Horizon Scanning Series
The Future of Precision Medicine in Australia

Immunotherapy

This input paper was prepared by Professor Rajiv Khanna (QIMR Berghofer Medical Research Institute)

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1. Abstract

Emergence of precision medicine as a tool for diagnosis and treatment of human diseases is dramatically transforming clinical management of patients. Indeed, in 2015, President of the United States, Barack Obama, launched a dedicated initiative on precision medicine diseases (The White House 2016). This initiative includes a framework of synergistic strategy based on an integrated analytics including genomic, phenotypic, environmental, and societal data. It is anticipated that precision medicine will complement personalized medicine to improve overall clinical responses but also reduce any adverse impact of standard treatments. One classic example is the emergence of immunotherapy as a therapeutic strategy for cancer patients. While chemotherapy and radiotherapy haves remained mainstays of cancer treatment for many decades, the adverse impact of these treatments can significantly impair the quality of life of the patient. Novel immunotherapeutic strategies are also emerging for the treatment of autoimmune and allergic diseases, and infectious complications in transplantation. Most importantly, development of novel platform technologies which allow careful evaluation and validation of patient-specific biomarkers in responsive and non-responsive patients will help us to develop new immunotherapeutic drugs to improve clinical response.

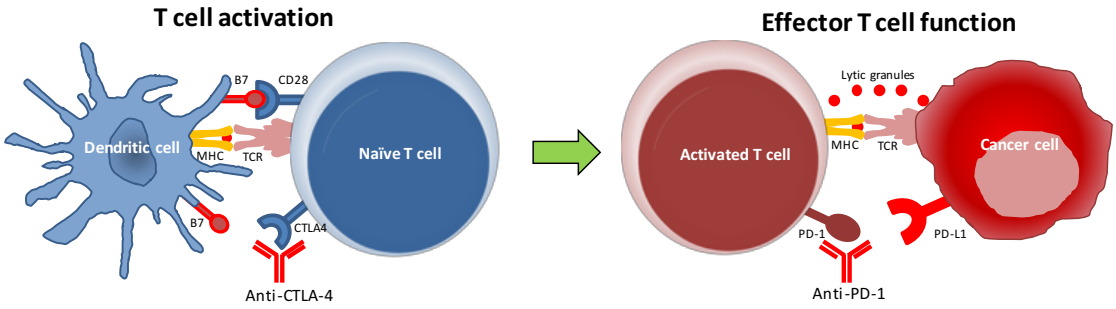
2. Advances in Immunotherapy

Immune dysregulation plays a crucial role in many diseases including cancer, autoimmune disorders and infectious complications in transplant recipients. Furthermore, immune-mediated chronic inflammation and associated disruption of tissue homeostasis has been linked to cardiovascular diseases, type 2 diabetes, obesity and Alzheimer's disease. While our immune system is primarily designed to block invading pathogens, its components also contribute in the maintenance of homeostatic regulations which help in tissues repair and limit organ damage due to acute or chronic inflammation. Thus immune cells and various proteins secreted by them, while controlling infectious complications, also maintain a series of counter-regulatory pathways to prevent collateral damage. Immunotherapy is designed to exploit these immunological pathways to modulate the blood and tissue microenvironment with an aim to restore an effective control of homeostatic processes (McDonald-Hyman, Turka & Blazar 2015; Lesokhin et al. 2015; June, Riddell & Schumacher 2015; Suurmond et al. 2015; Akdis & Akdis 2015). Our knowledge of immune tolerance and self/nonself-immune regulation which emerged in early 1950s, has provided an important platform for the development of new immunotherapeutic strategies. Indeed, this understanding has allowed progressive development of highly specific immune-based therapies from traditional treatment protocols which were developed to cause mass destruction of malignant cells. These traditional treatments often cause debilitating off-target toxicities and, in some cases, even new malignancies. Cancer immunotherapy in combination with cancer genomics has emerged as a powerful tool for cancer treatment and there are already early signs of significant success over certain cancers (e.g. melanoma and lung cancer)(Pardoll 2012).

