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Invited Review

Cancer Immunotherapy: Current Progress and Applications

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ABSTRACT

Cancer immunotherapy is a form of treatment protocol for cancer patients that has been studied intensively over the last two decades. The undesirable side effects during the course of conventional treatment has lead to the development of immunotherapy as an alternative treatment modality. This approach encompasses the use of three different strategies with various immunotherapeutic modalities including (i) cytokines and monoclonal antibodies; (ii) activation of antigen presentation cells (APC) by using antigen-specific peptides or sources of antigens such as tumour lysate; and finally (iii) adoptive transfer of *ex vivo* activated autologous cytotoxic T-cells. Due to specific-targeting by antigen-specific monoclonal antibodies, dendritic cells and activated $CD8^+$ T-cells, immunotherapy can eliminate tumour cells efficiently but the normal tissues are unaffected. Despite years of investigation, the outcome of immunotherapy-based clinical trials are inconsistent with very low response rates from patients. Several mechanisms have been proposed to contribute to this failure including the presence of regulatory T-cells (Treg), immunomodulatory cytokines, and aberrant gene expression in tumour cells. This review summarises information from about 140 articles and review papers. In addition, it also provides an update on recent trends in combinational immunotherapy with conventional therapy and encouraging results have been obtained. Re-evaluation of previous studies is necessary to fine-tune the design and approach of immunotherapy to ensure better treatment outcomes.

Keywords: Immunotherapy, cancer, combinational immunotherapy

INTRODUCTION

Immunotherapy is a form of clinical management procedure to enhance the defence mechanism of patients either specifically or non-specifically to fight against disease. Historically, the idea of immunotherapy was initiated a long time ago in the 1890s by Coley who attempted to use bacterial toxins to treat cancer patients.[1] Tumour regression was observed in those patients who received the injection. This could have been due to the effect of cytokines upon induction of a non-specific stimulation of the immune response towards the foreign antigen, the bacterial toxin.[1] Until today, Coley's idea of a general stimulation of the immune system by using bacterial agents has been applied to malignancy. For example, Bacillus Calmette Guerin (BCG) has been tested and used as an adjuvant together with either immunotherapy or chemotherapy to treat patients with superficial bladder cancers.[2] In addition, the potency of using virus-derived substances and various chemicals have also been investigated.[3, 4]

There are three different approaches of immunotherapy to enhance the cellular immune system for immunosurveillance (*Fig. 1*). Firstly, synthetic cytokines, interleukins or antibodies are used to enhance the immune system in general. For example, interleukin-2 (IL-2) and interferon γ and α have been used to treat systemic malignancy such as multiple myeloma[5] and breast cancer.[6] Secondly, the immune system can be activated specifically by optimising antigen presentation. Administration of biologic modifiers such as antigen-specific peptide or whole tumour cells are able to enhance endogenous antigen presentation of the epitope by professional antigen presenting (APC) cells to $CD8^+$ T cells. The third approach is to restore the immune system by adoptive transfer of *ex vivo* activated effector cells. The effector cells will first be stimulated against the tumour antigens of tumour cells *in vitro* before being infused back into the patients. Examples of immunotherapeutic agents that have been used in various immunotherapeutic settings are shown in Table 1.

BENEFITS OF IMMUNOTHERAPY

Radiotherapy, chemotherapy and surgery are the standard protocols to treat or to ensure better quality of time for patients with neoplasms. Surgery helps to reduce tumour burden, radiotherapy eliminates residual tumour cells in the surgical field and chemotherapy eliminates micro-metastatic disease both systematically as well as in the surgical field.[7] Despite their roles in tumour tissue eradication, these conventional protocols are associated with a number of side effects that may slowly deteriorate the normal physiological functions especially the immune defence mechanism of the patients. Besides eradication of tumour cells, both radiotherapy and chemotherapy can kill normal proliferating cells, including those in the bone marrow, hair follicles and lining of the gastrointestinal tract. During the course of conventional treatments, patients are vulnerable to infection as the immune system of the patient is weak due to toxicity.

To date, immunotherapy has moved from test tubes in the laboratory to the pre-clinical and clinical level.[8] Table 2 shows the list of clinical trials that involve immunotherapy. Immunotherapy helps to overcome several obstacles faced by conventional treatments, including, lack of specificity and toxicity.[9] Furthermore, immunotherapy has the ability to provide memory response to combat tumour reoccurrence.[7] Immunotherapy may provide a better quality of time and reduce the psychological burden for the patients. As for those patients who fail in their conventional therapy, when death appears to be inevitable, immunotherapy may provide better treatment outcomes for them.

