Review Article

# Cancer Immunotherapy – The Target is Precisely on The Cancer and Also Not

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## Abstract

**In recent years, the impressive number of cancer immunotherapy drugs approved has been unprecedented—building on over a century of understanding on how the immune system combats cancer, and how cancer evades it. Leading the charge are the immune checkpoint inhibitor monoclonal antibodies, and adoptive cell therapy with chimeric-antigen-receptor (CAR)-T cell therapy. These breakthrough therapies have led to improved survival in patients with many advanced cancers. Some of the clinical outcomes have been striking, and may even be potentially curative in some terminal cancer patients. While immune checkpoint inhibitors work by blocking regulatory immune checkpoint signals between cancer and the immune cells to awaken an effective anticancer immunity, CAR-T cell therapy targets specific molecules on cancer cells. Tumour antigens as cancer targets take many forms and may not necessarily be proteins related to known functional cellular mechanisms. The convergence of cutting edge omics, bioinformatics, protein synthesis, immunobiology and immunotherapy have led to novel, potentially highly effective cancer targeting against neoantigens, hence reviving the quest for anticancer vaccines. Early clinical trials of neoantigen vaccines have provided proof-of-principle efficacy, especially in melanoma patients. Combinations of immunotherapies through rational design are underway aiming to further improve clinical outcomes. Moving forward, cancer immunotherapy will gain even more momentum from the discovery of more cancer targets—both on the cancer itself and in the tumour microenvironment as well as the identification of biomarkers of treatment resistance and efficacy.**

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## Introduction

The immune system is intricately designed with unerring sophistication to identify—and if necessary—destroy invading pathogens, classically by distinguishing "self" from "non-self" proteins. The immune system can also attack "self" proteins such as when immune dysregulation results in autoimmune disease and/or when a danger signal triggers an immune response to "self" proteins. A role for the immune system in cancer surveillance and control has long been suggested, supported by evidence such as the observation of higher incidence of malignancies in immunocompromised patients (e.g. organ transplant patients on immunosuppressive drugs and acquired immunodeficiency).

Cancer immunotherapy harnesses the immune system to target cancer—either directly or indirectly. Although cancer cells originate from the patient's own cells, they may be potentially recognised as foreign and be targeted by the immune system due to aberrant expression of tumour-associated antigens (TAA) that are not normally expressed or at significantly lower expression levels by normal cells. These antigens could be viral antigens in virus-associated cancers, such as Epstein-Barr virus (EBV) antigens in nasopharyngeal carcinoma (NPC) and post-transplant lymphoproliferative disease or human papillomavirus (HPV) in HPV+ oropharyngeal cancer and cervical cancer; or "self" antigens such as the cancer/

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testis antigens, whose expressions are found in germ cell cancers like testicular cancer, colorectal cancer, non-small cell lung cancer (NSCLC) and melanoma. Targets on cancer cells for immunotherapy need not have known mechanistic functions in the cancer cell machinery. Conversely, they could be critical receptors of oncogenic signalling such as cerb-B2 (HER2/neu) in breast cancer.

Immune recognition of TAAs is commonly impaired in cancer patients largely due to immune tolerance and an inhibitory immune suppressive tumour microenvironment.[1,2] The immune suppressive components include regulatory T lymphocytes (Tregs), tolerogenic dendritic cells (DCs), myeloid-derived suppressor cells and angiogenic factors, all of which contribute to counteract specific cancer immunotherapy.[3] Immune evasion is now recognised as one of the major hallmarks that contributes to cancer emergence.[4] Immunotherapy strategies for cancer are therefore designed to directly target cancer proteins or restore and activate effective immunity against the cancer cells.[5,6] In the last few years, monoclonal antibodies specifically targeting immune checkpoints between cancer and immune cells such as the fusion protein cytotoxic T-lymphocyte-associated antigen 4-IgG1 (CTLA4Ig), programmed cell death protein 1 (PD-1) and programmed death-ligand 1 (PD-L1) inhibitors, and adoptive T cell therapy with CAR-T cells targeting the CD19 protein on B cell malignancies have shown convincing and often striking clinical benefit[7]. Monoclonal antibodies that target other tumour stromal and immune components, such as anti-vascular endothelial growth factor (VEGF) and anti-CD25 antibodies that target the vasculature and regulatory T cells, respectively, are also being actively explored (the former already established in clinical practice across tumour types).[8,9] These immunotherapies have led to cancer immunotherapy being hailed as "Breakthrough of The Year" by one of the leading scientific journals, 'Science', in 2013.[7]