2.1. Immune Checkpoints as immunotherapeutic targets

The human immune system has a remarkable capacity to protect us from infections and malignancies. Our understanding of the molecular basis of immune activation has provided the foundation for successful development of new immunotherapy drugs. It is now well established that malignant cells and many invading pathogens evade immune control by hijacking various immune pathways which block either activation of killer T cells or immune recognition by these effector cells. This intricate system of checks and balances also provide us protection from any damage caused by our own immune system which we now know is often disrupted in autoimmune diseases leading to tissue damage. Of particular interest are co-stimulatory molecules which are either involved in the early activation of the immune system or immune recognition by effector T cells. The monoclonal antibody ipilimumab, which is directed against CTLA-4, has been approved as an immune checkpoint–blockade therapy for the treatment of several different cancer

types (Drake, Lipson & Brahmer 2014). Antibodies directed against the programmed cell death–1 receptor (PD-1) molecule (Pembrolizumab and nivolumab), remove the immune block imposed by inhibitory molecules to allow more efficient recognition of malignant cells by effector T cells (Drake, Lipson & Brahmer 2014). Other immunotherapeutic antibodies directed to the CD28 molecule (referred to as belatacept and abatacept) have been approved for the treatment of organ transplantation and rheumatoid arthritis. A number of other potential immune checkpoint inhibitors are currently under clinical development and a graphical summary of these potential target molecules is presented in Figure below.