It is without doubt that surgery is a useful approach for cancer treatment. However, it has been shown that surgical procedures can lead to post-operative immunosuppression, which may facilitate dissemination of tumour cells and outgrowth of minimal residual disease or micro metastasis.[10] Therefore, strategies for stimulation of anti-tumour immune responses are particularly crucial to prevent metastasis formation and reduce risk of relapse. In patients with esophageal cancer who underwent trasthoracic esophaectomy, *ex-vivo* activated lymphokine-activated killer (LAK) cells were transferred just after surgery to overcome the post-operative immunosuppression.[11] According to Yamagchi and colleagues, LAK cells transfer can significantly increase the helper and cytotoxic T-cell populations in post-operative patients. In addition, the LAK cell treated group had a trend of reduction for post-

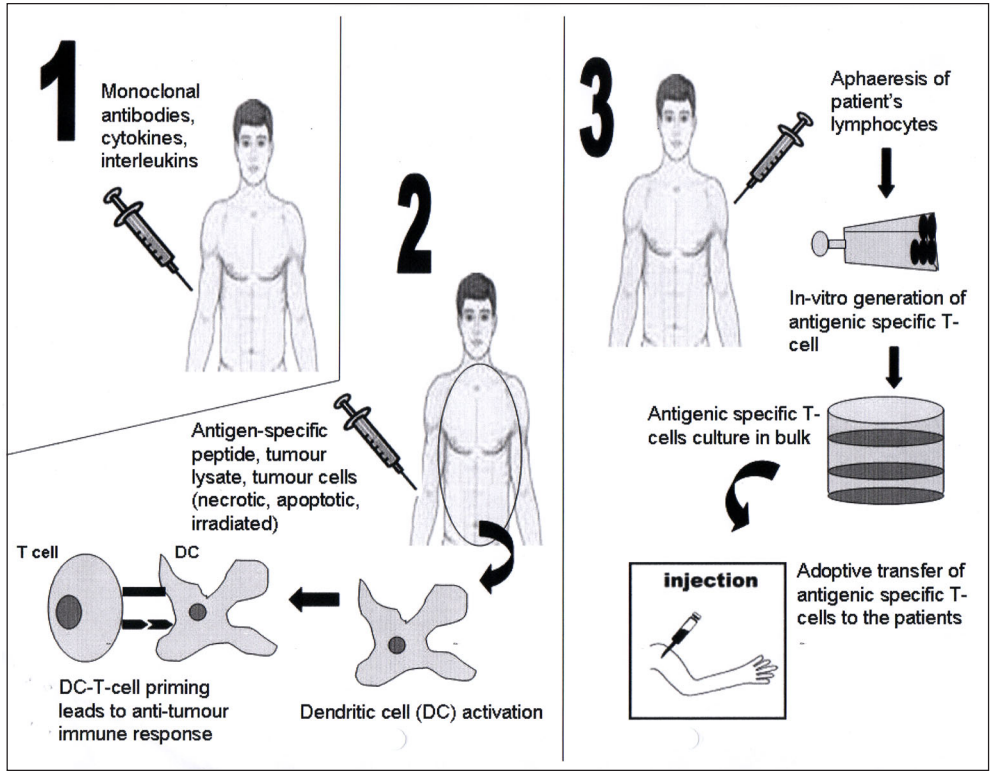

Figure 1. The three main approaches in immunotherapy: (1) Injection of immunostimulatory molecules such as cytokine to activate the immune system against tumour. (2) A tumour -specific immune response will be evoked by administration of antigen sources such as tumour-associated peptides to activate the dendritic cells (DC) and later activation of anti-tumour immune response via DC-T-cell priming. (3) Adoptive transfer of *ex vivo* activated T-cells to the patients. Autologous T-cells, which are obtained from the patients will be activated *in vitro* in an optimum condition in order to yield sufficient amounts of tumour antigen-specific T-cell clones for adoptive transfer.

Table 1. List of Immunotherapeutic agents used

Immuntherapeutic agents	Reference
Dendritic cells	
- loaded with peptide	[98]
- loaded with tumour lysate	[99]
- pulse with necrotic tumour cells	[100]
- loaded with irradiated tumour cells	[101]
- pulsed with recombinant viral vector	[102]
- pulsed with fusion protein	[103]
- transfected with recombinant viral vector	[104]
Monoclonal antibody	
- antibody alone (anti-CCR4, anti-CD25, anti-CD20, etc)	[105]
- fused with bacterial toxin	[98,106]
- bispecific	[107]
- genetically engineered	[62]
- radioactive labeled	[17]
Whole cell vaccine	
- irradiated tumour cells	[108]
- irradiated tumour cells transduced with viral vector	[109]
- modified by virus	[110]
Gene-modified	
- cytotoxic T-cells (Supernatural T-cell)	[54,111]
- dendritic cells	[63]
Bacterial gene therapy	
- recombinant idiotype Fab fragment	[112]
- recombinant viral vector	[87]
Immune cells	
- peripheral gamma delta T cells	[113]
- autologous cytokine induced killer (CIK)	[114]
- natural killer (NK)	[97]
- lymphokine activated killer (LAK)	[11]
- activated $CD8^+$ or $CD4^+$ or $CD3^+$ lymphocyte	[115,116]
Peptide	[117, 118]
Non-specific immunostimulatory factor (IL-2)	[19]