In 2017, the Food and Drug Administration (FDA) approved CAR-T cell therapy tisagenlecleucel (Kymriah) targeting the protein CD19 for the treatment of relapsed acute B cell leukaemia in children and young adults, and similarly, CAR-T cell therapy axicabtagene ciloleucel (Yescarta) for the treatment of advanced B cell lymphoma. Kymriah achieves complete remission in 80% to over 90% of relapsed cases (mostly heavily pretreated acute B cell leukaemias). Yescarta renders 50% of pretreated advanced B cell lymphoma into remission. The basis of CAR-T cell technology is single-chain variable fragment (scFv) portion of an anti-CD19 immunoglobulin transduced by a lentivirus into T cells; and this receptor has an integrated signalling domain that unleashes a potent costimulatory signal to in vivo activate and expand CAR-T cells in patients with B cell leukaemia and lymphoma[10]. T cell therapy differs from classical antineoplastic drugs such as cytotoxic chemotherapy, antibodies and small molecules. There are regarded as "living therapy", can expand exponentially in vivo, and potentially persist in the body for months and years. Immune checkpoint inhibitors generally have a half-life of a few weeks.

Immune checkpoint inhibitor antibodies are the other major paradigm shift in the war on cancer—that immunological targets against cancer do not come from targets on the cancer itself. Instead, these antibodies target the axis of immune checkpoints that regulate specific T cell-mediated immune responses against cancer. Landmark clinical trials of CTLA4Ig, PD1 and PDL1 Inhibitors proving improved survival in cancer patients have led to a quick succession of FDA regulatory approvals in the last few years for cancers including malignant melanoma, Hodgkin's lymphoma, bladder cancer, head and neck cancer, NSCLC, gastric cancer, Merkel cell carcinoma, renal cell cancer, hepatocellular carcinoma, microsatellite instable ($MSI^{high}$) colorectal cancer and any cancer with a high mutational burden.[11]

## The Early Years and Fears

The earliest observation that the immune system may be stimulated to attack cancer (demonstrated in pioneering clinical experiments) can be dated back to the 1860s (Fig. 1). Dr Rudolf Virchow, an eminent 19th century German physician, observed and described infiltration of leukocytes in cancer tissues and was the first to hypothesise a connection between the immune system and cancer.[12,13] Also around the same time, 2 other German physicians, Drs William Busch and Friedrich Fehleisen, noticed in some cancer patients that their tumours regressed following accidental infection by erysipelas caused by *Streptococcus*. Soon after, in 1868, Dr Busch became the first physician to treat cancer by deliberately infecting patients with bacteria. He infected a patient with an inoperable soft tissue sarcoma of the neck with erysipelas and reported noticeable shrinkage of the tumour.[14] In the 1890s, American orthopaedic surgeon, Dr William Coley, treated terminal bone and soft-tissue sarcoma patients with "Coley's toxins"—a vaccine comprising attenuated *Streptococcus pyogenes* and *Serratia marcescens*. The vaccine which aimed at non-specifically stimulating the immune system against the patients' cancers[15] had been provided to him by German bacteriologist, Dr Robert Koch. A significant number of his patients achieved tumour regression—fever and chills as side effects notwithstanding—and there were even reported cures![16] At that time, Russian physician and renowned writer Anton Chekhov, was also convinced that bacterial infection like erysipelas could activate an immune response against cancer.[17] The field waned, and active research in