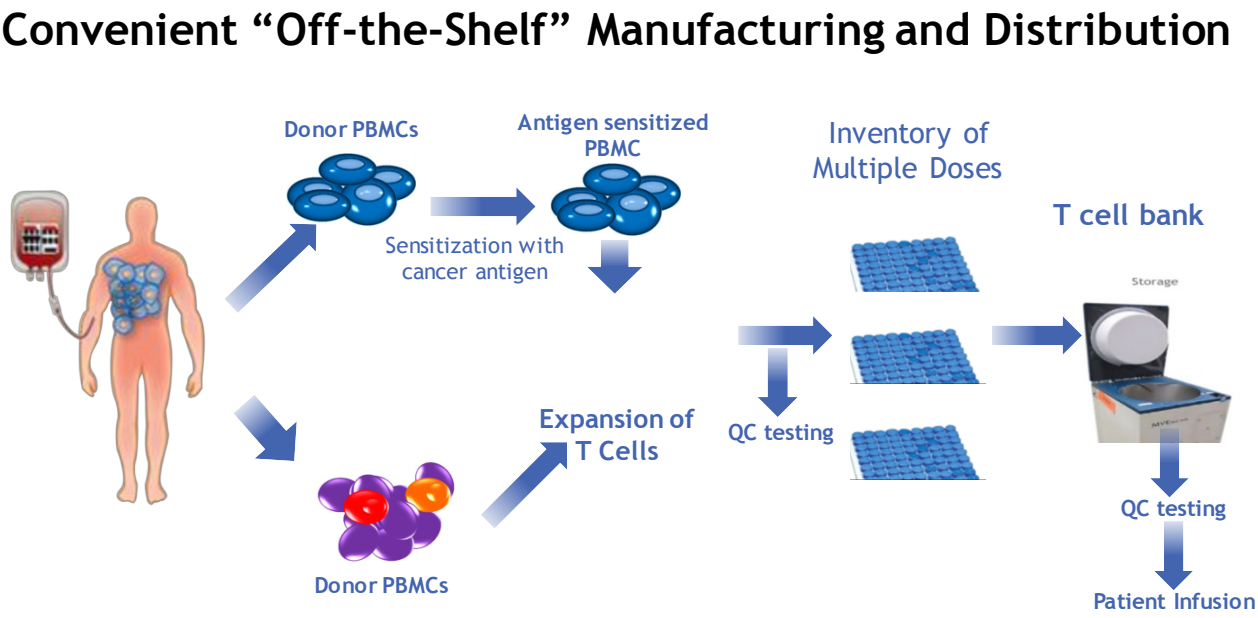


Target molecule`	Activation phase	Effector phase	Disease application (approved or under testing)	Combination target molecule	Immune signatures for therapeutic response	
					Responders	Non-responders
PD-1		✓	Melanoma, squamous NSCLC, GBM, gastric cancer, RCC, HNSCC, HL, bladder, prostate, TBNC	CTLA-4	**Pre/On**: ↑CD4+, ↑CD8+, ↑GzmB **On/Post**: ↑CD4+, ↑CD8+, ↑GzmB, ↑LAG3, ↑PD-1	**Pre/On**:↓CD4+, ↓CD8+ ↓GzmB **On/Post**: ↓CD4+, ↓CD8+ ↓GzmB, ↓PD-1, ↓LAG-3
PD-L1		✓	PD-L1+ve Bladder cancer, NSCLC, RCC, TNBC	CTLA-4	**Pre/On**:↑PD-L1, ↑CD8+, ↑GzmB, ↑IFNγ **On/Post**:↑PD-L1, ↑CD4	**Pre/On**:↓PD-L1, ↓CD8, ↓GzmB, ↓IFNγ **On/Post**: ↓PD-L1, ↓CD4
CTLA-4	✓		Melanoma, NSCLS, SCLC, CRPC, GBM, RCC	PD-1	**Pre/On:** ↑ICOS+ T cells **On/Post:** ↓CD4+FoxP3+, ↑CD4+CD69+, ↑CD8+, ↓MDSC	**Pre/On:** ↓ICOS+ T cells **On/Post:** ↓CD20+ B cells, ↑CD4+FoxP3+
LAG-3	✓		Phase I (solid cancers) NCT01968109 NCT02381314	PD-1	**Pre/On**: ↑CD4+, ↑CD8+ **On/Post**: NA	**Pre/On**:↓CD4+, ↓CD8+ **On/Post**: NA
IDO		✓	Melanoma and ovarian cancer - NCT02460367	PD-1	**Pre/On:** ↓CD4+FoxP3+ **On/Post:** NA	**Pre/On:** ↑CD4+FoxP3+ **On/Post:** NA
B7-H3	✓		Phase I NCT02628535	CTLA-4	**Pre/On**: ↑B7-H3 tumor Vasculature	**Pre/On**: ↓B7-H3 tumor Vasculature
TIM-3	✓	✓	Phase I (solid cancers) NCT02608268	PD-1, CTLA-4 or PD-L1	**Pre/On**: ↑TIM-3 + TADC, ↑CD8+TIM-3+	**Pre/On**: ↓TIM3+ TADC

2.2. Cellular therapies as immunotherapeutic tool

Over the last decade, a new class of biological therapy against cancer has emerged. Referred to as adoptive cellular therapy, this approach generates a cancer-specific immune response by the selection and modification of specific immune subsets through cell enrichment and gene-editing (Fischbach, Bluestone & Lim 2013). Today, cellular immunotherapy includes Chimeric Antigen Receptor (CAR)-modified T cells in which a receptor is inserted that is specific for an antigen expressed by a specific tumour, other gene-edited T cells (e.g. to express suicide genes or specific T cell receptors) and T cells enriched in antigen specificity by a variety of culture expansion and selection techniques. This cellular modification results in the targeting of malignant or pathogen-infected cells, leading to their elimination. Indeed, the infusion of CAR-T cells targeting the antigen CD19 has resulted in dramatic single-agent responses in refractory B cell acute leukaemia, with more than 90 per cent of patients achieving complete remission (Fischbach, Bluestone & Lim 2013). Innovative cell selection and genetic modification approaches have resulted in effective prevention and treatment of rejection responses after transplantation. Targeting of proteins that control cellular responses (either cytokines or checkpoint inhibitors) have also had dramatic responses in cancer. Safety and efficacy are two major challenges in developing cell-based immunotherapy. Newer technologies are being developed to further improve the safety of cellular therapies. One such technology is based on the transduction of 'safety switch' known as inducible caspase 9 (iCasp9) (Di Stasi et al. 2011). This safety switch

