Cancer Immunotherapy

Master Thesis by

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Abstract

Cancer is the leading cause of death in economically developed countries and the conventional anti-cancer therapies fail to meet the criteria of an ideal, safe and highly effective therapy especially against the metastatic cancer types. Cancer immunotherapy is an up-coming anti-cancer therapy that uses the patient's own immune system to target and kill the cancer cells. There are several different strategies and approaches to the cancer immunotherapy in respect to the diverse hallmarks of cancer. While some of these immunotherapeutic agents are approved and made their way into practice, some needs further development and assessment in trials. This study aims to review the already accepted and newly developing immunotherapies against cancer and also to shed light on the issues associated with cancer immunotherapy that determine its failures, successes and future in clinical studies and medical practice.

Cancer Immunotherapy

About Cancer

Highly complex and miscellaneous series of genetic and molecular factors, both intrinsic and extrinsic, create a class of fatal disease called Cancer in which the humankind is giving its one of the biggest fights in science and medicine in the last century to overcome this disease. With about 12.7 million cancer cases and 7.6 million cancer deaths having occurred worldwide in 2008, Cancer is the leading cause of death in economically developed countries and the second leading cause of death in economically developing countries (World Health Organization, Geneva, 2008 and Jemal, A. et al, 2011). Above all the problems associated with the treatment of cancer, metastasis, the spread of cancer cells from the primary tumour to seed (colonize) other distant tumours, is one of the greatest challenges that we are facing in cancer treatment today (Schroeder, A. et al, 2012). Although the conventional anti-cancer therapies are improving on effectively managing the primary tumours, more systemic, specific and targeted cancer treatments (discussed below) are needed to control the metastatic cancer cells (Steeg P.S., 2006).

The complexity of cancer biology has been recently simplified by defining the hallmarks of most, if not all, types of cancer (Hanahan, D. and Weinberg, R.A., 2011); Self-sufficiency in growth signals, resistance to growth suppressors, avoiding apoptosis, limitless proliferation, inducing angiogenesis, invasion and metastasis, reprogramming energy metabolism and lastly, evading the host's immune system. Further, Hanahan and Weinberg emphasis that these acquired functional properties of cancer cells can be made possible by two characteristics; development of genomic instability which generates random mutations, and tumour-promoting inflammation in which, the infiltrating immune cells (mostly innate immune cells) have paradoxical effect by providing growth factors, survival factors, proangiogenic factors, extracellular matrix-modifying enzymes and inductive signals in to the tumour microenvironment that cancer cells may benefit.

There is no doubt that defining these hallmarks of cancer is already opening a gate for the development of new, more specific and effective anti-cancer agents which targets a particular key hallmark to act on. Cancer immunotherapy, which is the focus area of this study, is one of these new inventions that is a result of these new developments.

Conventional and Newly Developing Therapies against Cancer

Surgery, chemotherapy and radiation therapy have long been considered as the traditional treatments of cancer. Even thought there have been major improvements in these treatments for the past decade, radiation and chemotherapy still can only kill a fraction of tumour cells with an adverse effect of a high level of cytotoxicity against the healthy cells (Urruticoechea, A. et al, 2010). Despite the effectiveness of surgery to cure cancer, this treatment is highly restricted to benign and confined metastases which are only 10-15% of the cancer cases

(Ohlsson, B. et al, 1998). Combination of these treatments, such as Chemoradiotherapy, has also been commonly used to treat many tumour kinds such as breast and central nervous system (Urruticoechea, A. et al, 2010). Bone Marrow Transplantation and Peripheral Blood Stem Cell Transplantation is mostly used for the patients that receive these high doses of chemotherapy and/or radiation therapy in order to restore the ability of the body to produce blood cell (National Cancer Institute, 2010). The drawbacks of these traditional anti-cancer treatments are their severe adverse effects due to the lack of selectivity and their tendency to cause drug-resistance cancer cells (Guillemard, V. and Saragovi, H.U., 2004). Apart from the surgery, chemotherapy and radiation therapy, another classical treatment, endocrine therapy has been improved for specially breast and prostate cancers, in which these two diseases share a common hormone dependence to grow. This selective therapeutic option includes: Aromatase inhibitors, anastrozole letrozole and exemestane (Smith, I.E. and Dowsett, M., 2003), selective oestrogen receptor modulators (SERMs), mostly tamoxifen (Shiau, A.K. et al, 1998), anti-androgens and central endocrine ablation with LHRH (lutheinizing hormone releasing hormone). During the last decade, our increasing knowledge about the molecular biology of the cancer in terms of its hallmarks has also started a new era of 'Targeted Cancer Therapy'. Targeted Cancer Therapy uses an agent that selectively attacks or binds to a specific alteration in the cancer cells where the same alteration/factor would not be encountered in the normal healthy cells. By this way, the therapy wields it cytotoxicity only to the cancer cells, sparing the patients from the most of the undesirable side-effects (Gaguski, M.E. and Begyn P., 2008). Such targets can include growth factors, signalling molecules, cell-cycle proteins, modulators of apoptosis and angiogenesis related molecules (Urruticoechea, A. et al, 2010). There are several different strategies to address these targets such as monoclonal antibodies, small molecule inhibitors, antisense oligonucleotides and liposomes (Guillemard, V. and Saragovi, H.U., 2004). Eventhough these targeted cancer therapies can provide a significant tumour regression, their anti-cancer effects are generally short-lived due to the emergence of resistant cancer cells.

Other kinds of cancer therapy includes Photodynamic Therapy where a laser beam can either be used to shrink or destroy various benign tumours or it can be used to locally illuminate a previously administered agent called photosensitizer which in return activates a specific drug that kills the tumour cells (Triesscheijn, M. et al., 2006). Hyperthermia Treatment, the use of increased temperatures to kill cancer cells (either locally/regionally or whole-body), is also a therapeutic option for cancer where the hypoxic and low pH environment of the solid tumours makes them more susceptible to hyperthermia than the normal tissue cells (J. van der Zee, 2002). This treatment is also under improvement in terms of heating techniques and targeting drugs to tumours and can also be used in combination with chemotherapy, radiation therapy or gene therapy (J. van der Zee, 2002). A number of approaches have also been developed in Gene Therapy to treat cancer such as replacing the missing or altered genes with healthy genes or introducing genes to the surrounding cell to slow the cancer cell growth, introducing "suicide genes" in to the tumour cells or Oncolytic Virotherapy, which uses viral particles that replicate within the cancer cell to cause cell death (Cross, D. and Burmester, J.K., 2006). Another Gene Therapy approach as an immunotherapy has also been developed which will be discussed later in this study.

In the light of all these developments for anti-cancer therapeutics, it is obvious that we still have a long way to discover the ideal anti-cancer therapy which is systemic, specific, and highly effective to both benign and metastatic tumours. In this manner, one of the most promising fields in the cancer therapeutics that has a huge potential to cure most, if not all, kinds of cancer is the up-coming Immunotherapy.

Immunotherapy

Cancer immunotherapy, also called biological therapy of cancer, means the modulating and using of the patient's own immune system to target the cancer cells rather than using an extrinsic means of therapy. In that manner, cancer immunotherapy focuses on developing agents that activates or enhances the immune system's recognition and killing of the cancer cells (Sharma, P. et al, 2011).

The origin of immunotherapy goes back to 1774, where a Parisian physician injected pus into the leg of a patient with advanced breast cancer and observed the suppression of the tumour growth as the infection that pus caused worsened (Ian D. Davis, 2000). However, the first documented potential of cancer immunotherapy was the use of extracted mixture of soluble toxins from *Streptococcus* and *Serratia* (Coley's toxins) by an American surgeon, William Coley, from 1893 to 1936, who treated over 800 patients with soft tissue sarcoma with these toxins which was the only known systemic treatment for cancer at that time (Ian D. Davis, 2000 and Kirkwood, J.M. et al, 2008).

After these first attempts in cancer immunology, the hypothesis of immune surveillance has originated decades ago and dictates that the immune system can control the cancer development, up until a point, by suppressing the development or progression of spontaneous tumorigenesis and malignancies (Dunn, G.P. et al, 2002 and Sharma, P. et al, 2011). Recent studies show increasing evidences in favour of this original concept that the immune system can indeed prevent tumour formation (Dunn, G.P. et al., 2002). However at the same time, these studies has also shown that the immune system can also partly functions to provide a mechanism to tumours to escape immunologic elimination by selecting tumour variants with reduced immunogenicity (Dunn, G.P. et al., 2002). All these findings led to the reformation of the immune surveillance idea to a concept called immunoediting, which suggests that cancer cells and immune system stays in a dynamic state of equilibrium between the two extreme ends; removal of the tumour cells by the immune system and development of characteristics that allow the tumour cells to avoid the immune response, which was defined as one of the hallmarks of cancer by Hanahan and Weinberg as mentioned above (Kirkwood, J.M. et al, 2008).

Our knowledge about the molecular and cellular principles underlying the immune system's role on cancer has expanded considerably nowadays, leading to the development of diverse strategies ranging from Immunostimulants to cancer vaccines (Table 1) to use the different aspects of the immune system as anti-cancer therapeutics. In this study, the mechanism of action of these immunological anti-cancer strategies will be reviewed and further, their progression in clinical trials for cancer treatments will be discussed.

Table 1: General Overview of Some of the Different Cancer immunotherapy Strategies

Type of Immunotherapy	E.g. of Agents or Strategies	Description
Immunostimulants	Interleukin-2 (IL-2) Alpha-Interferon (IFN-α)	A potent growth factor for T-cells Activates T and B cells and has apoptotic, antiangiogenic and antiproliferative properties
Immunomodulators	Ipilimumab Tremelimumab MDX-1106 PF-3512676	Antibody to CTLA-4 Antibody to CTLA-4 Antibody to PD-1 TLR-9 Agonist
Monoclonal Antibodies	Rituximab Trastuzumab Bevacizumab Cetuximab	Against the CD-20 Against the HER-2 Against the VEGF Against the HER1/EGFR
Radioimmunotherapy	^{90}Y-ibritumomab- tiuxetan ^{131}I-tositumomab	CD-20 Antibody conjugated to radioactive isotope yttrium-90 CD-20 Antibody conjugated to radioactive isotope iodine-131
Autograph or Allograph Transfer of Lymphocytes	Adoptive Cell Therapy (ACT) ACT + Genetically modified T-cells	Infusion of *ex vivo* grown tumour infiltrating or peripheral lymphocytes Genetic modification of the lymphocytes before infusion
Cancer Vaccines	Sipuleucel-T Vitespen BiovaxID DCVax	Infusion of autograph mononuclear cells with a tumour antigen and GM-CSF Peptide-based vaccine using heat shock proteins from patient's tumour Anti-idiotype vaccine targeting B cell lymphomas Dendritic cells pulsed with tumour lysates or antigens

CTLA-4, Cytotoxic T-lymphocyte antigen-4, PD-1, Programmed death-1, CD-20, B-lymphocyte antigen, HER1 and 2, Human epithermal growth factor-1 or 2, VEGF, Vascular endothelial growth factor, EGFR, Epidermal growth factor receptor, GM-CSF, Granulocyte-macrophage colony-stimulating factor.

Immunostimulants

The use of immunostimulants for cancer therapy is one of the earliest approaches in immunotherapy. It is a non-specific approach that aims to enhance, in general, the activity of the lymphocytes that are already attacking to the tumour cells but are insufficient to produce a full-powered immune response. In this manner, this strategy uses the patient's own immune system as the effecting factor. In the late 1980s and early 1990s, the most important cytokines for cancer therapy, Interleukin-2 (IL-2) and Alpha-Interferon (IFN-α), demonstrated their anti-cancer properties and were approved by the U.S. Food and Drug

Administration (FDA) for the treatment of diverse types of cancers including metastatic melanoma and renal cell carcinoma (Kirkwood, J.M. et al, 2008).

Alpha-Interferons are proteins belonging to the type-I IFN family which was discovered decades ago for its anti-viral properties (Belardelli, F., 1995). The human IFN-α family consist of at least 13 functional subtypes which share the same receptor system and very similar biological functions (Mogensen, K.E. et al, 1999). These diverse biological functions include the activation and regulation of both innate and adaptive immune system by enhancing the effects of macrophage and natural killer (NK) cells, increasing the expression of MHC class I antigens, and regulating the proliferation and survival of both helper and cytotoxic T-cells (Belardelli, F. et al, 2002). IFN-α has also direct effects on cancer cells by its apoptotic, antiangiogenic and antiproliferative properties (Belardelli, F. et al, 2002). In today's immunotherapy regimes, IFN-α is the most used cytokine for the treatment of more than a dozen types of cancer, such as hairy cell leukemia, chronic myeloid leukemia, B and T cell lymphomas, melanoma, renal carcinoma and Kaposi's sarcoma (Pfeffer, L.M. et al, 1998 and Belardelli, F. et al, 2002).

Interleukin-2 is a glycoprotein which is a strong T-cell and Natural Killing (NK) cell growth factor that plays a key role in immune regulation and lymphocyte proliferation (Smith, K.A., 1988). Unlike IFN-α, IL-2 has only indirect anti-cancer effects through the activation of the effector lymphocytes which are also called lymphokine-activated killer cells (Fang, L. et al, 2008). Clinical trials with systemic administration of high-dose IL-2 demonstrated that this regime provides consistent, however low, overall response rate of ~13–17% (Atkins, M.B. et al, 1999). The drawbacks of this immunotherapy are its high cost and its severe but reversible adverse effects. Never the less, to this date, IL-2 remains to be an indispensible immunotherapeutic agent for the treatment of metastatic melanoma (Fang, L. et al, 2008). Molecular modification of IL-2 is also being developed at the moment which is called BAY 50-4798. This agent has two modified amino acids that aims to have the same response as IL-2 but without the adverse cytotoxicity to healthy cells (Margolin, K. et al, 2007).

