IMMUNOTHERAPY OF CANCER: TOWARDS A NEW ERA

*John B.A.G. Haanen

The Netherlands Cancer Institute, Amsterdam, the Netherlands
*Correspondence to j.haanen@nki.nl

Disclosure: No potential conflict of interest.
Received: 22.04.14 Accepted: 22.07.14
Citation: EMJ Oncol. 2014;2:76-82.

ABSTRACT

In the past two decades, immunotherapy of cancer has developed into an established treatment option. At first, the development of monoclonal antibodies - targeting overexpressed cell surface molecules on tumour cells - resulted in improved survival when combined with standard chemotherapy or radiotherapy. More recently, T cell immunotherapy has impacted on survival of certain cancer types. In melanoma especially, but now also in renal cell cancer and non-small cell lung cancer, immune checkpoint inhibitors, such as cytotoxic T lymphocyte-associated antigen-4 (anti-CTLA4) and blockade of programmed death receptor-1-PD-ligand 1 (PD1-PD-L1) interaction, represent a completely new treatment paradigm, lowering the threshold for an anticancer immune response and breaking self-tolerance. Adoptive T cell transfer using tumour-infiltrating lymphocytes or genetically modified T cells are under development, but have shown impressive clinical efficacy in several Phase II studies. These emerging but highly promising treatments can give rise to durable tumour control in diseases that were lethal in all patients only a few years ago.

Keywords: Immunotherapy, cancer, T cell, checkpoint inhibitor, adoptive cell therapy, cytokine, monoclonal antibodies, melanoma.

INTRODUCTION

Immunotherapy of cancer goes back to the beginning of the previous century with a famous pioneer in the field, Dr William Coley, who observed that occasionally cancer could regress following a severe bacterial infection (erysipelas). Based on these observations, he started treating cancer patients with bacterial toxins, sometimes with great success. Despite these early observations and treatments, however, immunotherapy of cancer remained in its infancy for a very long time. Only very recently, immunotherapy of cancer made a breakthrough when immune checkpoint inhibitor ipilimumab (Yervoy®) demonstrated an improvement in overall survival (OS) in pretreated metastatic melanoma patients, and was approved for this disease.

T CELL IMMUNOTHERAPY

T cell based immunotherapy has been promising for decades as a minority of patients appear to benefit from this strategy. However, studies testing vaccines, for example, never showed any improvement in survival in large randomised controlled clinical trials. One may ask the question: why has T cell immunotherapy been tried for so many years? Firstly, some cancers are associated with spontaneous regressions, even metastatic disease. Well-known examples are melanoma and renal cell carcinoma. In melanoma, around 5% of patients with metastatic disease present without a primary tumour. Based on a highly similar genetic make-up as cutaneous primary melanomas, it is likely that these primary tumours have regressed spontaneously by an effective immune response. Secondly, for many tumour types, the presence of lymphocytic infiltrates in the primary tumour or metastatic lesions has been correlated with improved outcome. These observations have been clearly shown for melanoma, colorectal cancer, and ovarian cancer. Especially in the case of melanoma, many tumour-associated antigens that are recognised by these infiltrating T cells have been defined.
These antigens are mostly derived from tumour-associated proteins that are shared between patients. Examples of these antigens are the melanocyte differentiation antigens (MDAs), such as: MART-1, gp100 and tyrosinase, the cancer/testis gene products from (for example) the MAGE family, from NY-eso-1, or from the SSX family, and overexpressed proteins such as MELOE-1 and telomerase. Now that genomes of many human cancers have been sequenced, one has learned to appreciate that some tumours harbour many more mutations than others. Of all tumour types, melanomas have the highest somatic mutation rate. These mutations have been induced by exposure to ultraviolet irradiation. Next come non-small cell lung cancer (NSCLC) and other smoking induced cancers. When a mutation occurs in an expressed gene, this may lead to single amino substitution, and therefore, a potentially truly foreign neoantigen for the immune system. Recently, CD8 T cells, specific for these mutation-induced, tumour-specific antigens, have been found within tumour infiltrating lymphocytes. It is tempting to speculate that tumour types with the highest mutation rate are the most immunogenic tumours. Evidence is accumulating that supports this hypothesis.