enables the T cells to be deleted in the event of life-threatening adverse events, such as graft-versus-host disease, with the administration of a non-toxic synthetic drug. Cellular adoptive immunotherapy using both natural and genetically engineered has now been successfully extended beyond oncology with potential application for autoimmune diseases and stem cell transplantation by using immunomodulating regulatory T cells, mesenchymal stem cells, and/or regulatory dendritic cells. While autologous T cell therapies have been successfully used for the treatment of various diseases, especially haematological malignancies, manufacturing these autologous therapies is often highly laborious and time consuming. This delayed manufacturing can take 4-6 weeks, resulting in the exclusion of many patients who may need these therapies urgently. Development of allogenic "off-the-shelf" T cell therapies from healthy volunteers have provided new exciting opportunities (Leen et al. 2013). A schematic layout for the manufacture of off-the-shelf therapies is shown in the figure below. Many patients have been successfully treated with these allogeneic antigen-specific T cells with minimal side effects. Furthermore, new gene editing tools such as CRISPR may be able to provide a universal cell therapy strategy for off-the-shelf use (Gaj, Gersbach & Barbas 2013).



2.3. Immunomodulation as immunotherapeutic tool

The complexity of the clinical manifestation of many autoimmune diseases often makes it very difficult to precisely delineate the primary instigating factor. While diseases like psoriasis, inflammatory bowel disease and arthritis are managed by clinical experts with distinct expertise, it is important to appreciate that all these diseases are intrinsically linked by a single immunological phenomenon i.e. immune inflammation. In spite of multiple drivers of tissue inflammation in these diseases, there are common molecular pathways which directly contribute towards disease pathogenesis in different organs and individuals. Laboratory-based immunological tools can be employed to identify precise immune-cell subsets within the inflamed tissues and can provide a deeper understanding of the heterogeneous nature of inflammation. This knowledge can be used for developing new drugs that selectively target the molecular pathways responsible for the disease and can suppress inflammation while leaving the larger immune system intact. This is best exemplified by studies carried out by Jacques Banchereau's group who identified interleukin-1 (IL-1) as one of the major culprit in the autoimmune disease systemic onset juvenile idiopathic arthritis (Pascual et al. 2005). Immunotherapy with the IL-1 antagonist (Anakinra) offers a dramatic clinical response in paediatric patients who have refractory systemic onset juvenile idiopathic arthritis.

3. Immunotherapy in Australia

Immunotherapy is rapidly emerging as one of major areas of basic and clinical research in Australia. Currently, there are 265 active clinical trials primarily focussing on cancer immunotherapy (http://www.australiancancertrials.gov.au/). These trials are broadly focussed on either therapeutic

vaccines, immune checkpoints, cellular immunotherapies or immune modulators. The majority of these clinical trials are sponsored by the pharmaceutical industry while a small proportion of these trials are investigator driven studies sponsored by publically funded organizations. Industry sponsored clinical trials are primarily focussed on checkpoint blockers, mesenchymal cells and dendritic cell-based therapeutic vaccines. Unfortunately, there are very few new innovative trials sponsored by public institutions which remains a major roadblock for the development of newer therapies. Development of technological advances in adoptive cellular immunotherapies are one of the major strengths of Australian research and development. The earliest uses of T-cell-based immunotherapy in Australia revolved around post-transplant lymphoproliferative disease, which arises in organ and stem cell transplant recipients partly as a result of the immunosuppressive therapy needed to maintain the engrafted organ (Khanna, Moss & Gandhi 2005). The clinical studies at the QIMR Berghofer (Brisbane) revolved used the adoptive transfer of autologous polyclonal cytotoxic T lymphocytes that had been activated and expanded in vitro using autologous lymphoblastoid cell lines. While this adoptive immunotherapy has been successfully adopted at various clinical centres worldwide, the question of long-term viability of autologous T cell immunotherapy as a practical treatment option is an important consideration. In the case of the use of autologous T cells, there is a significant delay in activation and expansion of cells in vitro of about 1-2 months. This is expensive and may be clinically unacceptable in patients with rapidly progressive disease. Hence the establishment of a bank of HLA-typed T cells established from healthy individuals and stored centrally for distribution to hospitals has emerged as a powerful tool for adoptive immunotherapy. These T cells would be specifically activated to viral and cancer-associated antigens and could potentially be used for the treatment of various diseases. This strategy has been successfully used for patients in Brisbane (Princess Alexandra Hospital) and Sydney (Westmead) (Haque et al. 2007). Despite HLA disparity, there was negligible toxicity, with early in vivo antiviral efficacy and reconstitution of EBV peptide-specific immunity. Over the last decade a number of cell-based immunotherapy trials have been conducted in Australia. Development of CAR-T cell technology has also progressed successfully in Melbourne (PeterMac) and Sydney (Westmead). A brief list of some of these trials is provided below.