Apart from Interleukin-2 and Alpha-Interferon, Bacillus Calmette-Guerin (BCG) (Morales, A. et al, 1976), Levamisole (Renoux, G., 1980) and Granulocyte-macrophage colony-stimulating factor (GM-CSF) (Waller, E.K., 2007) have also being used as immunostimulants over the years for immunotherapy but mostly in combinations with other immunotherapies or other strategies for anti-cancer therapeutics.

Eventhough the usage of immunostimulants proved to be useful as an anti-cancer therapy, limitations do exist in their applications in this field. It seems that immunostimulants, on their own, are not sufficient to sustain a fully active, effective and selective anti-cancer immune response. However, new developments are underway to overcome the problems and increase the potential of these cytokines as mentioned above by molecular and genetic modifications. An even better use for these cytokines can be combining them with other cancer therapies such as chemotherapy, or with other immunotherapies such as cancer vaccines and adoptive cell transfer therapy (Dimberu, P.M. and Leonhardt, R.M., 2011).

Cancer Therapy with Monoclonal Antibodies

A century ago, an idea was set forth by Paul Ehrlich that suggested the use of antibodies to selectively target tumours. Over the years, this concept became applicable with the development of hybridoma technology by Kohler and Milstein and further by the generation of chimeric and humanized monoclonal antibodies (mAbs) to increase their immunogenicity and their ability to activate and channel the effector immune mechanisms (Adams, G.P. and Weiner, L.M., 2005). Today, the monoclonal antibodies play a crucial role in cancer immunotherapy through their diverse range of effects and targets (Table 2). The mechanisms of mAb actions (Figure 1) include Direct toxicity which is consist of antibody-dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC), directing and enhancing the activity of effector immune cells, slowing tumour growth and delivering radioactive isotopes, toxins or chemotherapeutic drugs to tumour cells (Bisht, M. et al, 2010).

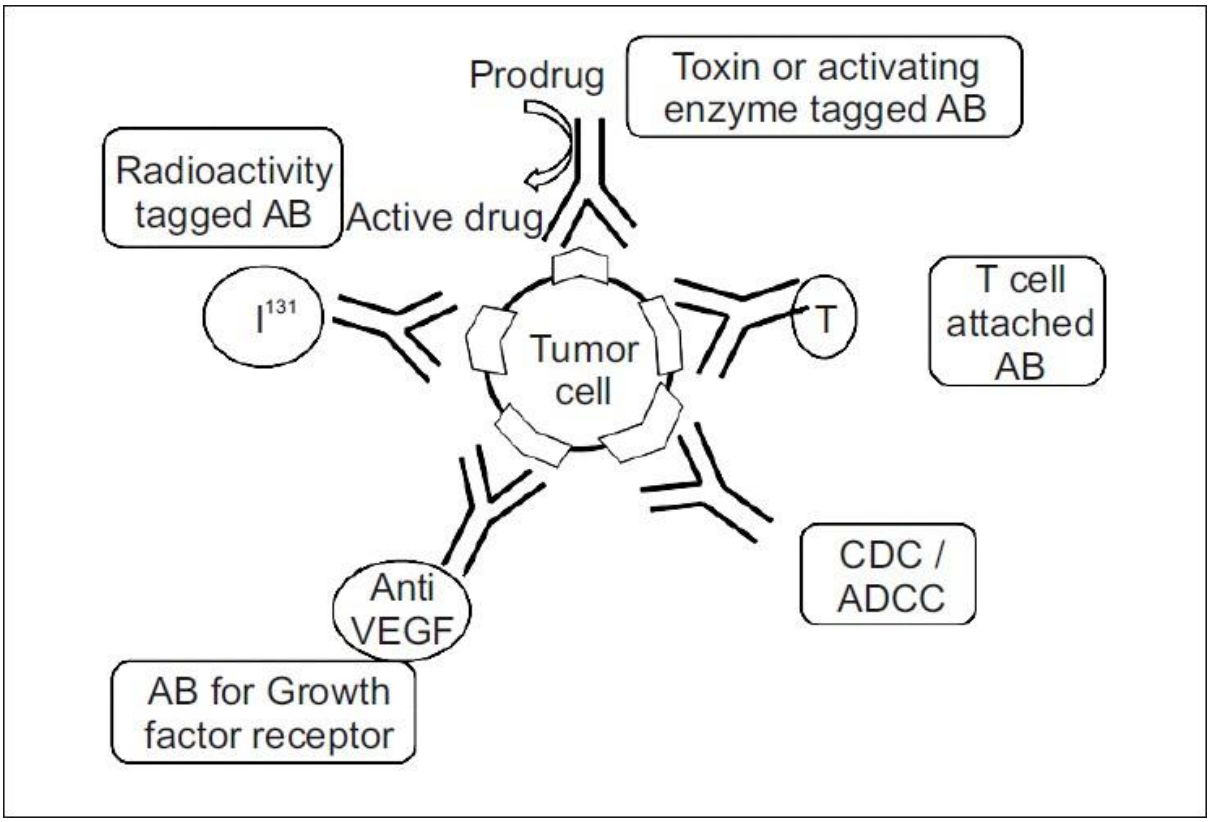

Figure 1: Different mechanisms of action of mAbs in cancer immunotherapy (From Bisht, M. et al, 2010). AB, Antibody, CDC, Complement Dependent Cytotoxicity, ADCC, antibody-dependent cellular cytotoxicity, VEGF, Vascular Endothelial Growth Factor.

Commonly, the chosen mAbs for cancer therapy use a combination of mechanisms to cause cytotoxic effects to tumour cells. This section focuses on the use of unconjugated (naked) mAbs. ADCC and CDC outline one of the common mechanisms of the mAbs. ADCC can be considered as a mechanism to directly provoke an acute tumour destruction in variable levels which also leads to antigen presentation and the activation of adaptive immune components

against cancer cells (Adams, G.P. and Weiner, L.M., 2005). The contribution of ADCC to the anti-cancer effects of the Rituximab, Trastuzumab and Cetuximab has been indicated in the clinical studies (Cartron, G. et al, 2002 and Adams, G.P. and Weiner, L.M., 2005).

Table 2: Unconjugated monoclonal antibodies in use for cancer immunotherapy

Antibody	Target Antigen	Cancer Type	Mechanism
Rituximab	CD-20	B-cell lymphoma, CLL	Direct cytotoxicity
Alemtuzumab	CD-52	B-cell/T-cell lymphomas, CLL	Direct cytotoxicity
Cetuximab	HER-1/EGFR	Colorectal, Head and Neck	Inhibit EFGR growth
Bevacizumab	VEGF	Colorectal, Metastatic Breast, NSCLC, metastatic RCC	Inhibit angiogenesis
Trastuzumab	HER-2	Breast	Inhibit signal growth
Panitumumab	EGFR	Colorectal	Inhibit EFGR growth
Tositumomab	CD-20	Non-Hodgkin's Lymphoma	Direct cytotoxicity
Ofatumumab	CD-20	CLL, B-cell lymphoma	Direct cytotoxicity
Gemtuzumab	CD-33	Acute Myelocytic Leukaemia	Double stranded DNA breaking
Epratuzumab	CD-22	Non-Hodgkin's Lymphoma	Direct cytotoxicity
Edrecolomab	17-1A	Colon/rectal	Direct cytotoxicity
Daclizumab	CD-25α	T-cell mycosis	Inhibit protein synthesis

(Modified from Weiner, L.M. et al, 2010 and Bisht, M. et al, 2010). CLL, Chronic Lymphocytic Leukaemia, NSCLC, Non-Small Cell Lung Cancer, RCC, Renal Cell Cancer, HER, Human Epithermal Growth Factor, VEGF, Vascular Endothelial Growth Factor, EGFR, Epidermal Growth Factor Receptor.

CDC acts on cell membranes where the complement cascade ends up forming a 100 Å pores that result cell death because of the uncontrolled passage of contents into and out of the cell (Gelderman, K.A. et al, 2004 and Adams, G.P. and Weiner, L.M., 2005). Eventhough CDC is not considered as a dominant factor in the anti-cancer properties of the mAbs, it is accepted that the clinical efficacy of Rituximab and Alemtuzumab has a major benefit from CDC and that it can also enhance ADCC (Di Gaetano, N. et al. 2003 and Gelderman, K.A. et al, 2004).

Majority of the anti-cancer targets for the therapeutic antibodies are the growth factor receptors that are being over expressed by the cancer cells during tumorigenesis (Weiner, L.M. et al, 2010). Cetuximab and Panitumumab physically block the interaction between the growth factor receptor and its ligand and therefore also blocks the receptor dimerization

(Sunada, H., 1986 and Li, S. et al, 2005) where as Pertuzumab allows ligand binding to the receptor but inhibits the heterodimerization of the receptor which is necessary for signal transduction (Franklin, M.C. et al, 2004). Bevacizumab also blocks binding of VEGF to its receptor and mostly used together with chemotherapy (Ellis, L.M. and Hicklin, D.J., 2008). Different from these mechanisms, Trastuzumab enters into the cell, binds it target antigen and is passively recycled back to the cell surface along with its payload, a mechanism that requires further understanding (Austin, C.D. et al. 2004). Furthermore, Trastuzumab also inhibits receptor dimerization, causes the endocytic degradation of the HER-2 (Hudis, C. A., 2007). Clinical activities of the these mAbs are promising and they provide high response and cure rates with increased survival advantages and also increased overall survival in patients with recurrent or metastatic disease (Ferris, R.L. et al, 2010). The Clinical efficacies of Rituximab, Trastuzumab, Cetuximab and Bevacizumab for several types of cancer are well indicated by Winter, M.C. and Hancock, B.W. 2009, Hall, P.S. and Cameron, D.A. 2009, William, W.N. Jr. et al 2009, and Norden, A.D. et al 2008 respectively.

The applications and efficacy of the mAbs for anti-cancer therapies can be further improved by administrating them in combination with other anti-cancer therapies such as chemotherapy, radiotherapy, targeted therapy agents and cancer vaccines (Weiner, L.M. et al, 2010). Such techniques are already being used for some mAbs such as Trastuzumab, Bevacizumab and Cetuximab which are often used in combination with chemotherapeutic regimens (Weiner, L.M. et al, 2010). Apart from the applications of unconjugated (naked) mAbs that are discussed above, the mAbs are also being conjugated to radioactive isotopes, toxins or chemotherapeutic drugs in which the toxicity of these agents is exclusively targeted to tumour cells.

Radioimmunotherapy

As mentioned above, one of the techniques to extend the use of monoclonal antibodies is to couple a radioactive atom to a mAb which is targeting a tumour specific antigen. This approach is called Radioimmunotherapy in which, the goal is to limit the application of the deadly radiation to those of tumour cells and keep the toxicity at minimal for the healthy cells (Dimberu, P.M. and Leonhardt, R.M., 2011). There are currently two FDA approved Radioimmunotherapy agents that are being used for the treatment of B-cell malignancies; the radioactive isotope yttrium-90 with an IgG1 mAb against CD20 antigen on B-cells, ^{90}Y-ibritumomab tiuxetan (Zevalin®), and the radioactive isotope iodine-131 with an IgG2a mAb that is also against CD20, ^{131}Itositumomab (Bexxar®) (Waldmann, T.A., 2003).

^{90}Y-ibritumomab tiuxetan, to this date, is mostly used for the treatment of non-Hodgkin's lymphoma where it has shown higher response rates and decreased progression of disease in the clinical trials compared to the Rituximab alone approach (Milenic, D.E. et al, 2004). ^{131}Itositumomab is generally used for the treatment of refractory or relapsed, low-grade lymphomas where it shows almost the same efficacy as ^{90}Y-ibritumomab tiuxetan but less adverse effects such as decrease in the platelets levels (Jacene, H.A., et al 2007). Time will

reveal whether this approach can be further developed and provide even better clinical outcomes.

Immunotherapy with a Chemo-conjugated mAb

In this method, a mAb is labelled with a chemotherapeutic drug and the antibody is targeted to the tumour cells to deliver the drug's high cytotoxicity selectively. Brentuximab Vedotin (SGN-35 or Adcetris) is a FDA approved chemolabeled monoclonal antibody that is being used for Hodgkin's lymphoma and Anaplastic large cell lymphoma (Katz, J. et al, 2011). The mAb used in this treatment is targeted to the CD30 antigen, expressed highly in the Hodgkin's and Anaplastic large cell lymphomas (Younes, A. and Kadin, M.E., 2003), and is coupled to the chemotherapeutic drug called mono-methylauristatin E (MMAE), a powerful inhibitor of microtubule polymerization (Alley, S.C. et al, 2010). The clinical trials show success of this approach due to its high response rates and also due to its mechanism of dual action which is the direct cytotoxic effect of MMAE to tumour cells and to the tumour microenvironment (Katz, J. et al, 2011).

Immunotoxins

Immunotoxins are generated by coupling plant-derived or bacterial toxins to mAbs that target specific antigens on the surface of cancer cells. The first developed toxins for this purpose included gelonin, ricin, abrin, pokeweed antiviral protein, Pseudomonas exotoxin and Diphtheria toxin (Coombes, R.C. et al, 1986). However, due to several drawbacks of these techniques such as rapid clearance from blood stream and immunogenicity led to the generation of the second cohort immunotxins such as BL22 and moxetumomab pasudotox (Teicher, B.A. and Chari, R.V.J., 2011). Both immunotoxins are anti-CD22-Pseudomonas exotoxins that are recently being tested in clinical trials for the treatment of B-cell malignancies and other hematological malignancies (Kreitman, R.J. and Pastan, I., 2011).

