Immunotherapy of cancer

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Background

For 50 years, cancer physicians have relied on just three primary treatment modalities: surgery, radiation therapy and chemotherapy. Over that time, enormous progress has been made in understanding cancer biology, targeted anti-cancer drugs have emerged, and thousands of clinical trials have taught us how best to craft treatment combinations that improve clinical outcomes. Only five years ago, a fourth and radically different form of therapy finally emerged: immune-based cancer therapies.

Objective

This review briefly outlines the history and theoretical framework underpinning cancer immunotherapy, and recent progress on several immunotherapeutic approaches.

Discussion

Immune-based cancer therapies are already revolutionising the management of several types of hitherto intractable cancer, while offering immense hope that the burden of personal suffering and community cost due to cancer will diminish appreciably over the coming decades. At least two immunotherapeutic approaches, checkpoint inhibition and cellular therapy with autologous ('self') chimeric antigen receptor T cells (CAR T cells), now show indisputable evidence of efficacy in several cancer types, and promise yet more rapid progress as they are refined and we learn to combine them with existing conventional therapies and each other.

The idea of using the immune system to treat cancer is traceable to the 1890s, the time of Pasteur. The first clinician to use an immunological approach was the surgeon William Coley of Memorial Hospital, New York (now Sloan Kettering Cancer Institute). Coley noted that, rarely, cancers would regress spontaneously, but only when local abscesses or other purulent bacterial infections were present. 'Coley's toxins' (cocktails of live bacteria) were repeatedly injected to produce prolonged pyrexia, but the associated high mortality first led to vaccines with killed bacteria and, ultimately, abandonment of the approach in the 1930s.[1]

Australian Nobel Laureate Macfarlane Burnet was a devotee of the theoretical links between cancer causation and immunity, and advanced his radical hypothesis of 'tumour immune surveillance' in the 1950s. Burnet proposed that, as well as protecting against pathogens, the immune system routinely detects and destroys pre-malignant cells and that clinical cancer, therefore, reveals 'failed immune surveillance'. However, it took 50 years for this view to gain acceptance.[2] In support of Burnet, very rare spontaneous regressions of advanced melanoma were typically accompanied by vitiligo (loss of normal skin pigmentation), suggesting the immune system was responding to antigens shared by normal and malignant melanocytes. By the 1990s, advanced technologies proved that clones of CD8+ cytotoxic T lymphocytes (CTLs) could kill both normal and malignant cells presenting fragments of abundant melanocyte differentiation antigens such as tyrosinase or Melan A/Mart-1.[3]

Further evidence of cancer immune surveillance came from international registries of chronically immunosuppressed, solid organ transplant patients; by far the greatest relative risks were for cancers caused directly by viruses.[4] Formal demonstration of 'immune surveillance' in the absence of oncogenic viruses came in the 1990s when Schreiber showed that mice lacking the immune effector molecule interferon-gamma (IFNγ) have a high incidence of spontaneous sarcoma and lung adenocarcinoma.[5] Our laboratory reported that mice congenitally lacking perforin, a protein toxin essential for CTL and natural killer (NK) cells to kill target cells, develop fatal B cell lymphoma.[6] In humans with colorectal cancer, Galon et al made the remarkable finding that

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infiltration of cancerous tissue with activated CD8+ T cells is a better predictor of long-term survival than the extent of anatomical spread of the tumour.[7] More recently, we also showed that patients who inherit mutations of the perforin (PRF1) gene develop a variety of blood cancers during childhood/adolescence.[8–10]

Immune checkpoint inhibitors

Being derived from self, malignant cells express few 'foreign' antigens (those arising from mutation), so most approaches over the decades aimed to make cancer cells visible to the immune system (immunogenic). Numerous vaccines combined patient antigen presenting cells (APC, commonly dendritic cells), purified cancer antigens and an adjuvant such as a cytokine to activate CTL responses, but a reproducible benefit was not achieved in any disease. In retrospect, the dramatic success of immune checkpoint blockade, even in advanced melanoma, non-small cell lung cancer and renal cancer indicates that many cancers are actually spontaneously immunogenic, but the immune response is inhibited by factors in the tumour microenvironment.[11]

What is an immune checkpoint?