operative remote infection such as pneumonia and surgical site infection when compared to the control group.[11] In a study conducted on patients who underwent radical surgery for colorectal cancer (Duke' B and C), it was found that pre-operative interleukin-2 (IL-2) administration had greatly reduced post-operative immunodeficiency with improved prognosis. [12] This would be due to early activation of the antineoplastic immune system to counteract the growth of minimal residue disease and prevent late disease progression.[12]

Besides surgery, radiotherapy is also an important treatment modality for cancer patients. However, ionising radiation acts both as a carcinogen as well as a therapeutic agent.[13] Besides the killing of cancer cells, it usually causes induction of intensive stress and DNA damage to the surrounding tissues that lead to production of pro-inflammatory cytokines including TGF-β,[14] reactive oxygen species (ROS),[13] epidermal growth factors and fibroblast growth factors.[15] The series of these events may lead to persistence of inflammatory reaction in the microenviromment and later carcinogenesis. [13] Thus, targeted radiotherapy is essential to ensure the efficacy of treatment. Conjugation of tumour-associated antigens (TAAs)-specific monoclonal antibodies with radioisotopes helped to achieve a better response and prognosis.[16] Radioimmunotherapy of prostate cancer using radiolabeled anti-prostate-specific membrane antigen monoclonal antibody (J591), showed excellent targeting of soft-tissue and bone metastasis with limited toxicity of manageable myelotoxicity.[17] A phase I/II clinical trial using the novel $^{[131]}$I (iodine)-labeled Hab18G/CD147-specific monoclonal antibody Fab'$_2$ fragment on patients with hepatocellular carcinoma has yielded an encouraging clinical outcome with improved survival rate of progression-free in the patients after either one or two cycles of treatment ($p<0.0001$).[18]

CHALLENGES IN THE FIELD OF IMMUNOTHERAPY

The first mentioned immunotherapeutic approach using the non-specific immunostimulatory agents including IFN-γ, IFN-α and rIL-2 has been associated with systemic toxicity and autoimmune-like symptoms due to the over-activated immune system.[19, 20] Administration of IL-2 in patients with advanced melanoma has shown no evidence of improved survival or disease remission. [21] Toxicity complications caused by systemic administration of cytokines such as IL-2 and IFN-α have been documented in patients with metastatic renal cell carcinoma, [19] melanoma,[21] low-grade lymphoid neoplasm,[22] and prostate cancer.[23]

Despite the use of imuunostimulatory cytokines, monoclonal antibodies (mAbs), which mainly target tumour associated antigens (TAAs), are also widely used in immunotherapy. Tumour cells that are 'tagged' by the TAAs specific mAbs may help to recruit and provoke host effector mechanisms to eradicate the targeted tumour cells.[9] However, some mAbs fail to respond with expected outcomes such as, trastuzumab (anti-Her/2neu).[24] The failure of antibody-based immunotherapy may mainly be due to low or loss of TAAs expression and alteration of the immunodominant epitope.

The evolution of the immune evasion during tumorigenesis further complicates the situation. Cancer immunoediting is a concept that focuses on the shaping of neoplasm and has been proposed by Dunn *et al.* in 2002.[25] In this process, the immune system will first undergo vigorous tumour killing, then come to the process of immune sculpturing to allow the survival of low immunogenic tumour variants and finally lead to tumour escape mechanism.[25] Down regulation or loss of TAA expression has been reported both in human neoplasm and animal models such as melanoma,[26] colorectal carcinoma [27] and murine retinoblastoma.[28] This phenomenon is often associated with repeated treatments [29] and the presence of *in vivo* immunoselection pressure for immunoresistant tumor variants.[30, 31] According to Matsui and colleagues, aggressive immunotherapy with peptide-stimulated DUC18 T cells in tumours bearing BALB/c mice led to relapse of the disease with re-growth

Table 2. List of recent clinical trials that are related to applications of immunotherapy