Antibody-Directed Enzyme Prodrug Therapy (ADEPT)

Another approach to use mAbs as an anti-cancer therapy is called ADEPT where an antibody is used as a vector to transfer an enzyme that is capable of activating a initially nontoxic drug, called a "prodrug," to a potently cytotoxic agent for tumour cells (Melton, R.G. and Sherwood, R.F., 1996). In this method, an antibody-enzyme conjugate is injected and allowed to localize at the tumour cells depending the specificity of the antibody. Then, the prodrug is administered that should be converted to a cytotoxic agent only within the tumour tissue where the activating enzyme resides (Senter, P.D., 1990). Eventhough the initial reaction towards the ADEPT technology was promising, this approach has not been further developed due to its drawbacks, such as immunogenicity of the enzyme components, short half-life of the conjugates and the observed little anti-tumour activity from *in vivo* studies (Teicher, B.A. and Chari, R.V.J., 2011).

Immunomodulators

There are several key regulatory elements, also called 'immune-checkpoints', in the immune system that manages the level of immune response by the means of downregulation and inhibition to restore the homeostasis. These critical elements are absolute necessity for the development of self-tolerance and to prevent autoimmunity, however, tumour cells constantly benefit from this property of the immune system in order to escape from its destructive power (Dimberu, P.M. and Leonhardt, R.M., 2011). There are several approaches to prevent this inhibition of immune response and to enhance the duration and activation of the T-cell mediated immunity; increasing the expression of co-stimulatory factors on the surface of dendritic cells (DC) by the CD40 or toll-like receptor 9 (TLR9) stimulation of DCs (Krieg A.M., 2006), and enhancing and prolonging the T-cell activation by inhibiting the cytotoxic T-lymphocyte antigen-4 (CTLA-4) or Programmed death-1 (PD-1) binding to B7 or to PD1 ligand (PDL-1) respectively (Ribas, A. et al, 2005). Newly developed immunotherapeutic agents such as oligodeoxynucleotides as TLR9 agonists and mAbs to block CTLA-4 or PD-1 can indeed provide more effective immunotherapies as discussed below.

CTLA-4, a homolog of CD28 that functions as an inhibitory receptor for B7 co-stimulatory molecules on mature antigen presenting cells (APC), is the main negative regulatory element of the T-cell mediated anti-tumour immune response since its binding to B7 downregulates the T-cell activation (Figure 2) (Krummel, M.F. and Allison, J.P. 1995 and Kirkwood, J.M. et al, 2008). Two anti-CTLA-4 mAbs that are in clinical trials now, Ipilimumab and Tremelimumab, therefore were developed to block the CTLA-4 binding to B7 through their higher affinities for the CTLA-4 than the B7 and so, competitively inhibiting the downregulation of T-cells (Ribas, A. et al, 2005). Ipilimumab binds and blocks CTLA-4 and has recently shown striking clinical successes against metastatic melanoma that has led to its FDA approval in 2011 (Dimberu, P.M. and Leonhardt, R.M., 2011).

Due to its potentially synergetic activity with other anti-cancer agents, Ipilimumab has also been tested in combination with chemotherapy and IL-2 that showed increased overall survival, higher survival and complete response rates (Robert, C. et al, 2011 and Prieto, P.A. et al, 2010). Another anti-CTLA-4 mAb, Tremelimumab, is a fully human immunoglobulin G-2 mAb that has been studied in a Phase III clinical trial for patients with melanoma where Tremelimumab failed to show improved benefit (Ribas, A., 2010). Nevertheless, treatments with both Ipilimumab and Tremelimumab have shown increased activation and prolongation of the both helper and cytotoxic T-cells as well as antibody responses to the tumour specific antigens (Carthon, B. C. et al. 2010 and Sharma, P. et al, 2011). Eventhough the studies with anti-CTLA-4 mAbs were focused on patient with melanoma, the use of these immunotherapeutic agents were also studied with other solid tumour types where, for example, treatments with Ipilimumab shows tumour regression in lung, liver, brain, lymph nodes and skin (Kirkwood, J.M. et al, 2008 and Mittendorf, E.A. and Sharma, P., 2010).

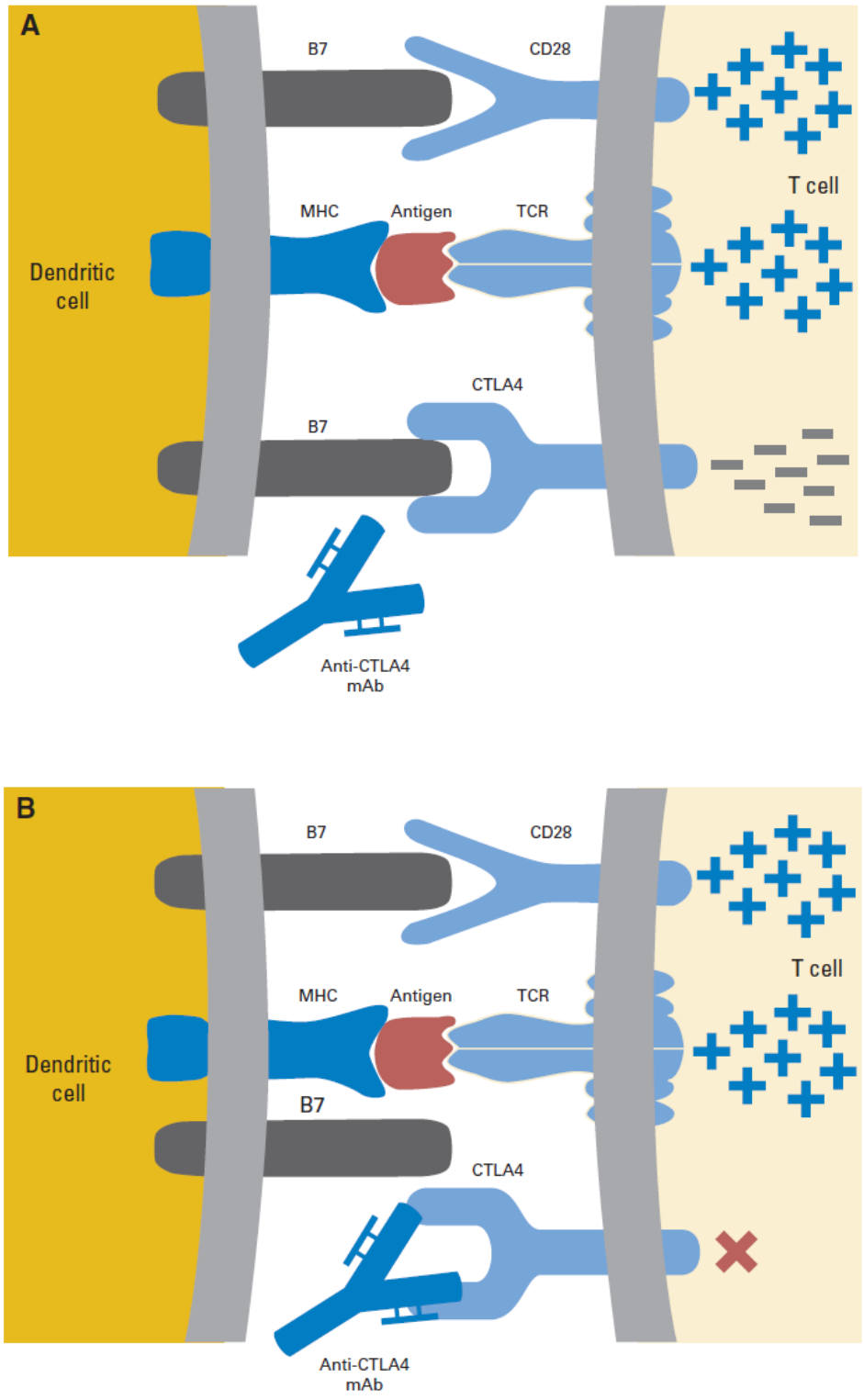

Figure 2: The CTLA-4 blockade by an Anti-CTLA-4 mAb prevents T-cell downregulation. (A) Activation of T-cells initiates the upregulation and binding of CTLA-4 receptors to B7 receptors on the dendritic cells sending inhibitory signals to downregulate the T-cell activation. (B) Anti-CTLA-4 mAb binds to CTLA-4 receptor and blocks the B7 binding therefore prolonging the T-cell activation. (From Kirkwood, J.M. et al, Journal of Clinical Oncology 2008 Jul 10;26(20):3445-55).

Similar to the CTLA-4 blockade, another approach to enhance the T-cell activity is the PD-1 or IDO blockade by the anti-PD-1 mAb. PD-1 is also an inhibitory receptor expressed on T-cell surface that upon binding to PD-L1 it downregulates the T-cell activation, a mechanism that is frequently enhanced by tumour cells in order to escape from the cytotoxic T cell activity (Dimberu, P.M. and Leonhardt, R.M., 2011). MDX-1106 is a newly developed anti-PD-1 mAb that has been recently studied in Phase II clinical trials for melanoma, renal cell carcinoma, prostate cancer, non-small-cell lung cancer or colorectal cancer where it showed a similar outcome to anti-CTLA-4 treatments by providing a high objective response rate for metastatic melanoma and renal cell carcinoma (Sznol, M. et al., 2010). Furthermore, the patients in these early clinical trials showed relatively less immune-related adverse effects than the anti-CTLA-4 treatment (Brahmer, J.R. et al, 2009). In time, the progressing clinical trials will reveal the anti-cancer therapeutic potential of this immunotherapy.

As mentioned above, apart from the CTLA-4 and PD-1 blockade, another recently developed approach to avoid the suppression and to enhance the activation of immune response is the increase of the expression of co-stimulatory factors on the surface of DCs by a TLR9 agonist. TLR9 is an intracellular receptor that recognizes un-methylated cytosine-guanine (CpG) dinucleotides which are frequently found in viral and bacterial DNAs (Krieg, A.M., 2002). A stimulation by TLR9 agonist, induces the activation/maturation of the DCs by resulting an increase in surface expression of co-stimulatory molecules, secretion of cytokines/ chemokines, activation of natural-killer cells, and antigen presentation (Figure 3) (West, M.A. et al, 2004 and Pashenkov, M. et al, 2006). PF-3512676, also called CPG 7909, is a synthetic TLR9 agonist oligodeoxynucleotide that imitate un-methylated CpG single-stranded DNA, therefore providing the effects explained above which sustain an enhanced immune response (Kim, Y. et al, 2004). This synthetic TLR9 agonist has established anti-tumour activity for advanced renal cell carcinoma, recurrent/refractory T-cell lymphoma, non-Hodgkin's lymphoma, advanced non–small-cell lung cancer, and metastatic melanoma in Phase I and II studies (Thompson, J.A. et al, 2004, Kim, Y. et al, 2004 and Pashenkov, M. et al, 2006). So far no autoimmune reaction is associated with the treatment of PF-3512676, demonstrating its high potential in immunotherapy for the treatment of various types of cancer (Kirkwood, J.M. et al, 2008).

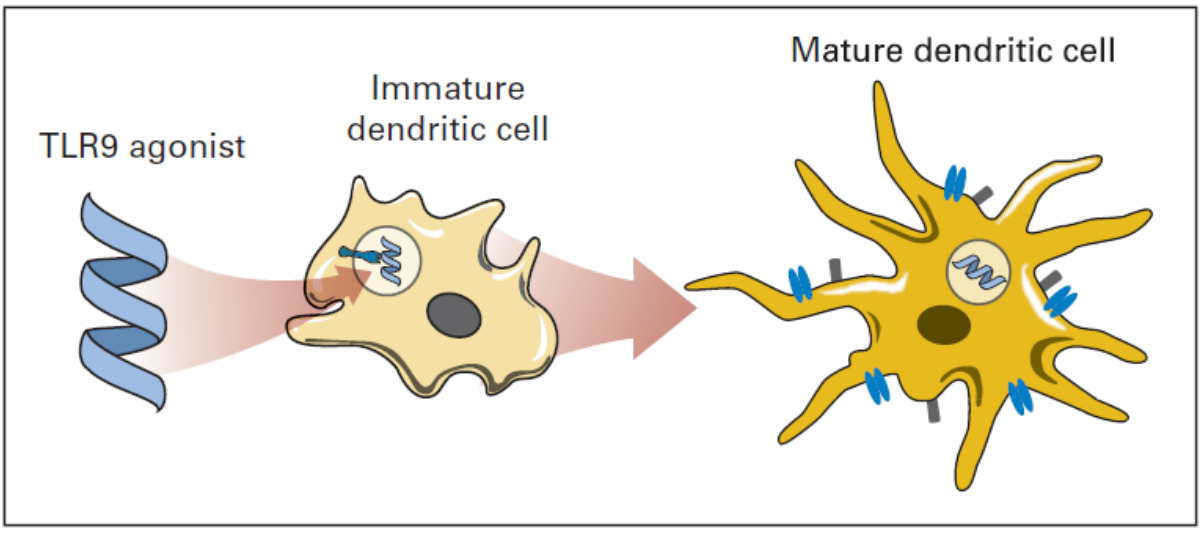

Figure 3: Toll-like receptor 9 (TLR9) agonists induce the activation and maturation of dendritic cells which increases the expression of co-stimulatory molecules on the surface that also causes the production of the adaptive immune response. (From Kirkwood, J.M. et al, Journal of Clinical Oncology 2008 Jul 10;26(20):3445-55).

Considering these recent developments discussed above, it seems safe to speculate that the Immunomodulators have one of the greatest potential to prevail in the future immunotherapeutic regimes.