Checkpoints enable us to limit normal immune responses, both to pathogens and self-antigens. Two signals are required to activate a cellular immune response. Each individual CD8+ CTL expresses a clonotypic receptor that binds a peptide fragment presented on major histocompatibility complex (MHC) molecules on the target cell surface (signal 1). A second, co-stimulatory signal is also required for a robust, sustained response leading to target cell death, cytokine secretion and the formation of memory T cells. Signal 2 is generated by binding of CD28 with ligands B7-1 (CD80) or B7-2 (CD86) on the target. Some days later, a 'checkpoint' is applied: stimulatory CD28 is replaced on the T cell surface by inhibitory CTLA-4, and the signal is switched off.

Many cancers aberrantly express ligands for CTLA-4 at high levels, thus imposing a strong negative signal. Therapeutic 'checkpoint inhibitor' antibodies such as anti-CTLA-4 (epilumumab, 'Yervoy') block this interaction, to disinhibit and amplify a pre-existing immune response to cancer. The second critical inhibitory receptor/ligand combination targeted by checkpoint blockade is between T cell PD-1, and PD-L1 or PD-L2 on cancer cells and APCs. This interaction occurs later in the immune response and leads to inactivation through 'exhaustion' or programmed death (thus 'PD') of the CTL.

Figure 1. In May 2010, when Emily Whitehead was 5 years of age, she was diagnosed with acute lymphocytic leukaemia (ALL). Her cancer resisted several rounds of chemotherapy (left). In April 2012, she was enrolled in a Phase I trial of CD19-CAR T cell therapy and within a month, her disease went into remission and she is still cancer free today (right) (PMID: 25317870).

Photos reproduced from Emily 'Emma' Whitehead: My journey fighting leukemia, with permission from Emily Whitehead Foundation

Table 1. Published reports of CAR T cells in clinical trials

Cancer type	Target antigen	Year reported	Number of patients	Responses	Reference
ALL	CD19	2014	30	27 CR	20
AML	Lewis Y	2013	4	0	21
Colorectal and breast	CEA	2002	7	Minor response in 2 patients	22
Colorectal	TAG-72	1998	16	1 SD	23
Neuroblastoma	CD171 GD2	2007 2011	6 19	1 PR 3 CR	24 25
Ovarian	αFR	2006	12	0	26
RCC	CAIX	2011	11	0	27, 28
Prostate	PSMA	2013	5	2 PR	29
Sarcoma, primitive neuroectodermal tumour, desmopastic small round cell tumour	HER2	2015	17	4 SD	30

αFR, alpha folate receptor; ALL, acute lymphocytic leukaemia; AML, acute myeloid leukaemia; CLL, chronic lymphocytic leukaemia; CR, complete response; OR, objective response; PR, partial response; RCC, renal cell carcinoma; SD, stable disease

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Immune checkpoints targeting the PD-1 pathway have generated high interest, with overall response rates across tumour types averaging 20–30%. This includes responses in melanoma, non-small-cell lung cancer, small-cell lung cancer, kidney cancer, Hodgkin lymphoma, hepatocellular cancer, bladder cancer and breast cancer. Early clinical data in patients with melanoma indicate that combined blockade of both CTLA-4 and PD-1 (ipilimumab/nivolumab) pathways leads to even better survival.

