: clonal (blue) and subclonal neoantigens (accompanying colour)

Multi-peptide vaccine or adoptive cellular therapy targeting subclonal or clonal neoantigens

Tumour neoantigen landscape following subclonal or clonal neoantigen targeted immunotherapy. Grey circles indicative of eliminated tumour clones