Adoptive Cell Therapy

Adoptive cell therapy (ACT) is a new and promising immunotherapy that is especially highly effective against metastatic melanoma (Rosenberg, S.A. and Dudley, M.E., 2009). In ACT, the T cells of a patient that have anti-tumour activity are identified, isolated, grown *ex vivo,* further stimulated by the tumour-antigen presenting cells and infused back to the same patient (Figure 4) (Dimberu, P.M. and Leonhardt, R.M., 2011). Before this infusion of the high amounts of tumour-infiltrating lymphocytes (TILs), the host can be manipulated in order to increase the effectiveness of the transferred cells. Patients normally undergo a lymphodepletion with either chemotherapy or body irradiation before the infusion that not only provides the elimination of the regulatory T cells which have immunosuppressive activities, but also eliminates the other lymphocytes that can compete with the transferred cells for cytokines that are essential for T-cell survival such as interleukin 7 (IL-7) and interleukin 15 (IL-15) (Rosenberg, S.A. and Dudley, M.E., 2009). For the same purpose, vaccines or growth factors, such as IL-2, can also be infused along with the transferred cells (Rosenberg, S.A. et al, 2008).

A recent improvement of ACT that has high potential involves the use of genetically modified T-cells. As mentioned above, ACT depends on the identification and isolation of the pre-existing autologous anti-tumour lymphocytes. Eventhough, these TILs can be commonly found in the patients with melanoma, they are not abundant in other types of cancer (Park, T.S et al, 2011). This particular problem has been overcome by the use of lentiviral or retroviral vectors to introduce the genes for selected high-affinity T cell receptors into the patient's lymphocytes which provides the generation of large quantities of tumour-antigen specific T cells for ACT treatment (Rosenberg, S.A. et al, 2008 and Park, T.S et al, 2011). This strategy greatly enhances the effectiveness of ACT since it enables a much greater application range for the therapy in terms of the type of cancer. Another alternative strategy that is being used in clinical trials for ACT is called 'young TILs' in which the patient's lymphocytes are grown *ex vivo* only for a short time and introduced back in to the patient without testing the effectiveness for the particular cancer cells (Dudley, M.E. et al, 2010).

Clinical successes of ACT are promising especially because of the improvement discussed above. In the initial clinical trials of ACT, objective anti-tumour responses were observed however, these effects were short termed due to the very low persistency of the infused lymphocytes (Rosenberg, S.A. et al, 1990). Nevertheless, the potential of these early studies

led to the developments mentioned above and consequently increased the success rates in trials. In this manner, a series of clinical studies were performed subsequently with the increasing levels of lymphodepletion. In this case, anti-tumour responses were long-lasting and objective tumour regressions were observed for 56% of the patients in all around the body including lung, liver, brain, lymph nodes and bone (Dudley, M.E. et al, 2008). In later clinical trials for metastatic melanoma, the observed 49 to 72% objective response rates with the use of specifically isolated TILs, has marked this particular ACT approach as the best available treatment for the metastatic melanoma (Goff, S.L. et al, 2010 and Dudley, M.E. et al, 2010). The clinical studies of ACT along with the use of genetically modified T-cells were recently reviewed in detail that demonstrates the clinical benefits of this approach against a variety of cancers such as, 50% response rate with no toxicity to Synovial sarcoma, almost complete response to Lymphoma, responses with on-target/off-tumour toxicity to Colorectal cancer, 30% response rate with on-target/off-tumour toxicity to Melanoma and a better persistency of infused cells were demonstrated in Neuroblastoma with the use of virus-specific cytotoxic T lymphocytes (Park, T.S et al, 2011).

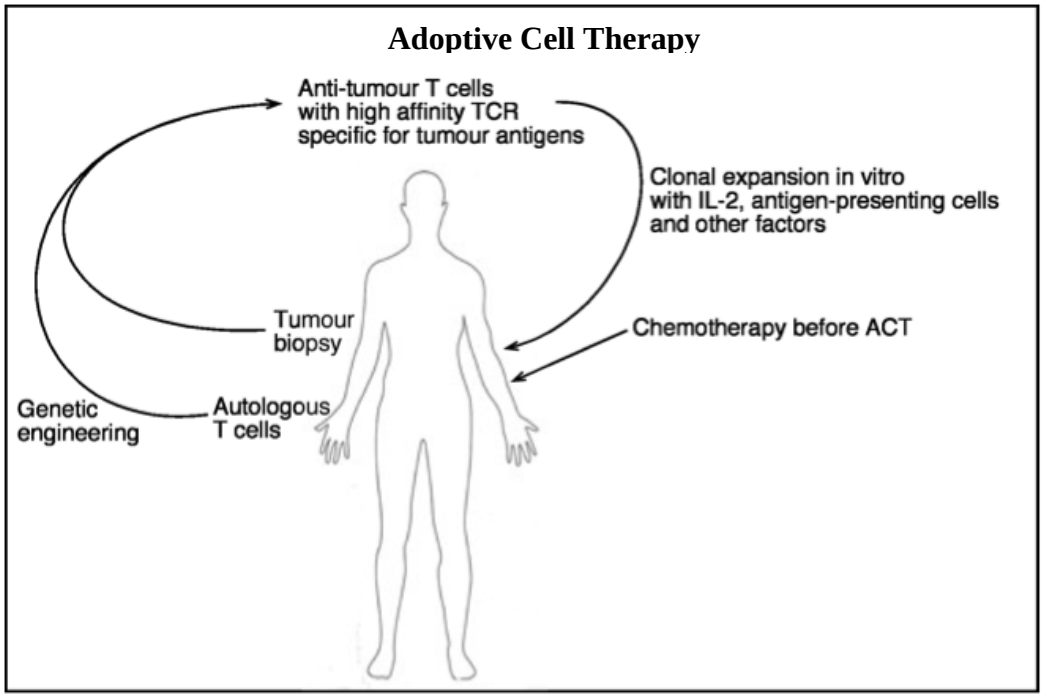

Figure 4: A schematic explaining the basic principles of ACT as discussed above. (From Research projects - Villadangos Laboratory – The University of Melbourne – Department of Microbiology and immunology, 2011. Understanding the mechanisms that impair anti-tumour Adoptive Cell Therapy. [online] (Updated on 25 October 2011 15:14:24) Available at <http://www.microbiol.unimelb.edu.au/research/immunology/villadangos/villadangos_research_proj_p2.html#vp6 > [Accessed on 9 March 2012]).

There are several different future aspects for the improvement of ACT that are currently underway due to its high potential in immunotherapy. Specific lymphodepletion of CD4+ or T regulatory cells, administration of Toll-like receptor agonists, anti-CTLA-4, anti-PD-1, alternative cytokines such as IL-15, IL-12, IL-21, or vaccines along with the ACT to further

support and stimulate transferred cells, generation of less differentiated lymphocytes, and genetic engineering of lymphocytes to introduce new recognition specificities by chimeric T cell receptors are among many opportunities to enhance the effectiveness of this immunotherapy (Rosenberg, S.A. et al, 2008).

Eventhough the adoptive cell therapy is a promising and effective immunotherapeutic regime, it is not without its drawbacks. One of the disadvantages of ACT is its demanding, labour-intensive laboratory applications which make the treatment hard to produce and not vial (Rosenberg, S.A. et al, 2008). However, the biggest issue with ACT is the fact that it is a highly personalized treatment where a new and different reagent has to be created each time for each patient. While this makes it very effective and optimized treatment for an individual, hence the term 'the era of personalized medicine', it does not fit well with the currently common clinical practice and makes it hard to commercialize the regime (Rosenberg, S.A. et al, 2008). It is important to emphasise that this bottle neck in the application of ACT is not a result of its own but rather an issue that the current strategy and procedures in care delivery are in need of change to accommodate this kind of highly individualized treatments.

Cancer Vaccines

Cancer vaccines probably create one of the most diverse classes in the immunotherapeutic approaches where it is also the case for the use of monoclonal antibodies. The development of cancer vaccines can be divided into two groups; preventative, also called prophylactic, and therapeutic. These groups are also further sub-grouped and some examples of each are briefly discussed here (Table 3).

Table 3: The classification of diverse cancer vaccines in immunotherapy

Vaccine type	Name of the Agent	Against to
Preventative		
Virus-based	Hepatitis B virus vaccine	Hepatocellular Carcinoma
	Human Papilloma virus vaccines: Gardasil and Cervarix	Cervical Cancer
Therapeutic		
Peptide or Protein-based	Vitespen	Melanoma and locally advance renal cell carcinoma
	Gp100	Melanoma
Autologous or Allogeneic Whole-Tumour-Cell	GVAX	Prostate Cancer
Dendritic-Cell-based	Sipuleucel-T (Provenge)	Advance metastatic prostate cancer
	DCVAX-Prostate	Prostate Cancer
	DCVAX-Brain	Glioblastoma
Gene Therapy-based	ProstVac-VF	Prostate Cancer
Idiotype Immunoglobulin-based	BiovaxID	Non-Hodgkin's lymphoma

Preventative cancer vaccines are being used with relative success for more than 30 years to prevent the increased chance of tumorigenesis caused by various viral infections. Currently, there are six human viruses identified which are indicated as carcinogenic to humans: hepatitis B virus (HBV), human papilloma virus (HPV), Epstein–Barr virus (EBV), human immunodeficiency virus type-1 (HIV-1), hepatitis C virus (HCV) and Kaposi's sarcoma-associated herpes virus (KSHV) (Sarid, R. and Gao, Shou-Jiang 2011). However, there are currently no vaccines against these viruses with the exception of first two viruses.

The very first such preventative cancer vaccine was the hepatitis B virus vaccine in which, it was approved by FDA in 1981 and since then it has been used as one of the standard agents in scheduled routine vaccinations for infants (Dimberu, P.M. and Leonhardt, R.M., 2011). The common use of this HBV vaccine not only dramatically reduced the rates of HBV infections but also reduced the number of incidences of Hepatocellular Carcinoma (HCC) where the immunization provided by this vaccine continued well for vaccinated individuals even in later ages (Chang, M.H. et al, 2009 and Dimberu, P.M. and Leonhardt, R.M., 2011). The initial development of these HBV vaccines was involved with inactivated and purified hepatitis B surface antigen (HBsAg) particles from plasma of the asymptomatic carriers of HBV infection (Michel, M.L. and Tiollais, P. 2010). Later on, the improvements in genomics and biotechnology led to the production of second generation HBV vaccines that are DNA recombinant which can be produced either in yeast cells (Saccharomyces cerevisiae) (Recombivax) or mammalian cells (GenHevac B) (Michel, M.L. and Tiollais, P. 2010).

The second preventative cancer vaccine is the human papilloma virus vaccine. In the 1980s, it was demonstrated (by Harald zur Hausen) that certain HPV types, HPV16 and HPV18, were present in most cervical cancer biopsies and also in cervical cancer-derived cell lines (Hausen, H.Z., 2009). Nowadays, HPV is known to be responsible for virtually all cases of cervical cancer in which the HPV16 and HPV18 are the high-risk HPVs that consist the almost 80% of the cervical cancer incidences (Sarid, R. and Gao, Shou-Jiang 2011). Currently, there are two HPV vaccines called Gardasil (Merck) and Cervarix (GlaxoSmithKline) that are targeted against these high-risk HPVs 16 and 18 where Gardasil also targets HPV6 and HPV11 which are the contributors to all the cases of genital warts (Lowy, D.R. and Schiller, J.T. 2006 and Dimberu, P.M. and Leonhardt, R.M., 2011). In phase III clinical trials, these two HPV vaccines succeeded to demonstrate their high effectiveness in preventing the high-grade abnormalities of cervix (Tay, Sun-Kuie, 2012), leading to their recommendation by World Health Organization (WHO) in 2009 to be used as primary preventative vaccines against cervical cancer (World Health Organization, 2009).

Apart from preventative vaccines, the therapeutic cancer vaccines aim to raise an immune response to an existing cancer rather than trying to prevent it from forming. This approach has been developed due to realization that the cancer patients can indeed produce both cytotoxic and helper T cells specific to antigens expressed in their tumours (Boon, T. et al, 2006). Therapeutic cancer vaccines intent to trigger or enhance these pre-existing T cell responses against the tumour cells and there are several different approaches in the making of these vaccines (Table 3) (Mellman, I. et al, 2011).

Peptide or Protein-Based Vaccines

This type of cancer vaccines use a whole protein or short peptide derived from the tumour cells as a tumour cell-specific antigen for the immunization. A vaccine belongs to this type, called Vitespen, is a peptide-based vaccine which uses an autologous tumour-derived heat shock (chaperone) protein; glycoprotein (gp) 96–peptide complex (HSPPC-96) as an antigen (Hammerstrom, A.E. et al, 2011). In phase III clinical studies against Melanoma and locally advance renal cell carcinoma, Vitespen has failed to provide a significant increase in overall survival rates and showed no overall benefit in recurrence-free survival (Wood, C. et al, 2008 and Testori, A. et al, 2008). However, these studies showed an insignificant benefit with patient in earlier stages and also the subgroup analysis indicated that patients with higher doses of Vitespen survived longer than ones with the lower doses (Hammerstrom, A.E. et al, 2011).

Another peptide-based therapeutic cancer vaccine is called Gp100 (or Gp100-based) that uses peptides from this glycoprotein 100 as a melanoma associated antigen for the vaccination (Hammerstrom, A.E. et al, 2011). Eventhough this vaccine has succeeded to demonstrate its ability to establish an immune response against the tumour cells, no reduction in tumour size was observed (Hodi, F.S. et al, 2010). However a recent study, where Gp100 was co-administrated with the Immunostimulant IL-2, showed an anti-cancer immune response with a prolonged progression-free survival rate in patients with advanced melanoma (Schwartzentruber, D. J. et al, 2011). Eventhough there is some potential in the future of peptide or protein-Based cancer vaccines, these primary studies clearly indicate the difficulties associated with the use of them. These difficulties may arose from the fact that short and free peptides are likely to be discarded rather quickly from the body without having the chance to associate with a dendritic cell to cause an immune response. Following up from the same problem, another issue can be the lack of effective dendritic-cell-activating adjuvant that is suppose to assist the peptides to be loaded to dendritic cells and promote their activation and maturation (Rosenberg, S.A. et al, 2004). Circumventing these issues can indeed improve the therapeutic benefits provided by these cancer vaccines.