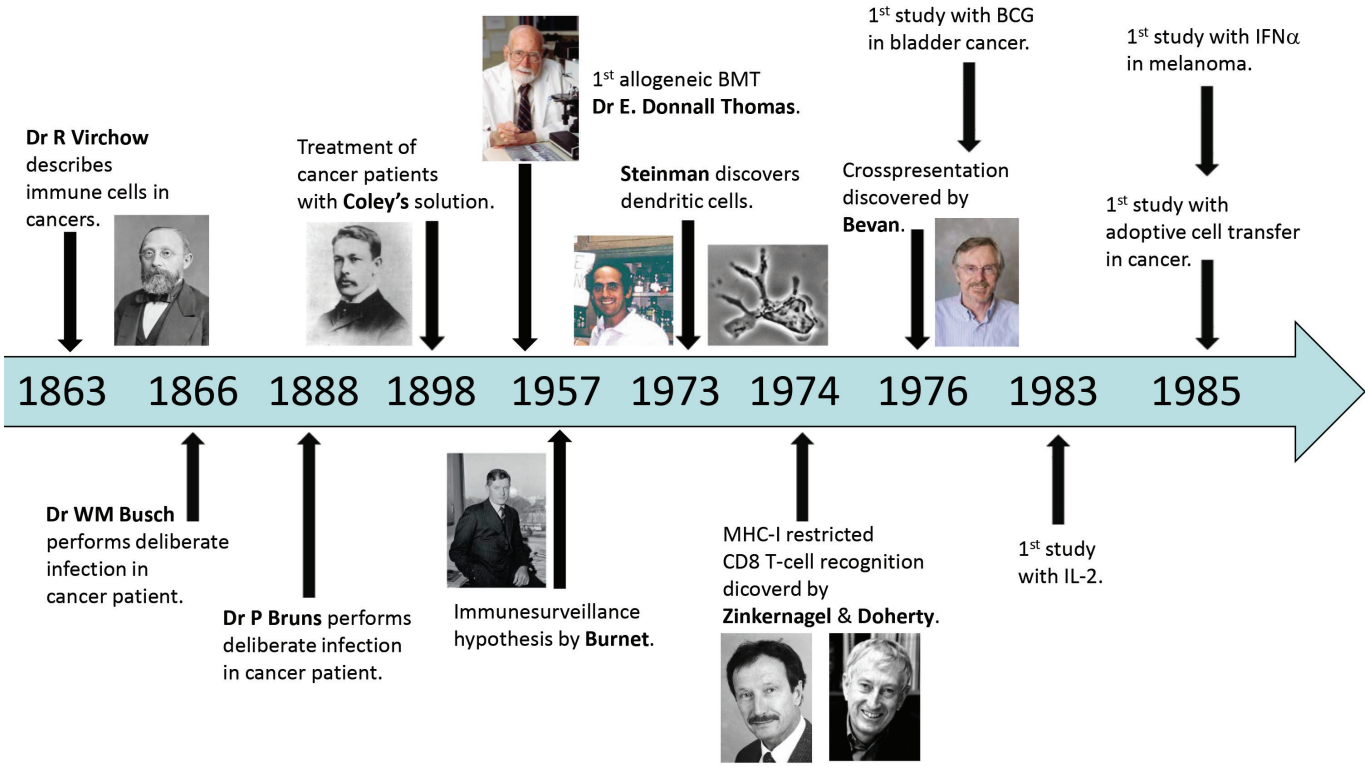


Fig. 1. Chart showing the key milestones in cancer immunotherapy development. (Reproduced with permission from Dr Jens Samol, Johns Hopkins Singapore International Medical Centre).

cancer immunotherapy only rekindled in the mid-twentieth century. Such earlier efforts successfully led to the regulatory approval of intravesicular Bacillus Calmette Guerin (BCG) vaccine for superficial bladder cancer in 1990.[18]

## Cancer Vaccines – Waning or Winning

Cancer vaccine development gained momentum in the 1990s. The rationale for this targeted cancer immunotherapy strategy is to boost anti-TAA immune response to activate and expand TAA-specific cytotoxic T lymphocytes (CTLs) to kill tumour cells expressing the tumour antigens. Therapeutic cancer vaccination can be achieved by TAA-pulsed antigen-presenting cells, such as DC derived from peripheral blood monocytes or designing peptides of the said TAA or encoding the TAA in a vector such as a replication-deficient virus to be delivered to the patient or given as a peptide vaccine by itself. There are many ways cancer antigens can be delivered on a cancer vaccine vehicle. Overall, therapeutic cancer vaccines have been proven safe with minimal adverse events.