VACCINE-BASED STRATEGIES

Several of the shared tumour-associated antigens have been part of a vaccine strategy. Many different vaccine-based strategies have been explored: short peptide vaccines, containing 8-10-mer peptides that can directly bind to major histocompatibility complex (MHC) Class 1 molecules, and thus, be presented to the T cell immune system; synthetic long peptides (20-40-mer) that require intracellular processing before presentation to the T cell immune system; viral vaccines engineered to express peptides from, or the tumour-associated antigens themselves; DNA vaccines, bacterial plasmids engineered to contain TAA sequences; and dendritic cell (DC) vaccines, consisting of autologous or allogeneic ex vivo cultured DCs that are loaded with TAA or tumour derived RNA, before injection or infusion into patients. Until recently, these strategies failed in randomised controlled trials (RCTs) despite the high number of promising early phase trials. In 2010, the FDA, because of a statistically significant impact on survival, approved Sipuleucel-T (Provenge®), an autologous DC vaccine for patients with castrate-resistant prostate cancer. One double blind, placebo controlled, Phase III study showed that Sipuleucel-T prolonged OS from 21.7 months to 25.8 months (HR: 0.775; CI: 0.61-0.98). This was confirmed in a smaller, second study with the same design.

CYTOKINE TREATMENT

The use of cytokines to treat cancer goes back >30 years. High-dose interleukin-2 (HD IL-2) was approved in 1992 for metastatic renal cell cancer (mRCC) and in 1998 for metastatic melanoma (MM). This was not based on results from randomised, controlled, Phase III clinical trials, but on Phase II data. In both mRCC and MM, the overall objective response rate (RR) is around 15%. 4-7% of patients treated with HD-IL-2 obtain a complete remission (CR). These patients tend to do extremely well and can be considered cured in the majority of cases. Interferon-alpha (IFN-α) was studied in many types of cancer and was approved for the treatment of melanoma (adjuvant setting in the US), mRCC, and haematological malignancies (including chronic myeloid leukaemia and hairy cell leukaemia). Cures during IFN-α, however, are rare. In mRCC, IFN-α has provided a 3-month improvement in OS compared to medroxyprogesterone acetate (considered a placebo). In melanoma, the debate has been ongoing for many years about the benefit of IFN-α in melanoma as adjuvant treatment, between study groups in the US and Europe. An OS benefit could not be demonstrated. Based on subgroup analyses, patients with ulcerating primary melanomas may benefit from adjuvant IFN-α. A Phase III trial in Stage 2 (ulcerating disease) will investigate the role of IFN-α in this patient population.

IMMUNE CHECKPOINT INHIBITORS

Anti-CTLA4

Almost 30 years ago, CTLA4 was discovered. CTLA4 is an inhibitory cell surface receptor expressed on activated CD4 and CD8 T cells. Mice deficient of CTLA4 succumb to severe lymphoproliferative disease a few weeks after birth, indicating that CTLA4 is required to dampen an ongoing immune response. CTLA4 binds two receptors present on antigen presenting cells (APCs), predominantly DCs in lymph nodes. For proper T cell activation, apart from the interaction between the T cell receptor - unique for every T cell - and the MHC-peptide complex on the APC, a second signal is required. This signal is delivered...
by the co-stimulatory molecule CD28 on the T cell, upon interaction with CD80 or CD86 on the APC. Hours after full T cell activation, T cells start expressing CTLA4, which also binds to CD80/CD86 on the APC. Due to a higher binding affinity, CTLA4 outcompetes CD28 for interaction with CD80/CD86, therefore resulting in an inhibitory signal, dampening the T cell response.\textsuperscript{32}