Autologous or Allogeneic Whole-Tumour-Cell Vaccines

Whole-tumour-cell cancer vaccines are prepared from either autologous tumour cells or allogeneic tumour cell lines. Eventhough the use of autologous tumour cells eliminates the antigen selection problem by providing the advantage of targeting the individual's own tumour associated antigens, this approached has been abandoned due to the motion that this kind of vaccine would not raise an effective anti-cancer immune response since it was not pre-existing in the first place (Hammerstrom, A.E. et al, 2011). Furthermore, the high complexity of the vaccine preparation for each individual patient additionally instigated the abandoning of this approach (Mellman, I. et al, 2011). In the other hand, the use of allogeneic tumour cell lines for the whole-tumour-cell vaccination was favoured because of its ability to introduce multiple antigens and therefore to stimulate a better immune response (Hammerstrom, A.E. et al, 2011). An example to this class of cancer vaccines is called GVAX which uses Allogeneic Prostate Cancer Cell Lines (VITAL) 1 and VITAL-2 that are

manipulated to secrete GM-CSF (Higano, C.S. et al. 2008). Despite its success in phase I and II clinical trials, the application of GVAX was terminated in phase III clinical trials against prostate cancer due to the increased rate of deaths and the low chance of reaching to its end point (Drake, C.G., 2009).

Gene Therapy-Based Vaccines

Gene therapy-based vaccines are also called vector or viral-vector vaccines since they use viruses to insert the vaccine (Hammerstrom, A.E. et al, 2011). In this approach, these viral vectors are engineered to encode for specific tumour antigens for the purpose of stimulating and enhancing the immune responses against cancer cells that carry the particular antigens. While advantages of using viruses as a delivery vehicle includes the easy gene insertion, low cost and ability to induce persistent immune response, the viruses belonging to the poxvirus family create an attractive candidate for this treatment due to their safe applications since the 1960s (Madan, R.A., et al 2009 and Hammerstrom, A.E. et al, 2011). The recombinant poxvirus vaccine, belonging to this class of cancer vaccines, is called ProstVac-VF that encodes for a prostate-specific antigen (PSA) and the adhesion molecules B7-1, ICAM-1 and LFA-3 to boost the T cell activation by resembling a specialized dendritic cell (Mellman, I. et al, 2011). Additionally GM-CSF is administrated along with the vector to further stimulate the immune response. In a phase II clinical trial against minimally symptomatic metastatic castration-resistant prostate cancer (mCRPC), ProstVac-VF failed to improve progression-free survival but succeeded to demonstrate a significant increase in overall survival rates and more than 40% of decrease in death rates, leading to its schedule to be used in a large phase III clinical trial (Kantoff, P.W. et al 2010 and Mellman, I. et al, 2011).

Idiotype Immunoglobulin-Based Vaccines

This type of cancer vaccines are prepared by fusing patient's malignant B lymphoma cells with a myeloma cell line in which the resulting heterohybridoma expresses antibodies that consist of patient's tumour-specific antigens called idiotypes (Hammerstrom, A.E. et al, 2011). Then the idiotypes are isolated from the produced antibodies from these heterohybridoma B cells, purified and are coupled to keyhole limpet hemocyanin (KLH) to enhance their immunogenic properties by providing specific T-cell responses (Reinis, M., 2008). The vaccine called BiovaxID was developed in such way as a cancer vaccine against the B-cell lymphomas. Three phase III clinical trials were performed with this vaccine in which one of them was for patients with follicular non-Hodgkin's lymphoma that the BiovaxID showed increased progression-free survival rates when administrated with GM-CSF (Schuster, S.J. et al, 2009). Unfortunately in the other two phase III clinical studies, BiovaxID failed to provide a significant clinical benefit which may be due to the differences between the populations of patients or due to the time and labour intensive manufacturing method of the BiovaxID (Mellman, I. et al, 2011).

Dendritic-Cell-Based Vaccines

Among all the cancer vaccines discussed here, perhaps dendritic-dell-based vaccines hold of the highest potentials in the field of therapeutic vaccination that still needs to be explored.

Considering the amount of information accumulated in the recent decades, the importance of dendritic cells is now known for a potent T-cell stimulation and therefore a persistent anti-cancer immune response (Mellman, I. et al, 2011). One of the dendritic-dell-based vaccines is called DCVAX-Prostate which is an autologous dendritic cell vaccine however it does not use a whole protein as in peptide or protein-based vaccines and it does not include GM-CSF in its administration. Its manufacturing follows an incubation of the patient's dendritic cells with a prostate-specific membrane antigen (PSMA) before it is infused back in to the same patient (Hammerstrom, A.E. et al, 2011). The phase I and II clinical trials in patients with prostate cancer, DCVAX-prostate proved to be able to induce an anti-cancer immune response against the prostate cancer cells (Fishman, M., 2009). Another dendritic-dell-based vaccine is called DCVAX-Brain which uses the exact same concept as in DCVAX-prostate but instead of PSMA the autologous dendritic cells are loaded with the patient's tumour cell lysates (Hammerstrom, A.E. et al, 2011). The DCVAX-Brain vaccine is used in patients with glioblastoma multiforme which the most aggressive, malignant and common brain tumour in humans (Van Meir, E.G. et al, 2010). As in the case of DCVAX-Prostate, the phase I and II clinical trials of the DCVAX-Brain vaccine also showed low toxicity and successful stimulation of an anti-tumour immune response (Wheeler, C.J. and Black, K.L., 2009).

Sipuleucel-T (Provenge)

The use of Sipuleucel-T for advance metastatic prostate cancer was approved by FDA in 2010, making Sipuleucel-T the first FDA approved therapeutic cancer vaccine (Hammerstrom, A.E. et al, 2011). It is an autologous personalized vaccine that is prepared from the patient's own peripheral blood mononuclear cells (Figure 5). After discarding platelets, monocytes, low-density lymphocytes and erythrocytes by leukapheresis, the remaining dendritic cells, T cells, B cells, and natural killer cells are incubated from 36 to 44 hour *ex vivo* with a fusion protein PA2024 which is composed of a prostate cancer antigen, prostatic acid phosphatise (PAP) and GM-CSF (Hammerstrom, A.E. et al, 2011 and Dimberu, P.M. and Leonhardt, R.M., 2011). After the *ex vivo* incubation, the cells are infused back into the same patient where the cells are thought to effectively present the antigen to host immune system and activate the cytotoxic T-cell responses against the tumour cells (Drake, C.G., 2010). Eventhough the Sipuleucel-T vaccine is considered as an autologous dendritic-cell-based vaccine, its mechanism of action is not fully comprehended since it has not been clearly demonstrated yet whether the complex mixture of the *ex vivo* incubated cells indeed contain the PAP-loaded dendritic cells or that the induction of PAP-specific T-cells by the infusion indeed exists (Pardoll, D. and Drake, C., 2012). Therefore there is still a need for further characterization of the incubated cells to fully understand the mechanism of this vaccine. Although the phase III clinical studies of Sipuleucel-T did not show reduction in tumour size or reduction in disease progression rate, it succeeded to provide a significant increase in the median survival rates that led to its FDA approval (Kantoff, P.W. et al., 2010). This appearance of increase in overall survival provided by the Sipuleucel-T vaccine without demonstration of an observable anti-tumour effect has led to the discussion that the tumour response criteria in clinical trials might be in need of modification for this kind of immunotherapeutic approaches.

In the light of these developments it is encouraging to see that cancer vaccines are finally emerging as an effective immunotherapy. However, there are several limitations that are associated with these developments. The selection of a suitable tumour antigen to target and work with still remains to be a problem for autologous cancer vaccines where the tumour cells can also have a diverse range of different antigens that can decrease the efficiency of a cancer vaccine that targets only one or two of them. The selected antigen can also be present in healthy cells or can be very similar to those in healthy cells therefore the vaccine can create undesired effects. Another limiting issue with cancer vaccines in that an appropriate adjuvant needs to be developed that can ensure the proper maturation of dendritic cells to facilitate an anti-tumour cytotoxic T-cell response. However, one of the most important points is the issue of immunosuppressive factors used by cancer cells that alters the effectiveness of the anti-tumour cytotoxic T-cell population that has been raised by these vaccines. This problem might be solved with the use of Immunomodulators that are discussed in this study such as anti-CTLA-4 mAb or anti-PD-1 mAb. This issue of immune checkpoints creating immunosuppressive factors for the immunotherapies is further discussed below.

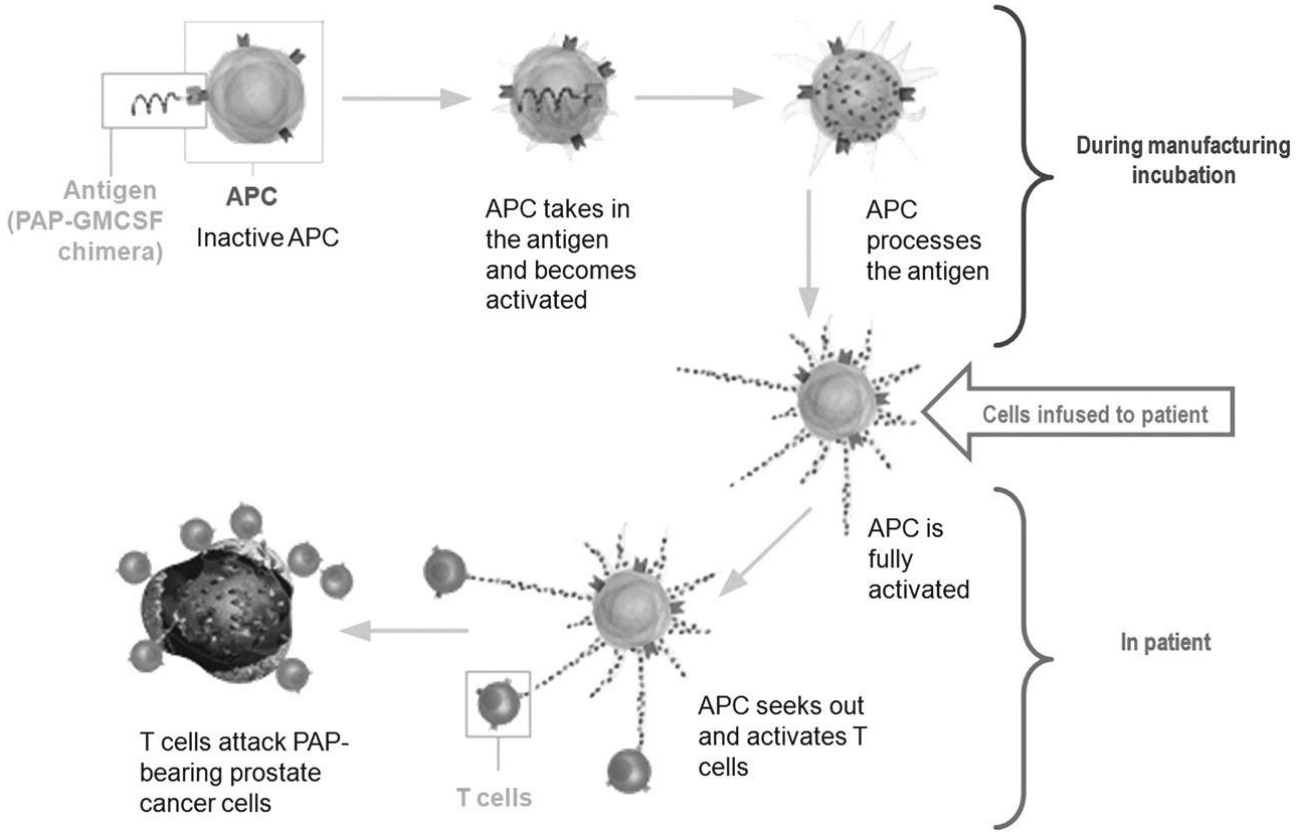

Figure 5: The preparation and proposed mechanism of action of Sipuleucel-T cancer vaccine. PAP: Prostatic acid phosphatise, GM-CSF: Granulocyte-macrophage colony-stimulating factor, APC: Antigen presenting cell. (Modified from Marie L. Huber, Laura Haynes, Chris Parker and Peter Iversen. Interdisciplinary Critique of Sipuleucel-T as Immunotherapy in Castration-Resistant Prostate Cancer. J Natl Cancer Inst 2012;104:1–7).