Over the decades, numerous cancer vaccines have been developed and tested in clinical trials—with the most promising results seen in lymphoma and hormone-refractory prostate cancer patients.[19,20] However, the clinical efficacy of especially the earlier generation of therapeutic cancer vaccines have in general been disappointing.[21,22] In a meta-analysis of all major cancer vaccine studies in colorectal cancer, an overall objective response rate of only 3.3% was observed in cancer vaccine treatments of over a thousand cancer patients with advanced disease.[22] The lack of efficacy is likely due to poor immunogenicity of the tumour, local and systemic immune suppression mediated by the growing tumour and its local environment, and tumour evasion of the immune system such as downregulation of the major histocompatibility antigen (MHC; or termed 'human leukocyte antigen', HLA, in humans).[3,23] An ideal cancer vaccine needs to target a uniquely expressed TAA, and also overcome immune tolerance. Examples of self TAA candidates include WT1, MUC-1 and the family of cancer/testis antigens such as NY-ESO-1, MAGE and SSX.[24] Strategies to further augment the specific anti-TAA immunity include adding immunoadjuvants and immune potentiating molecules into the cancer vaccine constructs.

A widely adopted cancer vaccine vehicle to optimise tumour antigen presentation is the DC vaccine. While the tumour-associated inhibitory mechanisms hamper the functions of endogenous DCs, ex vivo generated DCs are free from such inhibition during their development. DCs

are the most powerful professional antigen presenting cells (APC) capable of presenting relevant antigens to the adaptive immune system. We conducted a single-arm phase II MAGE-antigen (classic self TAA) lysate pulsed autologous DC vaccine study in advanced colorectal cancer patients who had received prior chemotherapy—many having been treated with multiple drugs. Twenty patients, biopsy-proven to express at least 1 of 6 MAGE antigen expression (identified as part of the tumour lysate), received up to 10 biweekly intradermal vaccinations of MAGE antigen-pulsed (an allogenic lysate) autologous DCs. The DC vaccine clinical benefit rate was 40% (1 partial response, 7 stable disease), with two stage 4 colon cancer patients surviving more than 6 years, 1 with multiple lung metastases remains alive after over 10 years.[25] These long-term survivors with advanced cancers are a small, select group characteristic of immunotherapy treatments including immune checkpoint inhibitors, and have been termed "supersurvivors".

We also completed a phase II clinical trial of an autologous DC vaccine transduced with an adenoviral vector to express the NPC-associated Epstein-Barr virus antigens LMP-1 and LMP-2 (classic non-self viral proteins) in 16 heavily pretreated advanced NPC patients. Two patients (12.5%) achieved disease stabilisation for over 18 weeks and 1 patient achieved partial response, leading to an overall clinical benefit of 19%.[26]

With innumerable clinical vaccine trials published since the 1990s, there is still only 1 FDA-approved therapeutic cancer vaccine—Sipuleucel-T (Provenge)—based on a landmark phase III study of in vivo infusion of activated monocytes (a DC-like approach) in 512 advanced hormone-refractory prostate cancer patients. Sipuleucel-T comprises autologous peripheral blood cells pulsed ex vivo with a fusion protein of prostatic acid phosphatase (PAP) antigen as target plus granulocyte-macrophage colony-stimulating factor (GM-CSF), included to activate endogenous DCs (PAP-GM-CSF). This cell-based vaccine's proven improved overall survival benefit in patients with advanced hormone-refractory prostate cancer led to it becoming the historic first-approved cellular therapy product in any cancer.[27]