It has been demonstrated that blocking the interaction between CTLA4 and CD80/CD86 results in potentiation of a T cell response, especially against self-antigens. In preclinical models, anti CTLA4 treatment showed anti-tumour activity, either as single agent or in combination with a vaccine depending on the tumour model that was used.\textsuperscript{33,34} Ipilimumab is a fully human monoclonal antibody (mAb) with high affinity for human CTLA4. Early phase clinical studies already showed activity of this drug as a single agent in patients with MM and mRCC.\textsuperscript{35} Indeed, side-effects observed in at least half of patients appeared immune related and resembled autoimmune diseases such as Crohn's disease, autoimmune hepatitis, thyreoiditis, uveitis, and an otherwise extremely rare disease, hypophysitis.\textsuperscript{36} Impressive objective responses were shown in 10% of patients. In 2010, the mature data from the first RCT were published, demonstrating a 4-month gain in median OS in favour of ipilimumab compared to the gp100 peptide vaccine as treatment for MM patients that had received one prior systemic therapy for advanced disease.\textsuperscript{37} Ipilimumab is the first drug after decades of clinical research to show improvement in survival in MM. Importantly, the Kaplan-Meier survival curves reached a plateau at 3 years after initiation of ipilimumab treatment. At that time, around 20% of patients were still alive, which was about 10% more than in the control group. Adverse events (AEs) were similar, as was already observed in Phase II trials, mostly immune related, with about 15-20% Grade 3-4 (colitis, hepatitis, etc.), but manageable mostly with high dose steroids. Only very few treatment-related deaths were reported. Based on these data, both FDA and European Medicines Agency approved ipilimumab for the treatment of MM. Ipilimumab is administered as four consecutive infusions at a dose of 3 mg/kg, every 3 weeks. The results from the first RCT were confirmed by a second trial in which dacarbazine was compared to the combination of dacarbazine and ipilimumab. In the ipilimumab-treated group of patients, the median OS was 2 months longer when compared to the control arm.\textsuperscript{38}

**Anti-PD-L1**

Several other immune checkpoint molecules have been discovered that may be displayed by T cells and other cells from the immune system during an immune response. Programmed death receptor-1 is expressed by activated CD4 and CD8 T cells.\textsuperscript{39} In contrast to CTLA4, which appears to play a role at an early stage during T cell activation, PD1 expression is important at the effector stage, within peripheral tissues or at tumour sites. PD1 can bind two ligands, PD-L1 and PD-L2.\textsuperscript{40,41} PD-L1 can be expressed on many cell types including tumour cells, whereas, so far as we know now, PD-L2 expression is limited to haematopoietic cells. Interaction of PD-L1 on tumour cells with PD1 on T cells results in an inhibitory signal to the T cell with diminished T cell receptor (TCR) signalling and the shutting down of cytolytic activity. Blockade of PD-L1-PD1 interaction can prevent this negative signalling and reinvigorate previously suppressed anti-tumour T cell activity. Several antibodies have been developed against PD1 and PD-L1 and all of these are either in early phase clinical trials or in Phase III RCT.

Nivolumab (Opdivo\textsuperscript{®}), a fully human IgG4 mAb, has been shown to effectively bind PD1, and has been tested in several cohorts of patients, including patients suffering from MM, mRCC, and NSCLC. In all three tumour types objective responses (18% in NSCLC and 27% in mRCC) have been observed.\textsuperscript{42} Interestingly, nivolumab is associated with fewer immune related AEs when compared to ipilimumab. The drug is well tolerated and is administered intravenously every 2 weeks. In melanoma, the objective RR observed in an extended Phase I study was 31%.\textsuperscript{43} In this cohort of 107 MM patients, the majority of whom were heavily pretreated, the median OS was 16.8 months, with impressive 1 and 2-year survival rates of 62% and 48%, respectively. Another anti-PD1 mAb, MK-3475 or pembrolizumab, a humanised IgG4 antibody, has demonstrated similarly impressive results in patients with MM and NSCLC.\textsuperscript{44} In a cohort of 113 MM patients, the objective RR was 40%. In NSCLC, monotherapy with pembrolizumab resulted in an objective RR of 21% (n=38), with a median OS of 12.8 months. Apart from anti-PD1, anti-PD-L1 antibodies have also been developed for clinical application. The first antibody, MDX-1105, was tested in a Phase I study in a large variety of cancer patients. In patients with MM, mRCC, NSCLC, and ovarian cancer, objective responses were observed. Also,
MDX-1105 was well tolerated and induced fewer immune related side-effects when compared to ipilimumab. MPDL3280A, another anti-PD-L1 antibody has been tested in patients with, among others, MM, mRCC, and NSCLC.\(^{45}\) Also, in these studies, objective responses were seen, some of which were durable. Initially, PD-L1 expression by the tumour appeared to be correlated with response to PD1/PD-L1 blockade. More recent (mostly unpublished) data indicate that patients with PD-L1-expressing tumours have a higher chance of responding to PD1/PD-L1 blockade, but that PD-L1 low or negative tumours can have objective responses as well. In addition, PD-L1 staining is complicated: several antibodies and companion diagnostic tests are being developed, but the inter and intra-test variability seems high, and apart from tumour cells expressing PD-L1, also stromal cells and lymphocytic infiltrates can stain positive. Next to CTLA4 and PD1/PD-L1, inhibitors of other immune checkpoint molecules such as LAG-3, TIM-3, BTLA, and others are (or will be) further developed.