Discussions and Perspectives

Eventhough the described diverse immunotherapies above have proved their potential in the war against cancer, there are several obstacles that prevent their complete triumph. Today, one of the biggest issues in the field of cancer immunotherapy is the immunosuppressive factors that inhibit the anti-cancer effects of the effector immune cells. These immunosuppressive factors can be both naturally occurring in the patient's immune system or can be induced by the cancer cells themselves or their immediate microenvironment. As mentioned before, these inhibitory factors, also called immune checkpoints, are normally crucial for the control of the immune response to sustain self-tolerance and prevent autoimmunity. Regulatory T (T_{reg}) cells are responsible for such actions since they are the pivotal players in controlling the immune responses. However, as an immune evasion strategy, it is known that the cancer cells or the normal cells in tumour microenvironment can indeed upregulate the expression of these inhibitory receptors and ligands therefore use this property for their benefits (Pardoll, D.M., 2012). High amounts of naturally occurring and antigen-specific T_{reg} cells have been detected in the tumour microenvironment of patients with various cancer types such as prostate, breast, lung, cervical cancers and melanomas (Rong-Fu Wang, 2008). This suppression of the effector immune cells creates the biggest problem for any immunotherapeutic strategy where the agent is fully equipped for specific and effective cytotoxic act on cancer cells but cannot deliver its effects because of its suppression by the immune checkpoints. Therefore, this effect of T_{reg} cells can be considered as the biggest obstacle for a successful cancer immunotherapy. The immunomodulators that are described in this study such as anti-CTLA-4 and anti-PD-1 mAbs, therefore, hold the key to solve this problem by blocking these immune checkpoints where they act on the patient's lymphocytes instead of targeting directly the cancer cells. This blocking of the inhibitory factors sets free the acquired effector T cells by the particular immunotherapy to act and kill the cancer cells. This new achievement, which has proved its effectiveness in clinical successes and by the FDA approval, revolutionized the field of immunotherapy and opened the gates for new opportunities such as new, different immunomodulators and effective combination therapies. Some of these newly developed immunomodulators that are in clinical and pre-clinical studies are well reviewed very recently (Pardoll, D.M., 2012). The use of these immunomodulators for blockade of immune checkpoints along with an agent or strategy that facilitates a population of anti-cancer T cells such as cancer vaccines or ACT can provide an ultimate cancer therapy that is highly effective. Such combinational strategies are already under way as some of them are already mentioned in this study. Further, our increasing understanding of the role of regulatory T cells in cancer may one day provide the tools to not only block them, which is the case with the Immunomodulators, but also reverse their suppressive effects to stimulatory effects that would enhance the anti-cancer effect of the effector T cells that are in the vicinity of the cancer cells. One issue that is still needed to be addressed is the adverse effects of the blocking immune checkpoints in the form of immune toxicity which can be manifested in 25-30% of the patients (Beck, K. E. et al, 2006). However, recently it is shown that a therapeutic window might be created where these immunomodulators can be safely administered without causing much of the adverse side effects (Pardoll, D.M., 2012).

Another obvious obstacle that stands in the way of the success of immunotherapy is the current clinical assessment approaches to validate the responses of patients to a particular immunotherapy. The common evaluation of the activity of cancer therapies follows the Response Evaluation Criteria In Solid Tumours (RECIST) and modified WHO criteria which depends on the measurement of tumour size (area or volume) after the administration of the anti-cancer agent (Therasse, P. et al, 2000 and Mellman, I. et al, 2011). While these criteria are appropriate for the assessment of classical cancer therapies such as chemotherapy and radiation therapy, they seem insufficient to accurately determine the true response of the cancer immunotherapies. This issue might have been elevated from the fact that the conventional cancer therapies show their response rather quickly in terms of few weeks which they cause tumour regression via apoptosis whereas according to the same criteria the response of immunotherapies (within a few months) might be seen as delayed or even shadowed by a further tumour growth (Mellman, I. et al, 2011). However, these immunotherapies such as in the case of ipilimumab (the anti-CTLA-4 mAb), show prolonged tumour control or regression in a long term and increased overall survival rates in patients eventhough they were marked as unsuccessful by the RECIST programme. These observations emphasises that the criteria of assessment for cancer immunotherapies should be indeed different from those of classical cancer therapies. Now it is apparent that a longer period of time might be required for the immunotherapies to show their anti-cancer responses and clinical benefits probably due to their systemic nature of action. Therefore, a new set of criteria was proposed to provide a flexibility in the assessment of tumour size during cancer immunotherapies which is called the immune-related response criteria (irRC) (Wolchok, J. D. et al, 2009). With this new set of guidelines, it is immunologists hope that the cancer immunotherapies can be accurately validated before they are marked as failures in the clinical studies.

A further improvement to the assessment of immunotherapies in clinical trials would be the identification of relevant biomarkers. Unfortunately, up this date, an immunological assay or a biomarker for both response and toxicity which correlate with the clinical outcomes of a particular immunotherapy has yet to be developed or identified (Sharma, P. et al, 2011). These improvements would not only provide the opportunity to follow the immunotherapy in real-time and make changes in the administration protocol and so forth, but would also provide the possibility to actually detect the individuals that are most likely to respond to the particular immunotherapy. The ability of complete monitoring of the immune system during a tumour development or an applied immunotherapeutic regime would enormously help to elucidate the exact mechanism underlying the responses we observe during these events. It has been suggested by some that the phase Ia and phase IIa steps should be integrated in to the common clinical trial steps where the both tumour tissues and peripheral blood samples are taken for the laboratory analyses by new novel immune monitoring assays such as the PI3K signalling pathway assay to validate T cells activation, monitoring gene expression and micro- RNA signatures (Sharma, P. et al, 2011). This possibility of monitoring the anti-cancer responses of a particular immunotherapy for a particular type of cancer in a subset of individuals at an intermediate level would ensure the optimum application regime and the highest possible efficiency of that agent.

Apart from these obstacles, the importance and influence of the immune system itself during a conventional cancer therapy has long been underestimated and ignored by oncologists. Our recently increasing knowledge about the relationship between immune system and cancer cells during both the tumorigenesis and cancer therapy has shed light to this issue and has proved that the immune system is also a crucial element in the success or failure of a cancer therapy especially for chemotherapy and radiation therapy. Now we know that most of the cancer therapies not only act on the tumour cells but also manipulates the immune system where they either initiate an anti-cancer immune response beside their cell toxicity or eliminate the immune suppressing elements (Zitvogel, L. et al, 2008). Many anti-cancer agents have shown to have these immune stimulatory effects. Chemotherapeutic agents can reduce the immunosuppressive properties of the tumours by simply reducing their sizes. Particularly, Cyclophosphamide has been shown not only to eliminate the suppressive effects of the regulatory T cells but also increase the proliferation of the effector T cells and the effectiveness of Natural Killer cells (Ghiringhelli, F. et al, 2007). This downregulation of the regulatory T cells has also been reported for the other chemotherapeutic agents such as 5-Fluorouracil (5-FU) and Oxaliplatin (Correale, P. et al, 2005). Immunostimulatory effects of Gemcitabine, in the other hand, has been demonstrated at clinical level where it was reported that it increases the number of IFN-γ–producing T cells in pancreatic cancer patients (Plate, J.M. et al, 2005). The hormonal therapy strategies for breast and prostate cancers where the androgen levels are suppressed, has shown not only cause to overcome the tolerance for tumour cells and induce anti-cancer immune response via effecting T cell populations but also via promoting the generation of B cells from bone marrow (Viselli, S.M. et al, 1995 and Roden, A.C. et al, 2004). It was also shown that the removal of primary tumours by surgery can diminish the immunosuppressive factors induced by the tumour cells and can trigger a support for anti-cancer effects of the immune system (Danna, E.A., et al. 2004).

The effect of radiation on immune system has already been investigated and its application has been integrated in to the practice of a certain cancer immunotherapy. As previously discussed in this study, the adoptive cell therapy (ACT) performs a lymphodepletion with a total or partial body irradiation to eliminate any suppressive immune factors that might compromise the activity of the infused effector immune cells. This approach has been proven to increase the effectives of the anti-cancer cytotoxic T cells in the patients (Gattinoni, L. et al, 2005). Another aspect to this issue is that it has also been shown that both radiation and chemotherapy can increase the immunogenicity of the tumour cells via increasing the MHC I expression therefore increasing the antigen presentation to the effector T cells (Reits, E.A. et al, 2006). Overall, these observations and many more, have clearly demonstrated that the effectiveness of most conventional cancer therapies including surgery, chemotherapy, hormonal therapy and radiation is influenced by their effect on the relationship between the immune system and tumour cells. It is now understood that the importance of immune system is much more then we previously anticipated in the war against cancer. This recent realization will not only be useful for the development of a better cancer therapy and the improvement of the conventional cancer therapies but will also create an opportunity for an effective combinatorial therapy where an up-coming cancer immunotherapy such as cancer vaccines can be merged with a classical but effective chemotherapy.

In the light of all these obstacles and new developments, combinational strategies with immunotherapies are emerging as a strong approach, as already mentioned before. In classical cancer therapies, especially chemotherapy, it is common to reach a more successful treatment by the combinational use of anti-cancer agents. It is only natural to assume that cancer immunotherapy can also benefit the use of combinational therapies either with another immunotherapeutic agent or with a classical approach such as a chemotherapeutic agent or a targeted cancer therapy. This assumption has been and is being verified by recent clinical and pre-clinical studies. For example, the combination of the immunotherapy, ipilimumab (the anti-CTLA-4 mAb), with the classical cancer therapies such as gemcitabine (chemotherapeutic agent) (Mellman, I. et al, 2011), radiation therapy, leuprolide acetate (horomonal therapy) and androgen-deprivation therapy is already in phase III and phase II clinical trials respectively (Sharma, P. et al, 2011). The use of immunotherapy along with the targeted cancer therapies is very well reviewed recently where it is revealed that the targeted agents such as mTOR inhibitors, JAK2 inhibitors and lenalidomide, which act on the molecular pathways that initiates and supports the cancer development, can be synergistically combined with the emerging successful immunotherapies such as dendritic cell and other cancer vaccines, ipilimumab and several anti-cancer antibodies (Vanneman, M. and Dranoff, G., 2012).

Combining two immunotherapies is also biologically logical since two different immunotherapeutic agents that have different effects on the relationship between the immune system and tumour cells can be used give a more beneficial cancer treatment. The use of Ipilimumab with an anti-PD-1 mAb is currently in a clinical trial with metastatic melanoma patients (Sharma, P. et al, 2011). Together, these immunomodulators are expected to function synergistically since they eliminate the immune suppression in two distinct pathways (Mellman, I. et al, 2011). However, combining these two immunomodulators might increase the adverse effects associated with them collectively. Therefore, a decisive assessment and planning would require during these studies to ensure that the unwanted toxic effects are eliminated. Other immunotherapy combinations can include the use of approaches that increases the T cell anti-cancer activation and numbers such as ACT or approaches that increases the antigen presenting to T cells and priming their effects such as cancer vaccines (Sipuleucel-T or ProstVac-VF) along with the immunomodulators such as anti-CTLA-4 and anti-PD-1 mAbs that suppresses the inhibitory immune checkpoints. Another combinational approach can be the use of immunostimulants such as IL-2 with the immunomodulators mentioned above. In this strategy, while the immunostimulant increases the overall activity of the lymphocytes that are attacking to the tumour cells, the immunomodulator can suppress the inhibitory factors that affect these lymphocytes, together providing a potent anti-cancer immune activity.

It seems to be that there are two major challenges for the development of the combinational therapies. One of these challenges is fine-tuning the correct sequence, time and dosage of the two combined agents during the administration to patients. While these factors are hard to determine prior to the clinical trials, they are the key players for the success of the combinational therapy. The other challenge is the preventing of the increased unwanted

adverse effects that might associate with combining the two particular agents, which might be the case for the combining of immunomodulators. However, this factor can be manageable once the appropriate administration of the agents is determined to ensure the safe usage of the therapy. Once these challenges are overcome, the combinational immunotherapies will prove to be the next step for an effective cancer immunotherapy.

A perfect cancer immunotherapy, which is the most specifically effective and the most long-lasting, would be the one that raises a very high number of tumour-antigen specific lymphocytes, directs them to the tumour sites and releases their destructive power to the cancer cells while it effectively blocks the immunosuppressive factors expressed by either normal or cancer cells that might inhibit the anti-cancer effects of the lymphocytes. This ideal cancer immunotherapy can be a result of either one type of immunotherapy strategy or a combination of two as discussed above, although the odds are in favour of the later. One of the reasons that the immunotherapy approach is so attractive for cancer treatment is the fact that immune system can create a memory that lasts for a very long time if not for the rest of the individual's life. After a successful cancer immunotherapy is managed, this memory of the immune system would ensure that the patient is immune and protected to the encountered type of cancer so that the common problem of reoccurrence of the cancer, which is associated with most of the conventional cancer treatments, would be resolved.

In the last decade, much has been learned about the immune system's role during both cancer manifestation and the fight against cancer. This increasing knowledge has already led to the development, approval and usage of several effective and high potential immunotherapies. There are numerous new studies and researches that are currently underway which very soon will extract the full potential of immunotherapy against the cancer. There is no doubt that the future of cancer treatments looks much brighter with the light of cancer immunotherapy.

References

Adams G.P. and Weiner L.M. Monoclonal antibody therapy of cancer. Nature Biotechnology 23, 2005, 1147 – 1157.

Alley S.C., Okeley N.M., Senter P.D. Antibody-drug conjugates: targeted drug delivery for cancer. Curr Opin Chem Biol 2010;14: 529–37.

Atkins M.B., Lotze M.T., Dutcher J.P., Fisher R.I., Weiss G., Margolin K., Abrams J., Sznol M., Parkinson D., Hawkins M., Paradise C., Kunkel L., Rosenberg S.A. High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. J Clin Oncol, 1999, 17:2105–2116.

Austin C.D., De Mazière A.M., Pisacane P.I., van Dijk S.M., Eigenbrot C., Sliwkowski M.X., Klumperman J., Scheller R.H. Endocytosis and sorting of ErbB2 and the site of action of cancer therapeutics trastuzumab and geldanamycin. Mol. Biol. Cell 15, 2004, 5268–5282.

Beck K.E., Blansfield J.A., Tran K.Q., Feldman A.L., Hughes M.S., Royal R.E., Kammula U.S., Topalian S.L., Sherry R.M., Kleiner D., Quezado M., Lowy I., Yellin M., Rosenberg S.A., Yang J.C. Enterocolitis in patients with cancer after antibody blockade of cytotoxic T‐lymphocyte‐associated antigen 4. J Clin Oncol. 2006 May 20;24(15):2283-9.

Belardelli F. Role of interferons and other cytokines in the regulation of the immune response. APMIS 1995;103:161–79.

Belardelli F., Ferrantini M., Proietti E., Kirkwood J.M.. Interferon-alpha in tumor immunity and immunotherapy. Cytokine & Growth Factor Reviews 13 2002 119–134.

Bisht M., Bist S.S. and Dhasmana D.C. Biological response modifiers: Current use and future prospects in cancer therapy. Indian journal of cancer, 2010, 47(4):443-451.