Checkpoint antibodies are administered by intravenous infusion and have half-lives of about three weeks. Repeated administration is necessary and we urgently need to decipher when treatment can safely be tapered, so that costs can be contained. Although well tolerated overall, about 10% of checkpoint blockade recipients develop serious autoimmune effects, such as inflammatory colitis, myocarditis, pneumonitis or skin rashes, that require specific management.[12,13]

Repurposing anticancer antibodies with chimaeric antigen receptor T cells

Therapeutic antibodies, either 'naked' or conjugated to cancer drugs or radioactive isotopes (both for imaging and therapy), have been used for many years, and can mediate their effects via the immune system. For example, trastuzumab (Herceptin), which binds the HER2 antigen on breast cancer, has two effects: apart from blocking growth signals to cancer cells, its Fc (non–antigen binding) domains also engage circulating NK cells, enabling them to directly kill cancer cells through antibody-dependent cellular cytotoxicity.[14]

In chimeric antigen receptor (CAR) T cell therapy, the payload delivered by antibodies consists of potent anticancer T cells rather than toxins or isotopes. This form of adoptive T cell immunotherapy sidesteps the need for pre-existing cancer immunity. Rather, an immune response is generated by manipulating a patient's own CD8+ T cells *ex vivo*, with subsequent re-infusion of the activated cells into the patient.

The technology that preceded CAR T cells was tumour infiltrating lymphocyte (TIL) therapy. TILs present in a fresh surgical specimen are isolated in the lab, induced to proliferate and become 'activated' with tumour antigen and growth factors such as interleukin (IL)-2, and then re-infused with IL-2 to sustain them *in vivo*. Encouraging responses were noted in patients with melanoma and renal cancer, particularly where patients had received 'pre-conditioning' chemotherapy, but complete response rates have rarely exceeded 7% in large clinical trials, and benefit is limited by toxicity of co-administered systemic IL-2.[15]

CAR T cell therapy has recently been shown to overcome these limitations. It uses replication-deficient retroviruses or

Table 2. Current immunotherapies for cancer

Immunotherapy	Target antigen	Clinical indication	Reported toxicities
*Ipilimumab (Yervoy)	CTLA-4	Various; Hodgkin lymphoma, myeloma, AML, CML, CLL, NHL, melanoma	Arthritis, hyperthyroidism, pneumonitis, colitis, bowel perforation, vitiligo, hypophysitis
*Nivolumab, pembrolizumab	PD-1	Melanoma, RCC, NSCLC, prostate	Colitis
*Alemtuzumab	CD52	CLL	Destruction of normal leukocytes, leading to susceptibility to infections
*Rituxumab	CD20	Lymphoma	Suppression of B cells leading to deficiency in immunoglobulin and infections
*Trastuzumab (Herceptin)	HER-2	Breast	Cardiac toxicity
*IFN-α		CML cutaneous T cell lymphoma	Thyroiditis, sarcoidosis, systemic lupus erythematosis associated with auto-antibodies
*IL-2		Renal cell carcinoma, melanoma	Acute cardiorespiratory failure, hypothyroidism, myasthenia gravis, type 1 diabetes mellitus and myositis
*Sipuleucel-T cancer vaccine (Provenge)	Prostate-specific antigen	Prostate	Fever, chills, fatigue, back and joint pain, nausea, and headache, high blood pressure
TCR gene-modified T cells	MART-1, gp100	Melanoma	Vitiligo
†CAR-modified T cells	Various antigens ie CD19	Lymphoma, ALL, CLL	B cell depletion

**FDA-approved for specific clinical indications*
†Refer to Table 1
ALL, acute lymphocytic leukaemia; AML, acute myeloid leukaemia; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; NHL, non-Hodgkin lymphoma; NSCLC, non-small cell lung cancer; RCC, renal cell cancer

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lentiviruses to express, on a patient's CD8+ T cells, antibody-like receptors capable of binding to a target antigen on cancer cells. Key for success are twin signalling motifs engineered into cytoplasmic domains of the CAR, which mimics the two signals (T cell receptor and co-stimulatory CD28), enabling the T cells to produce their own IL-2 *in vivo*. When a CAR T cell encounters a cancer cell, the result is antigen-driven proliferation, cytokine secretion, longevity and powerful perforin-mediated killing of cancer cells.[16]

Generally, 1–10 billion CAR T cells are expanded in specialised 'clean rooms' over 10–12 days. Single infusions of CAR T cells have been highly effective in lymphoid malignancies, particularly acute lymphocytic leukaemia, where the targeted antigen is the B cell differentiation antigen CD19 (Figure 1), but other B cell malignancies and solid cancers are currently being studied in extensive clinical trials (Table 1). In contrast to TILs, CAR T cells are achieving complete and durable remission in 50–80% of paediatric acute lymphocytic leukaemia, even after all other treatment options have failed.