Cellular immunotherapy trials in Australia

Multiple Myeloma (Peter MacCallum Cancer Centre) Phase I trial in refractory and relapsed multiple myeloma patients. Autologous T cells genetically modified to express humanized chimeric Ag receptors consisting of a single-chain Lewis-Y Ab attached to the intracellular domain of the T-cell zeta chain and the co-stimulatory molecule CD28. Recognition of target Ag enables initiation of T-cell signalling to the nucleus and recruitment of effector T-cell function, independent of HLA restriction. The technology is potentially applicable to other Lewis-Y-expressing cancers, including ovarian and other epithelial neoplasms
Nasopharyngeal carcinoma (NPC; QIMR Berghofer) Phase I trial at QIMR and Princess Alexandra Hospital in collaboration with University of Hong Kong. This malignancy is strongly associated with EBV and is common in parts of Asia and North Africa. This trial involved activation and expansion of polyclonal T cells from NPC patients with replication-deficient adenoviral vector encoding epitopes from latent membrane proteins (LMP1 and 2) and EBV nuclear antigen 1 and subsequent adoptive transfer back into the same patient. These studies have now been extended to Phase II clinical trial to assess T cell therapy as adjuvant treatment for NPC patients who have been diagnosed with distant metastatic diseases.
Cytomegalovirus in stem cell transplant patients (Westmead Hospital) A phase I study of CMV-specific T cells re-infused into leukemic patients following allogeneic hematopoietic stem cell transplant. CD4 and CD8 CMV-specific T cells were generated from an HLA-matched CMV-seropositive donor using DC infected with a replication-deficient adenoviral vector expressing the immunodominant CMVAg pp65.
Solid tumours (Greenslopes Research Institute) A phase I dose-escalation study of autologous gamma/delta T cells using zoledronic acid-based in vitro culture. In vivo zoledronic acid is administered prior to infusion to enhance tumour susceptibility. Seven patients have been enrolled to date. Advantages include widespread applicability to a broad range of tumours independent of HLA restriction.
Viral infections in stem cell transplant recipients (Westmead) Phase I/II clinical trial to assess the safety and biological efficacy of treatment with virus-specific, cytotoxic T-lymphocytes from partially matched third-party unrelated donors, in stem cell transplant patients with viral reactivation unresponsive to standard therapy (R3ACT trial)