Boon T., Coulie P. G., Van den Eynde B. J. and van der Bruggen P. Human T cell responses against melanoma. Annu Rev Immunol. 2006;24:175-208.

Brahmer J.R., Topalian S.L., Powderly J., Wollner I., Picus J., Drake C.G., Stankevich E., Korman A., Pardoll D. and Lowy I. Phase II experience with MDX-1106 (Ono-4538), an anti-PD-1 monoclonal antibody, in patients with selected refractory or relapsed malignancies [abstract]. J Clin Oncol 2009;27(suppl 15):abstract 3018.

Carthon B.C., Wolchok J.D., Yuan J., Kamat A., Ng Tang D.S., Sun J., Ku G., Troncoso P., Logothetis C.J., Allison J.P., Sharma P. Preoperative CTLA4 blockade: tolerability and immune monitoring in the setting of a presurgical clinical trial. Clin Cancer Res. 2010 May 15;16(10):2861-71.

Cartron G., Dacheux L., Salles G., Solal-Celigny P., Bardos P., Colombat P., Watier H. Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcgammaRIIIa gene. Blood 99, 2002, 754–758.

Chang M.H., You S.L., Chen C.J., Liu C.J., Lee C.M., Lin S.M., Chu H.C., Wu T.C., Yang S.S., Kuo H.S., Chen D.S. Decreased incidence of hepatocellular carcinoma in hepatitis B vaccinees: a 20-year follow-up study. J Natl Cancer Inst. 2009;101(19):1348-55.

Coombes R.C., Buckman R., Forrester J.A., Shepherd V., O'Hare M.J., Vincent M., Powles T.J., Neville A.M.. In vitro and in vivo effects of a monoclonal antibody-toxin conjugate for use in autologous bone marrow transplantation for patients with breast cancer. Cancer Res 1986;46: 4217–20.

Correale P., Del Vecchio M.T., Di Genova G., Savellini G.G., La Placa M., Terrosi C., Vestri M., Urso R., Lemonnier F., Aquino A., Bonmassar E., Giorgi G., Francini G., Cusi M.G. 5-fluorouracil-based chemotherapy enhances the antitumor activity of a thymidylate synthase-directed polyepitopic peptide vaccine. J Natl Cancer Inst. 2005 Oct 5;97(19):1437-45.

Cross D. and Burmester J.K. Gene therapy for cancer treatment: past, present and future. Clin Med Res. 2006 Sep;4(3):218-27.

Danna E.A., Sinha P., Gilbert M., Clements V.K., Pulaski B.A., Ostrand-Rosenberg S. Surgical removal of primary tumor reverses tumor-induced immunosuppression despite the presence of metastatic disease. Cancer Res. 2004 Mar 15;64(6):2205-11.

Di Gaetano N., Cittera E., Nota R., Vecchi A., Grieco V., Scanziani E., Botto M., Introna M., Golay J. Complement activation determines the therapeutic activity of rituximab in vivo. J. Immunol. 171, 2003, 1581–1587.

Dimberu P.M. and Leonhardt R.M. Cancer Immunotherapy Takes a Multi-Faceted Approach to Kick the immune System into Gear. Yale Journal of Biology and Medicine 84, 2011, pp.371-380.

Drake C.G. Immunotherapy for prostate cancer: walk, don't run. J Clin Oncol 2009;27:4035–7.

Drake C.G. Prostate cancer as a model for tumour immunotherapy. Nat Rev Immunol 2010;10:580–93.

Dudley M.E., Gross C.A., Langhan M.M., Garcia M.R., Sherry R.M., Yang J.C., Phan G.Q., Kammula U.S., Hughes M.S., Citrin D.E., Restifo N.P., Wunderlich J.R., Prieto P.A., Hong J.J., Langan R.C., Zlott D.A., Morton K.E., White D.E., Laurencot C.M., Rosenberg S.A. CD8+ enriched ''young'' tumour infiltrating lymphocytes can mediate regression of metastatic melanoma. Clin Cancer Res. 2010 Dec 15;16(24):6122-31.

Dudley M.E., Yang J.C., Sherry R., Hughes M.S., Royal R., Kammula U., Robbins P.F., Huang J., Citrin D.E., Leitman S.F., Wunderlich J., Restifo N.P., Thomasian A., Downey S.G., Smith F.O., Klapper J., Morton K., Laurencot C., White D.E., Rosenberg S.A. Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. J Clin Oncol 2008, 26:5233-5239.

Dunn G.P., Bruce A.T., Ikeda H., Old L.J. and Schreiber R.D. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002 Nov;3(11):991-8.

Ellis L. M. and Hicklin D. J. VEGF-targeted therapy: mechanisms of anti-tumour activity. Nature Rev. Cancer 8, 2008, 579–591.

Fang L., Lonsdorf A.S., and Hwang S.T. Immunotherapy for Advanced Melanoma. Journal of Investigative Dermatology 2008 128, 2596–2605.

Ferris R.L., Jaffee E.M. and Ferrone S. Tumor Antigen–Targeted, Monoclonal Antibody–Based Immunotherapy: Clinical Response, Cellular Immunity, and Immunoescape. Journal of Clinical Oncology, 2010, vol. 28 no. 28 p4390-4399.

Fishman M. A changing world for DCVax: a PSMA loaded autologous dendritic cell vaccine for prostate cancer. Expert Opin Biol Ther 2009;9:1565–75.

Franklin M.C., Carey K.D., Vajdos F.F., Leahy D.J., de Vos A.M., Sliwkowski M.X. Insights into ErbB signaling from the structure of the ErbB2- pertuzumab complex. Cancer Cell 5, 2004, 317–328.

Gaguski M.E. and Begyn P. Advances in cancer therapy: targeted agents. Nurs Manage. 2008 May;39(5):45-8.

Gattinoni L., Finkelstein S.E., Klebanoff C.A., Antony P.A., Palmer D.C., Spiess P.J., Hwang L.N., Yu Z., Wrzesinski C., Heimann D.M., Surh C.D., Rosenberg S.A., Restifo N.P. Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of adoptively transferred tumor-specific CD8+ T cells. J Exp Med. 2005 Oct 3;202(7):907-12.

Gelderman, K.A., Tomlinson, S., Ross, G.D. and Gorter, A. Complement function in mAb-mediated cancer immunotherapy. Trends Immunol. 25, 2004, 158–164.

Ghiringhelli F., Menard C., Puig P.E., Ladoire S., Roux S., Martin F., Solary E., Le Cesne A., Zitvogel L., Chauffert B. Metronomic cyclophosphamide regimen selectively depletes CD4+CD25+ regulatory T cells and restores T and NK effector functions in end stage cancer patients. Cancer Immunol Immunother. 2007 May;56(5):641-8.

Goff S.L., Smith F.O., Klapper J.A., Sherry R., Wunderlich J.R., Steinberg S.M., White D., Rosenberg S.A., Dudley M.E., Yang J.C. Tumor infiltrating lymphocyte therapy for metastatic melanoma: analysis of tumors resected for TIL. J Immunother. 2010;33(8):840-7.

Guillemard V. and Saragovi H.U. Novel Approaches for Targeted Cancer Therapy. Current Cancer Drug Targets, 2004, 4, 313-326.

Hall P.S. and Cameron D.A. Current perspective: trastuzumab. Eur J Cancer 45, 2009, 12-18.

Hammerstrom A.E., Cauley D.H., Atkinson B.J. and Sharma P. Cancer Immunotherapy: Sipuleucel-T and Beyond. Pharmacotherapy 2011;31(8):813–828.

Hanahan D. and Weinberg R.A. Hallmarks of Cancer: The Next Generation. Cell 2011;144:646-674.

Hausen H.Z. Papillomaviruses in the causation of human cancers: a brief historical account. Virology. 2009 Feb 20;384(2):260-5.

Higano C.S., Corman J.M., Smith D.C., Centeno A.S., Steidle C.P., Gittleman M., Simons J.W., Sacks N., Aimi J., Small E.J. Phase 1/2 doseescalation study of a GM-CSF-secreting, allogeneic, cellular immunotherapy for metastatic hormone-refractory prostate cancer. Cancer 2008;113:975–84.

Hodi F.S., O'Day S.J., McDermott D.F., Weber R.W., Sosman J.A., Haanen J.B., Gonzalez R., Robert C., Schadendorf D., Hassel J.C., Akerley W., van den Eertwegh A.J., Lutzky J., Lorigan P., Vaubel J.M., Linette G.P., Hogg D., Ottensmeier C.H., Lebbé C., Peschel C., Quirt I., Clark J.I., Wolchok J.D., Weber J.S., Tian J., Yellin M.J., Nichol G.M., Hoos A., Urba W.J. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711–23.

Hudis C. A. Trastuzumab: mechanism of action and use in clinical practice. N. Engl. J. Med. 357, 2007, 39–51.

Ian D. Davis. An overview of cancer immunotherapy. Immunology and Cell Biology 2000;78:179–195.

J. van der Zee. Heating the patient: a promising approach? Ann Oncol. 2002 Aug;13(8):1173-84.

Jacene H.A., Filice R., Kasecamp W., Wahl R.L. Comparison of ^{90}Y-ibritumomab tiuxetan and ^{131}I-tositumomab in clinical practice. J Nucl Med. 2007;48(11):1767-76.

Jemal A., Bray F., Center M.M., Ferlay J., Ward E., Forman D. Global Cancer Statistics. CA: A Cancer Journal for Clinicians 2011;61:69–90.

Kantoff P.W., Higano C.S., Shore N.D., Berger E.R., Small E.J., Penson D.F., Redfern C.H., Ferrari A.C., Dreicer R., Sims R.B., Xu Y., Frohlich M.W., Schellhammer P.F., IMPACT Study Investigators. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010 Jul 29;363(5):411-22.

Kantoff P.W., Schuetz T.J., Blumenstein B.A., Glode L.M., Bilhartz D.L., Wyand M., Manson K., Panicali D.L., Laus R., Schlom J., Dahut W.L., Arlen P.M., Gulley J.L., Godfrey W.R. Overall survival analysis of a phase II randomized controlled trial of a poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. J Clin Oncol 2010;28: 1099–105.

Katz J., Janik J.E., and Younes A. Brentuximab Vedotin (SGN-35). Clin Cancer Res. 2011 Oct 15;17(20):6428-36.

Kim Y., Girardi M., McAuley S. and Schmalbach T. Cutaneous T-cell lymphoma (CTCL) responses to a TLR9 agonist CpG immunomodulator (CPG 7909), a phase I study. Journal of Clinical Oncology, 2004 ASCO Annual Meeting Proceedings (Post-Meeting Edition). Vol 22, No 14S (July 15 Supplement), 2004: 6600.

Kirkwood J.M., Tarhini A.A., Panelli M.C., Moschos S.J., Zarour H.M., Butterfield L.H., and Gogas H.J. Next Generation of Immunotherapy for Melanoma. Journal of Clinical Oncology 2008 Jul 10;26(20):3445-55.

Kreitman R.J. and Pastan I. Antibody-fusion proteins: anti-CD22 recombinant immunotoxin: moxetumomab pasudotox. Clin Cancer Res 2011;17:6398–405.

Krieg A.M. CpG motifs in bacterial DNA and their immune effects. Annu Rev Immunol. 2002;20:709-60.

Krieg A.M. Therapeutic potential of Toll-like receptor 9 activation. Nat Rev Drug Discov 5, 2006, 471- 484.

Krummel M.F. and Allison J.P. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J Exp Med. 1995 Aug 1;182(2):459-65.

Li S., Schmitz K.R., Jeffrey P.D., Wiltzius J.J., Kussie P., Ferguson K.M. Structural basis for inhibition of the epidermal growth factor receptor by cetuximab. Cancer Cell 7, 2005, 301–311.

Lowy D.R. and Schiller J.T. Prophylactic human papillomavirus vaccines. J Clin Invest. 2006;116(5):1167-73.

Madan R.A., Arlen P.M., Mohebtash M., Hodge J.W., Gulley J.L. Prostvac-VF: a vector-based vaccine targeting PSA in prostate cancer. Expert Opin Investig Drugs 2009;18:1001–11.

Margolin K., Atkins M.B., Dutcher J.P., Ernstoff M.S., Smith II J.W., Clark J.I., Baar J., Sosman J., Weber J., Lathia C., Brunetti J., Cihon F. and Schwartz B. Phase I Trial of BAY 50-4798, an Interleukin-2–Specific Agonist in Advanced Melanoma and Renal Cancer. Clin Cancer Res June 1, 2007 13; 3312.

Mellman I., Coukos G. and Dranoff G. Cancer immunotherapy comes of age. Nature. 2011 Dec 21;480(7378):480-9.

Melton R.G. and Sherwood R.F. Antibody-Enzyme Conjugates for Cancer Therapy. J Natl Cancer Inst. 1996 Feb 21;88(3-4):153-65.

Michel M.L. and Tiollais P. Hepatitis B vaccines: Protective efficacy and therapeutic potential. Pathol Biol (Paris). 2010 Aug;58(4):288-95.

Milenic D.E., Brady E.D., Brechbiel M.W. Antibody- targeted radiation cancer therapy. Nat Rev Drug Discov. 2004;3(6):488-99.

Mittendorf E.A. and Sharma P. Mechanisms of T cell inhibition: implications for cancer immunotherapy. Expert Rev Vaccines 2010;9:89–105.

Mogensen K.E., Lewerenz M., Reboul J., Lutfalla G., Uze G. Type I IFN receptor: structure, function, and evolution of a family business. J Interferon Cytokine Res 1999;19:1069–98.

Morales A., Eidinger D. and Bruce A.W. Intracavitary Bacillus Calmette-Guerin in the treatment of superficial bladder tumors. The Journal of Urology, 1976, 116(2):180-3.