The immunosuppressive tumour microenvironment

Why are checkpoint blockade and CAR T cells not universally effective? There are many reasons, but local suppression of the immune system by cancer is clearly a major contributor. The genetic instability of cancers means they can stop expressing antigens targeted by T cells, or lack the mechanisms that present them. Cancer cells may 'switch on' immunosuppressive cytokines such as TGFß or IL-10, or induce local stromal cells to do so. The tumour may now attract suppressive 'regulatory' T cells (Treg) and myeloid-derived suppressor cells (MDSC), which normally prevent autoimmunity. Depleting these negative influences with other therapeutic antibodies is now being combined with checkpoint blockade or CAR T cells (Figure 2). In other instances, suppressive metabolic by-products of cancer cell metabolism (eg adenosine) can be neutralised with new drugs that block production or prevent their binding to receptors.[17]

Other immune-based cancer preventives and therapies

While these major advances have greatly spurred interest in cancer immunotherapy, some less-heralded approaches have contributed sporadically over many years. 'Non-specific' immune stimulation by repeated BCG immunisation can be effective in advanced bladder cancer. Vaccines against oncogenic viruses are potent cancer preventives, but ineffective therapeutically. Vaccines for hepatitis B (a major cause of hepatocellular cancer) and oncogenic human papillomavirus strains (cervical, anal, and some head and neck cancers) are notable, and further opportunities exist if Epstein–Barr virus (B cell malignancies, Hodgkin lymphoma, nasopharyngeal carcinoma), *Helicobacter pylori* (gastric cancer) and human

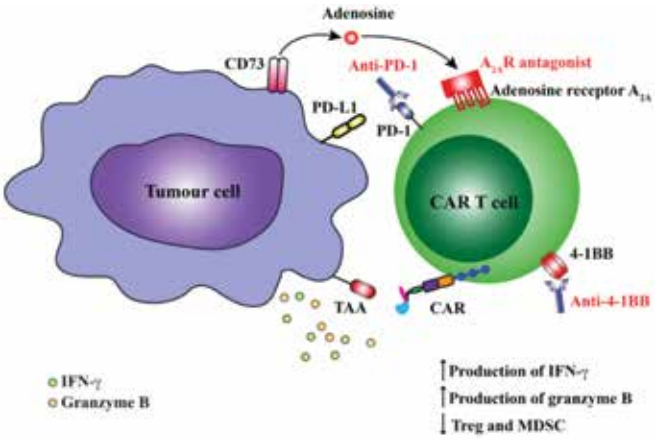

Figure 2. Blockade of immunosuppression or activation of co-stimulatory pathways augment anti-tumour responses by CAR T cells. CAR activation following engagement of tumour-associated antigen (TAA) leads to upregulation of both inhibitory receptors on CAR T cells including programmed death-1 (PD-1) and adenosine A_{2A} receptor (A_{2A}R) or activation receptors such as CD137 (4-1BB). Interaction of PD-1 with its ligand PD-L1 expressed on the tumour cell surface or binding of adenosine to A_{2A}R can reduce functional responses by T cells. Blockade of PD-1 using an anti-PD1 mAb or A_{2A} using a specific A_{2A}R antagonist can augment CAR T cell function. Similarly, the use of an immune agonist antibody such as anti-4-1BB can increase CAR T cell function and reduce frequency of regulatory cell types such as Treg and MDSCs within the tumour microenvironment.

immunodeficiency virus (Kaposi sarcoma) infections can be prevented. Finally, allogenic bone marrow transplantation, which restores haematopoiesis after high-dose chemoradiotherapy, also provides donor T and NK cells that recognise residual cancer cells as foreign, and induce a beneficial 'graft versus leukaemia' effect.