Glioblastoma multiforme (GBM; QIMR Berghofer) Phase I trial of autologous cytomegalovirus (CMV)-specific T cells as adjuvant therapy for primary glioblastoma multiforme. The investigational product is autologous CMV-specific T cells, stimulated with autologous peripheral blood mononuclear cells (PBMC) coated with synthetic CMV epitopes.
Nasopharyngeal carcinoma (NPC; QIMR Berghofer) Phase I/II clinical trial to assess T cell therapy as adjuvant treatment for NPC patients who have been diagnosed with distant metastatic diseases. This study is evaluating the effect of using T cell immunotherapy following standard chemotherapy for the treatment of nasopharyngeal cancer.
Multiple Sclerosis (MS; QIMR Berghofer) Phase I clinical trial to assess feasibility, safety and tolerability of autologous Epstein–Barr virus-specific T cell therapy as treatment for patients with progressive multiple sclerosis. The investigational product is produced by stimulation with gamma-irradiated autologous peripheral blood mononuclear cells infected with the recombinant adenoviral vector AdE1-LMPpoly. This vector encodes multiple CD8+ T cell epitopes from the EBV latent proteins EBNA1 and LMP1&2.
Drug-resistant cytomegalovirus in organ transplant patients (QIMR Berghofer) Phase I open label clinical trial of autologous T cell therapy for the treatment of cytomegalovirus reactivation and disease after transplantation.

4. Future Application of Immunotherapy

In 2013, the editors of the journal Science nominated cancer immunotherapy as breakthrough of the year. Over the last four years, remarkable progress has been made in the area of immunotherapy. Dramatic clinical responses in a significant number of metastatic melanoma patients with a combination of anti-CTLA-4 and anti-PD-1 has brought a new level of enthusiasm. One of the most impressive aspects of these clinical responses is that patients who do respond to checkpoint therapy often remain disease free for a very long time. This success has prompted many scientists to explore other potential checkpoint molecules as targets for immunotherapy. It will be important to expand this portfolio of combination drugs to improve progression free and overall survival of cancer patients. There are approximately 20-25 novel molecules (see a select list in Table under section 1.1) which are under preclinical or clinical development.

Another area that will need greater attention is to understand why some patients successfully respond to immunotherapy while others fail to show any clinical benefit. Future work should focus on identifying blood and tissue biomarkers which can allow clinicians to predict which patients are unlikely to respond. As these immunotherapy drugs are very expensive, we need to ensure that patients are offered these treatments diligently. More importantly, identification of response/nonresponse-specific biomarkers may also lead to new combination treatments that work for additional patients (Patel et al. 2017).

As discussed above, adoptive T cell therapy is another area which is likely to expand rapidly. Australia has played a lead role in this area and there are many groups who are actively developing new platform technologies to improve adoptive T cell therapies. One of the most exciting developments has been the CAR-T cell technology which has shown the most dramatic anti-tumour responses in patients with haematological malignancies. One future challenge in adoptive T cell therapy will be how to meet the global demand to treat patients in regional health centres. Development of allogenic “off-the-shelf” technologies will need to be further refined and expanded. We already have considerable success in using these effector cells in treating transplant patients with infectious complications. Research groups in Sydney and Brisbane are actively working on develop new technologies with an aim to offer T cell therapies beyond local hospitals.

In spite of the continuing challenge and limited clinical responses, therapeutic cancer vaccine research remains an important area of further research and development. Over the last many decades, cancer immunologists have worked extremely hard to identify unique cancer antigens (e.g. NY-ESO-1, MAGE) which have been extensively tested in both preclinical and clinical settings as vaccine formulation. Unfortunately, it has proven to be a very hard road to ride with many roadblocks. Identification of cancer-associated neo-antigens has renewed the interest in cancer vaccines again.

Here are a few specific areas that Australia needs to focus to further enhance progression of the immunotherapy field

(a) It is important to develop and enhance the capacity of immunotherapy manufacturing facilities which can provide rapid access to clinical grade material for clinical trials.

(b) Development of appropriate expertise within Australian regulatory agencies (e.g. TGA) in the area of immune-based therapies (especially cellular therapies)

(c) An appropriate funding structure to support rapid translation of novel immunotherapies from bench to bedside.

(d) Greater harmonisation of the regulatory approval process for clinical trials across clinical centres within individual states and between states

(e) Improved training opportunities for workforce in the area of GMP manufacturing, clinical trial design, regulatory processes.

(f) Harmonisation of regulatory approval process across continents (TGA, FDA and EU).

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