### ADOPTIVE CELL THERAPY

Anecdotally, infusion of ex vivo cultured T cells has been successful; however, it was not until 2002 that Dudley and colleagues\(^{46}\) published an objective RR of 50% in pretreated MM patients using autologous ex vivo grown tumour-infiltrating lymphocytes (TIL) in a Phase I/II trial.\(^{46}\) TIL were isolated from a resected metastatic lesion and cultured to high numbers (1-10\(^{10}\) T cells) before reinfusion. Prior to infusion, patients received non-myeloablative chemotherapy consisting of high-dose cyclophosphamide (Cytoxan\(^{\text{®}}\)) and fludarabine (Fludara\(^{\text{®}}\)). Apart from generating physical space for the infused cells, preclinical studies had shown that prior lymphodepletion also removed so-called cytokine sinks and suppressive cell types such as regulatory T cells, so that the infused cells could have a head start before repopulation of the normal lymphocyte pool.\(^{47}\) In later studies this protocol was amended either by adding total body irradiation to the chemotherapy, requiring peripheral stem cell support for bone marrow recovery, or by culturing the TIL for a much shorter period of time.\(^{48-51}\)

Despite these changes, the objective RR averaged around 50% (40-70%) with a median OS of about 16 months in patients with MM. Interestingly, about 10% of these patients achieved CR; they tend to have an excellent prospective. For many years, investigators have tried to culture TIL from tumour types other than melanoma. This has been successful only recently so that adoptive cell therapy with TIL can now be tested in other cancers as well.\(^{52,53}\)

As it is still difficult to culture TIL from tumour types other than melanoma, an off-the-shelf product to treat many patients over many tumour types would be a solution to this problem. By genetically transferring TCR genes, encoding a receptor specific for a certain tumour antigen, into peripheral blood T cells, one can create a large army of tumour-specific T cells.\(^{54}\) This so-called TCR gene therapy has been, and is being, studied in several clinical trials. In the first trials, TCR derived from T cell clones specific for MART-1 or gp100 (MDA) were used for genetic transfer.\(^{55,56}\) The used transfer platform was a retrovirus that is capable of transducung T cells upon division and insert the TCR genes into the genome. Thus, obtained T cells do express this novel TCR and are specific for tumours that express the tumour antigen in the context of mostly HLA-A2. The objective response rates obtained with this versatile strategy, however, were lower than what was seen in TIL trials but the transduced T cells appear to be able to persist in the treated patients. Next to the use of TCR, others have used chimeric antigen receptors (CAR) instead.\(^{57}\) CAR are single chain antibodies that are linked to TCR signalling molecules such as CD3 zeta and CD28 or CD137. T cell transduction with CAR specific for the B cell antigen CD19 were able to recognise and lyse CD19+ B cells and B cell malignancies. Early clinical trials targeting CD19+ haematological malignancies. Early clinical trials targeting CD19+ haematological malignancies have shown very promising results and will be further studied in larger patient cohorts.\(^{58-60}\) CAR targeting other cell surface tumour antigens are under development.