Future challenges and opportunities

After 120 years, countless setbacks and decades of scepticism, immune-based therapies for cancer are here to stay (Table 2). Despite major recent advances, we have barely commenced learning how to best apply these emerging treatments, rationally combine them, or combine them with established treatments. Some conventional anticancer drugs such as lenalidomide (for multiple myeloma) have immune-stimulatory activity that can synergise with other immune-based approaches.[18] Also exciting is the realisation that radiation therapy may amplify the immune response to cancer; we are still in the early stages of optimising the dose of radiation and understanding which patients are likely to benefit.[19]

Dozens of clinical trials for checkpoint inhibitors alone are open across Australia (www.australiancancertrials.gov.au) and demand is sure to grow. We urgently need biomarkers to predict which patient will respond, as a course of antibody-based treatment may cost the community or individual tens of thousands of

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dollars. Investment in molecular pathology and clinical trials infrastructure should be a priority for both government and pharmaceutical companies, as bottlenecks are already appearing. Ethically, new therapies must first be trialled in patients in whom the current standard-of-care treatment has failed, but eventually, immunotherapy may have an impact at earlier stages of disease, where cancer-related immunosuppression will present fewer obstacles.

For CAR T cells, there is great need to identify new target antigens for solid tumours. B cell cancers, where CD19/CD20 are effective targets, comprise about 5% of all cancer in Australia and the huge unmet clinical need in lung, oesophageal, brain and pancreatic cancers must be addressed. Currently, the cost of an autologous CAR T cell vaccine is comparable to bone marrow transplantation, but this would drop drastically if an 'off-the-shelf' solution, such as allogenic CAR T cells, becomes feasible, or with technological advances that simplify sterile production.

After decades of promise, there is much excitement for cancer immunologists and oncologists, and even greater hope and optimism in the cancer community.