New generation therapeutic cancer vaccination strategies include incorporating immune-modulating elements into the vaccine construct. In a first-in-human phase I clinical trial that we recently completed, 18 epithelial cancer patients with advanced disease were subcutaneously administered an adenoviral vector that encodes a fusion protein of the MUC-1 antigen and the extracellular domain of CD40L (Ad-sig-hMUC-1/ecdCD40L).[28] This vaccine via CD40L aims at activating the endogenous DCs that would potentially further improve MUC-1-specific immunity. MUC-1 is a polymorphic, type I transmembrane protein expressed at low levels on the apical surface of normal epithelial cells, which functions to stabilise the protective layer of mucous. It is highly expressed on neoplastic cells in 90% of epithelial cancers of the breast, ovary, colon, prostate, and lung.[29-31] In these epithelial cancers, MUC-1 overexpression disrupts E-cadherin function, leading to anchorage-independent tumour cell growth and metastases.[30,31] MUC-1 is hypoglycosylated in cancer cells, making it a prime TAA vaccine candidate. Several MUC-1-based cancer vaccine clinical trials had been conducted, including a recombinant MUC-1+IL-2 encoding vaccinia virus vector vaccine for advanced prostate cancer patients, as well as a viral vector-based vaccine clinical study in NSCLC patients.[32,33] CD40L is a strong adjuvant for induction of antigen presenting cell activation. It binds to DCs and induces cytokine production, leading to tumouricidal activity and proliferation of activated T cells.[34] In the preclinical murine model study, this Ad-sig-hMUC-1/ecdCD40L vaccine activated DCs and induced a potent CD8+ tumour suppressive immune response against hMUC-1 antigen, breaking tolerance in old mice where anergy exists to these antigens.[35,36] This vaccine, on an adenoviral backbone, was shown to be safe at all dose levels (tested with increasing viral titres and with no grade 3 or more toxicity). Clinical efficacy is observed and full evaluation is ongoing (unpublished).

One of the key reasons for the underperformance of therapeutic cancer vaccines is that it is normally given to heavily pretreated patients with large, aggressively growing tumour, an immunosuppressive network, and an anergic, exhausted ineffective immune system. To avoid this disadvantage, the landmark MAGRIT Trial aimed to evaluate the benefit of a MAGE-A3 peptide vaccine with an immunostimulant in surgically resected NSCLC patients. This randomised clinical trial recruited 2312 NSCLC patients—the largest therapeutic cancer vaccine trial ever conducted—to evaluate if the peptide-based cancer vaccine could significantly reduce cancer relapse (disease-free survival) in these cancer-free patients. It failed to meet its objective.[37] This negative phase III clinical trial dealt a big blow to the field of cancer vaccines.

**A New Dawn with Neoantigens**

In the decades that have been characterised with more disappointments than successes in identifying ideal tumour targets to optimise clinically impactful cancer vaccine strategies, a new window has opened. Genome instability that underlies the hallmarks of cancer allows tumour to acquire mutations that help it gain survival advantage.[4] These mutations, both driver (implicated in oncogenesis) and passenger (that do not confer a growth advantage), may generate proteins that are not part of the individual's proteome and are exclusively expressed by the tumour

cells.[38-40] When classically processed by the body's antigen-presenting machinery into short peptides, these antigens (referred to as tumour-specific neoantigens [TSAs]), are then presented by major histocompatibility complex (MHC) on the cell surface to the immune system. The recognition of these neoantigens as foreign thereby initiates an antitumour immune response. These ever-evolving neoantigens have not been subject to time-dependent immune tolerance. Therapeutic strategies that aim to identify the individual's TSAs, and utilise the ability of the immune system to recognise self and non-self, are hence the epitome of this new era of personalised and precision medicine that go beyond targeting oncogene addiction.

One of the earliest preclinical studies that demonstrated the ability of the immune system to recognise neoantigens was led by Boon et al.[41,42] In vitro mutagen-induced mouse tumour cell lines that expressed aberrant peptides failed to form tumours when injected into syngeneic mice, as opposed to the original tumour cell lines. Through a gene transfection method, they were able to identify the specific mutations that generated neoantigens. These neoantigens could be recognised by cytolytic T cells. In more recent years, clinical studies demonstrated the presence of T cells in melanoma patients that were able to recognise and generate antitumour response against TSAs.[43-45] Collectively, these studies helped to pave the way in developing better immunotherapy as the next cornerstone in cancer treatment. And so the field of cancer vaccines has been revived from its near-death journey. By the same principle, immune checkpoint inhibitors unleash T cells that can recognise neoantigens, as evidenced by its unique efficacy in cancers with a high mutational burden (such as $MSI^{high}$ colorectal cancer).[11]