National Cancer Institute, 2010. Bone Marrow Transplantation and Peripheral Blood Stem Cell Transplantation. [online] (Updated on 09/24/2010) Available at <http://www.cancer.gov/cancertopics/factsheet/Therapy/bone-marrow-transplant> [Accessed 15 February 2012].

Norden A.D., Young G.S., Setayesh K., Muzikansky A., Klufas R., Ross G.L., Ciampa A.S., Ebbeling L.G., Levy B., Drappatz J., Kesari S., Wen P.Y. Bevacizumab for recurrent malignant gliomas: efficacy, toxicity, and patterns of recurrence. Neurology. 2008;70(10):779-87.

Ohlsson B., Stenram U., Tranberg K.G. Resection of colorectal liver metastases: 25-year experience. World J Surg 1998; 22(3): 268-76; discussion 276-7.

Pardoll D. and Drake C. Immunotherapy earns its spot in the ranks of cancer therapy. The Journal of Experimental Medicine, 2012 February, 209(2):201-209.

Pardoll D.M. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012 Mar 22;12(4):252-64.

Park T.S., Rosenberg S.A. and Morgan R.A. Treating cancer with genetically engineered T cells. Trends in Biotechnology, November 2011, Vol. 29, No. 11, p550-557.

Pashenkov M., Goëss G., Wagner C., Hörmann M., Jandl T., Moser A., Britten C.M., Smolle J., Koller S., Mauch C., Tantcheva-Poor I., Grabbe S., Loquai C., Esser S., Franckson T., Schneeberger A., Haarmann C., Krieg A.M., Stingl G., Wagner S.N. Phase II trial of a toll-like receptor 9-activating oligonucleotides in patients with metastatic melanoma. J Clin Oncol. 2006 Dec 20;24(36):5716-24.

Pfeffer L.M., Dinarello C.A., Herberman R.B., Williams B.R., Borden E.C., Bordens R., Walter M.R., Nagabhushan T.L., Trotta P.P., Pestka S. Biological properties of recombinant alpha-interferons: 40th anniversary of the discovery of interferons. Cancer Res 1998;58:2489–99.

Plate J.M., Plate A.E., Shott S., Bograd S. and Harris J.E. Effect of gemcitabine on immune cells in subjects with adenocarcinoma of the pancreas. Cancer Immunol Immunother. 2005 Sep;54(9):915-25.

Prieto P.A., Yang J.C., Sherry R.M., Hughes M.S., Kammula U.S., White D.E., Levy C.L., Rosenberg S.A., Phan G.Q. The combination of ipilimumab and IL-2 appears to have an increased complete response rate. J Clin Oncol. 2010;28(15 Suppl): 8544.

Reinis M. BiovaxID, a personalized therapeutic vaccine against B-cell lymphomas. Curr Opin Mol Ther 2008;10:526–34.

Reits E.A., Hodge J.W., Herberts C.A., Groothuis T.A., Chakraborty M., Wansley E.K., Camphausen K., Luiten R.M., de Ru A.H., Neijssen J., Griekspoor A., Mesman E., Verreck F.A., Spits H., Schlom J., van Veelen P., Neefjes J.J. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. J Exp Med. 2006 May 15;203(5):1259-71.

Renoux G. The general immunopharmacology of levamisole. Drugs, 1980, 20(2):89-99.

Ribas A., Camacho L.H., Lopez-Berestein G., Pavlov D., Bulanhagui C.A., Millham R., Comin-Anduix B., Reuben J.M., Seja E., Parker C.A., Sharma A., Glaspy J.A., Gomez-Navarro J. Antitumor activity in melanoma and anti-self responses in a phase I trial with the anti-cytotoxic T lymphocyte-associated antigen 4 monoclonal antibody CP-675,206. J Clin Oncol. 2005 Dec 10;23(35):8968-77.

Ribas, A. Clinical development of the anti-CTLA4antibody tremelimumab. Semin Oncol. 2010 Oct;37(5):450-4.

Robert C., Thomas L., Bondarenko I., O'Day S., M D J.W., Garbe C., Lebbe C., Baurain J.F., Testori A., Grob J.J., Davidson N., Richards J., Maio M., Hauschild A., Miller W.H. Jr., Gascon P., Lotem M., Harmankaya K., Ibrahim R., Francis S., Chen T.T., Humphrey R., Hoos A., Wolchok J.D. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011 364(26):2517-26.

Roden A.C., Moser M.T., Tri S.D., Mercader M., Kuntz S.M., Dong H., Hurwitz A.A., McKean D.J., Celis E., Leibovich B.C., Allison J.P., Kwon E.D. Augmentation of T cell levels and responses induced by androgen deprivation. J Immunol. 2004 Nov 15;173(10):6098-108.

Rong-Fu Wang. CD8 regulatory T cells, their suppressive mechanisms, and regulation in cancer. Hum Immunol. 2008 Nov;69(11):811-4.

Rosenberg S. A., Yang J. C. and Restifo N. P. Cancer immunotherapy: moving beyond current vaccines. Nat Med. 2004 Sep;10(9):909-15.

Rosenberg S.A. and Dudley M.E. Adoptive cell therapy for the treatment of patients with metastatic melanoma. Current Opinion in Immunology 2009, 21:233–240.

Rosenberg S.A., Aebersold P., Cornetta K., Kasid A., Morgan R.A., Moen R., Karson E.M., Lotze M.T., Yang J.C., Topalian S.L. Gene transfer into humans: immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. N Engl J Med 1990, 323:570-578.

Rosenberg S.A., Restifo N.P., Yang J.C., Morgan R.A., Dudley M.E. Adoptive cell transfer: a clinical path to effective cancer immunotherapy. Nat Rev Cancer 2008, 8:299-308.

Sarid R. and Gao Shou-Jiang. Viruses and human cancer: From detection to causality. Cancer Lett. 2011 Jun 28;305(2):218-27.

Schroeder A., Heller D.A., Winslow M.M., Dahlman J.E., Pratt G.W., Langer R., Jacks T. and Anderson D.G. Treating metastatic cancer with nanotechnology. Nature Reviews Cancer, 2012;12:39-50.

Schuster S.J., Neelapu S.S., Guase B.L., Muggia F.M., Gockerman J.P., Sotomayor E.M., Winter J.N., Flowers C.R., Stergiou A.M., Kwak L.W. Idiotype vaccine therapy (Biovax ID) in follicular lymphoma in first complete remission: phase III clinical trial results [abstract]. J Clin Oncol 27:18s, 2009 (suppl; abstr 2).

Schwartzentruber D.J., Lawson D.H., Richards J.M., Conry R.M., Miller D.M., Treisman J., Gailani F., Riley L., Conlon K., Pockaj B., Kendra K.L., White R.L., Gonzalez R., Kuzel T.M., Curti B., Leming P.D., Whitman E.D., Balkissoon J., Reintgen D.S., Kaufman H., Marincola F.M., Merino M.J., Rosenberg S.A., Choyke P., Vena D., Hwu P. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. N Engl J Med. 2011 Jun 2;364(22):2119-27.

Senter P.D. Activation of prodrugs by antibody-enzyme conjugates: a new approach to cancer therapy. FASEB J 1990;4:188–93.

Sharma P., Wagner K., Wolchok J.D. and Allison J.P. Novel cancer immunotherapy agents with survival benefit: recent successes and next steps. Nature reviews. Cancer. 2011 Oct 24;11(11):805-12.

Shiau A.K., Barstad D., Loria P.M., Cheng L., Kushner P.J., Agard D.A., Greene G.L. The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. Cell 1998; 95(7): 927-37.

Smith I.E. and Dowsett M. Aromatase inhibitors in breast cancer. N Engl J Med 2003; 348(24): 2431-42.

Smith K.A. Interleukin-2: Inception, impact, and implications. Science 1988, 240:1169-1176.

Steeg P.S. Tumor metastasis: mechanistic insights and clinical challenges. Nature Medicine 2006;12:895 – 904.

Sunada, H., Magun, B.E., Mendelsohn, J. and MacLeod, C.L. Monoclonal antibody against epidermal growth factor receptor is internalized without stimulating receptor phosphorylation. Proc. Natl. Acad. Sci. USA 83, 1986, 3825–3829.

Sznol M., Powderly J. D., Smith D. C., Brahmer J. R., Drake C. G., McDermott D. F., Lawrence D. P., Wolchok J. D., Topalian S. L., Lowy I. Safety and antitumor activity of

biweekly MDX1106 (Anti-PD1, BMS936558/ONO4538) in patients with advanced refractory malignancies. J Clin Oncol 28:15s, 2010 (suppl; abstr 2506).

Tay Sun-Kuie. Cervical cancer in the human papillomavirus vaccination era. Curr Opin Obstet Gynecol 2012, 24:3–7.

Teicher B.A. and Chari R.V.J. Antibody Conjugate Therapeutics: Challenges and Potential. Clin Cancer Res 2011;17:6389-6397.

Testori A., Richards J., Whitman E., Mann G.B., Lutzky J., Camacho L., Parmiani G., Tosti G., Kirkwood J.M., Hoos A., Yuh L., Gupta R., Srivastava P.K., C-100-21 Study Group. Phase III comparison of vitespen, an autologous tumor-derived heat shock protein gp96 peptide complex vaccine, with physician's choice of treatment for stage IV melanoma: the C-100–21 study group. J Clin Oncol. 2008 Feb 20;26(6):955-62.

Therasse P., Arbuck S.G., Eisenhauer E.A., Wanders J., Kaplan R.S., Rubinstein L., Verweij J., Van Glabbeke M., van Oosterom A.T., Christian M.C. and Gwyther S.G. New Guidelines to Evaluate the Response to Treatment in Solid Tumors. NCI J Natl Cancer Inst, 2000, 92 (3): 205-216.

Thompson J.A., Kuzel T., Bukowski R., Masciari F., Schmalbach T. Phase Ib trial of a targeted TLR9 CpG immunomodulator (CPG 7909) in advanced renal cell carcinoma (RCC). Journal of Clinical Oncology, 2004 ASCO Annual Meeting Proceedings (Post-Meeting Edition). Vol 22, No 14S (July 15 Supplement), 2004: 4644.

Triesscheijn M., Baas P., Schellens Jan H. M., Stewarta F.A. Photodynamic Therapy in Oncology. The Oncologist 2006;11:1034–1044.

Urruticoechea A., Alemany R., Balart J., Villanueva A., Vinals F., Capella G. Recent advances in cancer therapy: an overview. Curr Pharm Des. 2010;16(1):3-10.

Van Meir E.G., Hadjipanayis C.G., Norden A.D., Shu Hui-Kuo, Wen P.Y., and Olson J.J. Exciting New Advances in Neuro-Oncology: The Avenue to a Cure for Malignant Glioma. CA Cancer J Clin. 2010 May–Jun; 60(3): 166–193.

Vanneman M. and Dranoff G. Combining immunotherapy and targeted therapies in cancer treatment. Nat Rev Cancer. 2012 Mar 22;12(4):237-51.

Viselli S.M., Stanziale S., Shults K., Kovacs W.J., and Olsen N.J. Castration alters peripheral immune function in normal male mice. Immunology. 1995 Feb;84(2):337-42.

Waldmann T.A. Immunotherapy: past, present and future. Nat Med. 2003;9(3):269-77.

Waller E.K. The Role of Sargramostim (rhGM-CSF) as Immunotherapy. The Oncologist October 2007 vol. 12 Supplement 2 p22-26.

Weiner L.M., Surana R. and Wang S. Monoclonal antibodies: versatile platforms for cancer immunotherapy. Nature Reviews Immunology 10, May 2010, 317-327.

West M.A., Wallin R.P., Matthews S.P., Svensson H.G., Zaru R., Ljunggren H.G., Prescott A.R., Watts C. Enhanced dendritic cell antigen capture via toll-like receptor-induced actin remodeling. Science. 2004 Aug 20;305(5687):1153-7.

Wheeler C.J. and Black K.L. DCVax-Brain and DC vaccines in the treatment of GBM. Expert Opin Investig Drugs. 2009 Apr;18(4):509-19.

William W.N. Jr., Kim E.S. and Herbst R.S. Cetuximab therapy for patients with advanced squamous cell carcinomas of the head and neck. Nat Clin Pract Oncol 6, 2009, 132-133.

Winter M.C. and Hancock B.W. Ten years of rituximab in NHL. Expert Opin Drug Saf 2009, 8:223-235.

Wolchok J.D., Hoos A., O'Day S., Weber J.S., Hamid O., Lebbé C., Maio M., Binder M., Bohnsack O., Nichol G., Humphrey R., Hodi F.S. Guidelines for the evaluation of immune therapy activity in solid tumors: immunerelated response criteria. Clin Cancer Res. 2009 Dec 1;15(23):7412-20.

Wood C., Srivastava P., Bukowski R., Lacombe L., Gorelov A.I., Gorelov S., Mulders P., Zielinski H., Hoos A., Teofilovici F., Isakov L., Flanigan R., Figlin R., Gupta R., Escudier B., C-100-12 RCC Study Group. An adjuvant autologous therapeutic vaccine (HSPPC-96; vitespen) versus observation alone for patients at high risk of recurrence after nephrectomy for renal cell carcinoma: a multicentre, openlabel, randomised phase III trial. Lancet 2008;372:145–54.

World Health Organization. The Global Burden of Disease: 2004 Update. Geneva: World Health Organization; 2008.

World Health Organization. WHO position on HPV vaccines. Vaccine 2009; 27:7236–7237.

Younes A. and Kadin M.E. Emerging applications of the tumor necrosis factor family of ligands and receptors in cancer therapy. J Clin Oncol 2003;21:3526–34.

Zitvogel L., Apetoh L., Ghiringhelli F., André F., Tesniere A., and Kroemer G. The anticancer immune response: indispensable for therapeutic success? J Clin Invest. 2008 Jun;118(6):1991-2001.

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