### IMMUNE ESCAPE

Despite these amazing recent successes in immunotherapy of cancer, it is clear that immunotherapy is not a panacea as not all patients will respond to immunotherapy. Additionally, patients who respond originally may show disease progression later on. Therefore, one can distinguish intrinsic and acquired resistance to immunotherapy. Intrinsically resistant tumours are characterised by either lack of tumour immune infiltrates, or by strong local immune inhibiting mechanisms. The tumour microenvironment (TME) that has been recognised as equally important as the cancer cells themselves in tumour progression, invasion,
and metastasis formation may be highly hostile for effector T cells and prevent their homing and infiltration. TME consists of endothelial cells, stromal cells, myeloid cells, and immune cells. It is now well-established that many of these cell types may have tumour growth-promoting and immunosuppressive properties, rendering these tumours invisible for, or unresponsive to, the immune system. Among these cells are myeloid derived suppressor cells, tumour associated macrophages, and DCs that are highly immunosuppressive by production of immunosuppressive factors, including arginase-1, nitric oxide synthase, and indoleamine 2,3-dioxygenase. Other cell types that appear to play a role in preventing an effective anti-tumour immune response are the recently described regulatory B cells or Bregs. These B cells that may reside in the TME invariably produce IL-10, an immune-inhibitory cytokine that impairs normal DC and T cell function. Next to Bregs, regulatory T cells (Tregs), CD4+CD25+FoxP3+ T cells, which inhibit normal effector T cell function, can reside within the TME. Oncogene induced expression of T cell inhibitory molecules by tumour cells such as PD-L1 can also paralyse tumour-infiltrating lymphocytes. In some tumour types, PD-L1 expression is associated with PTEN deletion or an activating mutation in the phosphoinositide 3-kinase signal transduction pathway.

With acquired resistance, there is no evidence for altered T cell activation or homing. This type of resistance is enforced by mechanisms that interfere with T cell function within the TME. Many inhibitory mechanisms can be involved - including the induced expression of T cell checkpoint molecules and their ligands - that reduce the immune response, such as LAG-3 and its ligand MHC Class 2, TIM-3 and its ligand galectin-9, BTLA, and PD-L1.39

---

Figure 1: Cancer immune escape mechanisms.
Within the tumour micro-environment (TME) several mechanisms that help tumours to escape immune attack: regulatory B cells (Bregs) produce immunosuppressant cytokines including interleukin (IL)-10 and transforming growth factor-beta (TGF-β); regulatory T cells (Tregs) directly inhibit the function of effector T cells (Teff); myeloid derived suppressor cells (MDSCs) suppress effector T cells through arginase-1; tumour-associated macrophages (TAM) inhibit effector T cells through nitric oxide produced by NO synthase (NOS); immature dendritic cells (immature DC), but also tumour cells highly express indoleamine 2,3-dioxygenase (IDO) leading to tryptophane deprivation, which inhibits effector T cell function.

PD1: programmed death receptor
The latter may be induced at the tumour cell surface as the result of IFN-α signalling upon recognition and binding of cognate tumour antigen by the infiltrating T cells, which limits further T cell effector function by engaging its ligand PD-1 on the T cells. In addition, an effective immune response may select for tumour cell subpopulations with loss or defects in the antigen processing and presentation machinery, like loss of MHC Class 1 expression, thereby hiding the tumour cell from the immune system. Similarly, immune evasion may occur through a process called immune-editing: selection of tumour subclones present within heterogeneous tumours, lacking one or multiple antigens that are subject to strong Darwinian selection.

CONCLUSION

Immunotherapy has developed from a promising treatment strategy to an adult and established cancer therapy. In the next decade, it is expected that immunotherapy will become a standard of care for many cancer patients beyond melanoma. First results coming from patients treated for mRCC and NSCLC are promising, and it is highly likely that other tumour types will follow. This can be achieved by single agent treatment, but more likely by combination therapy, such as combination of immune checkpoint inhibitors with chemo or targeted therapy, and ACT in combination with immune checkpoint inhibitors.

REFERENCES

27. Eggermont AM et al. Post-surgery adjuvant therapy with intermediate doses