Technological advancements in recent years have enabled identification of TSAs employing different strategies. A common in silico approach guided by exome sequencing of the individual's tumour and matched normal tissue, first identifies somatic mutations found within the tumour.[39,46-48] The mutated deoxyribonucleic acid (DNA) sequences are translated to their corresponding amino acid sequences and non-synonymous mutations are selected for. Using various filtering steps which may include transcriptomic data to identify expressed genes, proteosomal processing, peptide transportation and MHC-binding prediction algorithms, these candidate neoantigens are then identified and prioritised. The TSA landscape has not surprisingly been noted to be highly variable both within and across tumour types.[49]

Both preclinical and clinical studies have shown that only a small fraction of the predicted neoantigens is capable of eliciting T cell reactivity.[50,51] Using next generation sequencing data and the NETMHC-3.4 prediction algorithm, Yadav et al identified 170 and 6 predicted neoepitopes in MC-38 and TRAMP-C1 murine tumour models, respectively. Simultaneously, mass spectrometry analysis was performed on the tumours to identify MHC Class I presented epitopes and only 7 predicted neoantigens in MC-38 were identified. Of these, 3 neoantigens were validated to be immunogenic through in vivo immunisation of murine models. Through mining exome data of melanoma tumours derived from 3 patients and using NetMHC prediction algorithm, Robbins et al identified neoepitope candidates that were predicted to bind with their respective HLA with high affinity. Only 3% to 6% of these tumour-specific neoantigens were found to be recognised by corresponding tumour-infiltrating lymphocytes. With the understanding that the various in silico prediction algorithms have differing focus, strengths and weaknesses, a large consortium from more than 35 research groups united to help refine, validate and identify the best algorithms.[52]

As clinical proof-of-principle of this new translational technology, the Rosenberg group successfully treated a patient with metastatic cholangiocarcinoma and another with metastatic colon cancer using an adoptive T cell approach.[53,54] Through whole-exome sequencing of the tumours, TSAs were identified and evaluated for reactivity with the corresponding tumour-infiltrating lymphocytes. These neoantigen reactive T cells were then expanded ex vivo and infused back into the patients, resulting in remarkable objective tumour regression. Another therapeutic approach using highly personalised TSA vaccines has been reported in 3 recent phase 1 studies in melanoma.[55-57] With each study utilising a different vaccine delivery approach (i.e. DC vaccine, peptide vaccine and ribonucleic acid [RNA]-based poly-neo-epitope vaccine), early readouts to detect T cell responses against TSAs were seen across the studies. Several important observations are noteworthy from these studies. Such neoepitope vaccines may be used as powerful adjuvant treatment in cancer patients at high risk of relapse (as illustrated in the melanoma studies where the vaccines were delivered to already cancer-free melanoma patients at high risk of relapse). Compellingly, these high-risk patients did not relapse after several years postvaccination. Also, when the neoepitope vaccine is combined with an immune checkpoint inhibitor, the clinical efficacy appears even more potent, capable of inducing complete tumour remissions.[56,57] While early results are promising, larger studies are required to address the relevant therapeutic endpoints, optimal vaccine delivery methods and the potential synergism in combining vaccine with other treatments. A limitation is that the time from neoantigen discovery to vaccine delivery is still in months than days but this will improve with further advancements in technology, bioinformatics and production.

The route to personalised, precision cancer immunotherapy comes closer to reaching its destination. New biomarkers, and an even greater understanding into the tumour-immune interaction will be crucial in contributing to this eventual success.

## Conclusion

Historically, cancer therapy has followed the path of the proverbial William Tell[58]—with his bow and arrow aiming directly at the apple (cancer target) above his nervous son's head (normal cells). In those ancient days, the arrows were blunt tools that did not always fly straight. Today, arrows are much sharper, stronger and more precise. Also, another revolutionary paradigm has emerged—that cancer immunotherapy can also activate surrounding, previously inactive immune cells to hit the cancer target. This is like William Tell calling on nearby sleepy birds to swoop down and eat the apple. Combining 2 "arrows" is likely going to be even more effective in some cancers than using 1 "arrow", as long as the cumulative toxicities are manageable.

As Winston Churchill once said, "Now this is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning". So, too, with cancer immunotherapy.

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