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References

1. Coley WB. The treatment of malignant tumors by repeated inoculations of erysispelas: With a report of ten original cases. Clin Orthop Relat Res 1991;262:3–11.
2. Smyth MJ, Godfrey DI, Trapani JA. A fresh look at tumor immunosurveillance and immunotherapy. Nat Immunol 2001;2(4):293–99.
3. Romero P, Valmori D, Pittet MJ, et al. Antigenicity and immunogenicity of Melan-A/MART-1 derived peptides as targets for tumor reactive CTL in human melanoma. Immunol Rev 2002;188:81–96.
4. Engels EA, Pfeiffer RM, Fraumeni JF Jr, et al. Spectrum of cancer risk among US solid organ transplant recipients. JAMA 2011;306(17):1891–901.
5. Kaplan DH, Shankaran V, Dighe AS, et al. Demonstration of an interferon gamma-dependent tumor surveillance system in immunocompetent mice. Proc Natl Acad Sci U S A 1998;95(13):7556–61.
6. Smyth MJ, Thia KY, Street SE, MacGregor D, Godfrey DI, Trapani JA. Perforin-mediated cytotoxicity is critical for surveillance of spontaneous lymphoma. J Exp Med 2000;192(5):755–60.
7. Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006;313(5795):1960–64.
8. Chaudhry MS, Gilmour KC, House IG, et al. Missense mutations in the perforin (PRF1) gene as a cause of hereditary cancer predisposition. Oncoimmunology. 2016;5(7):e1179415.
9. Chia J, Yeo KP, Whisstock JC, Dunstone MA, Trapani JA, Voskoboinik I. Temperature sensitivity of human perforin mutants unmasks subtotal loss of cytotoxicity, delayed FHL, and a predisposition to cancer. Proc Natl Acad Sci U S A 2009;106(24):9809–14.
10. Trapani JA, Thia KY, Andrews M, et al. Human perforin mutations and susceptibility to multiple primary cancers. Oncoimmunology 2013;2(4):e24185.
11. Nair SK, Boczkowski D, Snyder D, Gilboa E. Antigen-presenting cells pulsed with unfractionated tumor-derived peptides are potent tumor vaccines. Eur J Immunol 1997;27(3):589–97.
12. Palucka AK, Coussens LM. The basis of oncoimmunology. Cell 2016;164(6):1233–47.
13. Tsirigotis P, Savani BN, Nagler A. Programmed death-1 immune checkpoint blockade in the treatment of hematological malignancies. Ann Med 2016;48(6):428–39.
14. Arnould L, Gelly M, Penault-Llorca F, et al. Trastuzumab-based treatment of HER2-positive breast cancer: An antibody-dependent cellular cytotoxicity mechanism? Br J Cancer 2006;94(2):259–67.
15. Rosenberg SA, Yang JC, Sherry RM, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res 2011;17(13):4550–57.
16. Voskoboinik I, Whisstock JC, Trapani JA. Perforin and granzymes: Function, dysfunction and human pathology. Nat Rev Immunol 2015;15(6):388–400.
17. Beavis PA, Slaney CY, Milenkovski N, et al. CD73: A potential biomarker for anti-PD-1 therapy. Oncoimmunology 2015;4(11):e1046675.
18. Hsu AK, Quach H, Tai T, et al. The immunostimulatory effect of lenalidomide on NK-cell function is profoundly inhibited by concurrent dexamethasone therapy. Blood 2011;117(5):1605–13.
19. Vacchelli E, Bloy N, Aranda F, et al. Trial watch: Immunotherapy plus radiation therapy for oncological indications. Oncoimmunology 2016;5(9):e1214790.
20. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med 2014;371(16):1507–17.
21. Ritchie DS, Neeson PJ, Khot A, et al. Persistence and efficacy of second generation CAR T cell against the LeY antigen in acute myeloid leukemia. Mol Ther 2013;21(11):2122–29.
22. Ma Q, Gonzalo-Daganzo RM, Junghans RP. Genetically engineered T cells as adoptive immunotherapy of cancer. Cancer Chemother Biol Response Modif 2002;20:315-41.
23. Warren RS, Bergsland EK, R. P-D, Nemunaitis J, Venook AP, Hege KM. Clinical studies of regional and systemic gene therapy with autologous CC49-z modified T cells in colorectal cancer metastatic to the liver. (Abstract, 7th International Conference on Gene Therapy of Cancer). Cancer Gene Ther 1998;5:S1–S2.
24. Park JR, Digiusto DL, Slovak M, et al. Adoptive transfer of chimeric antigen receptor re-directed cytolytic T lymphocyte clones in patients with neuroblastoma. Mol Ther 2007;15(4):825–33.
25. Louis CU, Savoldo B, Dotti G, et al. Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. Blood 2011;118(23):6050–56.
26. Kershaw MH, Westwood JA, Parker LL, et al. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clin Cancer Res 2006;12(20 Pt 1):6106–15.
27. Lamers CH, Sleijfer S, van Steenbergen S, et al. Treatment of metastatic renal cell carcinoma with CAIX CAR-engineered T cells: Clinical evaluation and management of on-target toxicity. Mol Ther 2013;21(4):904–12.
28 Lamers CH, Willemsen R, van Elzakker P, et al. Immune responses to transgene and retroviral vector in patients treated with ex vivo-engineered T cells. Blood 2011;117(1):72–82.
29. Junghans RP, Ma Q, Rathore R, et al. Abstract C13: Phase I trial of anti-PSMA designer T cells in advanced prostate cancer. Cancer Res 2012;72(4 Suppl):C13.
30. Ahmed N, Brawley VS, Hegde M, et al. Human epidermal growth factor receptor 2 (HER2)-specific chimeric antigen receptor-modified T cells for the immunotherapy of HER2-positive sarcoma. J Clin Oncol 2015;33(15):1688–96.

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