Disease	Agent used	Response	Toxicity	Reference
Non-Hodgkin's lymphoma	Epratuzumb (anti-CD22 MAb) + rituximab (anti-CD20)	47% response rate	-nil-	[105]
Prostate cancer	Provenge (DC pulsed with PA2024, a recombinant PSA) + bevacizumab	Induction of immune response towards PA2024	-nil-	[102]
Advance melanoma	Peptide, tumour lysate or both peptide & tumour lysate pulsed dendritic cells	Tumour reduction (1/16), DTH response (10/16)	Autosensitisation dermatitis-like eruption	[99]
Recurrent malignant glioma	Autologous whole cell tumour + GM-CSF + anti-CD3 activated lymphocyte	DTH response towards tumour (17/18)	-nil-	[108]
Metastasis, asymptomatic hormone refractory prostate cancer (Phase III)	Sipuleucel-T (APC8015)	Survival advantage	-nil-	[119]
Breast cancer (Phase III)	Oxidised mannan-MUC1 [ISRCTN71711835]	No recurrence (0/16), anti-MUC-1(9/13), MUC-1 specific response (4/10)	-nil-	[120]
Hormone-naive prostate cancer (Phase I/II)	Irradiated, allogeneic, prostate cancer cells with GM-CSF gene transduced	Decrease prostate-specific antigen (16/21), infiltration of DC and macrophage, oligoclonal antibodies	-nil-	[109]
Metastatic renal cancer (Phase I)	Autologous DC pulsed with HLA-A2 specific peptides + low dose IL-2	Regression (6/20), MUC-1 specific T-cells response at peripheral	-nil-	[98]
Gliomas and melanoma (Pilot trial)	Irradiated tumour cells tranduced with recombinant retroviral vector with B7-2 and GM-CSF	No specific anti-tumour immunity	Inflammation response	[121]
Advance B-cell lymphoma (Phase I)	Recombinant Fab fragment of *E coli* + lipid-based adjuvant	Vaccine specific mAb (5/17) anti-Fab specific T-cell response (8/17)	Mild	[112]
Solid malignancy (Phase I/II)	HLA-A2402- restricted modified WT1 (Wilms' tumour gene) peptide in Montanide ISA51 adjuvant	Potential anti-tumour effect	Acceptable	[117]
Acute myeloid leukemia (Phase I/II)	Autologous dendritic- like leukemic cells (DLLC)	Anti-leukaemic T-cells response	Autoimmunity, eczema (1/22)	[122]
Metastatic renal carcinoma	IL-2 + IFN-α	Only subset of patients responded	Fever, asthenia, nausea /emesis, skin disorder, hypotension, diarrhea	[19]

Malignant Mesothelioma (MM)	Autologous MM tumour lysate with recombinant GM-CSF	Anti-MM immune response (7/22), no major tumour regression	-nil-	[123]
Hormone refractory prostate cancer (HRPC) (Phase I)	DC loaded with HLA-A0201 restricted peptide derived from PSA, PSMA, surviving, prostein, trp-p8	Decrease serum PSA (1/8), antigen specific CD8 + activation against survivin, prostein and PSMA	Local skin reaction	[124]
Adenocarcinoma (Phase I)	Autologous DC pulse with mannan-MUC-1 fusion protein	Tumour stabilisation (2/10)	-nil-	[103]
Metastatic melanoma	Recombinant vaccinia virus with B7.1, ICAM-1 and LFA-3 (rV-TRICOM)	Anti-vaccinia antibody and T-cell response	Mild fatigue, myalgia, vitiligo	[87]
Cervical intraepithelial neoplasia	HPV 16 L1 VLP vaccine	High level protection against HPV16 infection for at least 3.5 years after immunisation	-nil-	[125]
T-cell large granular lymphocyte leukemia	Mikbeta1 (murine MAb against CD122)	Down-regulation of receptors from surface of leukemic cell.	-nil-	[126]
Advance non-small cell lung cancer (NSCLC) (Phase I)	Epidermal growth factor (EGF) – based vaccine	Good mAb response against EGF (15/43)	Fever, chills, nausea, vomiting, flushing	[127]
Stage II melanoma	DC pulsed with Melan/MART-1antigen	Increase antigen specific $CD4^+$ and $CD8^+$ T-cell response, long-live tumour antigen specific DTH reactivity	-nil-	[128]
Multiple melanoma	Idiotype-pulsed allogeneic DC (alloDC) + soluble protein Id conjugated with KLH (Id-KLH)	Anti-KLH antibody response, secretion of cytokines (IL-2, IL-10, TNF-α, IL-6, IFN-γ)	-nil-	[129]
Multiple metastasis (Phase I)	DC pulsed with autologous necrotic tumour cells + OKT3 and IL-2 activated lymphocytes	DTH hypersensitivity reported, prolonged survival time	Low grade fever	[130]
Respectable stage III melanoma (randomised double-blind trial)	Newcastle disease virus-modified autologous melanoma cell lysate + IL-2	No remarkable differences between verum and placebo group	-nil-	[110]
Metastatic melanoma (Phase I/II)	Anti-CTLA-4 +IL-2	Objective tumour response (8/36), no synergistic effect observed	Autoimmune toxicity (grade II/IV),	[20]
Prostate cancer	PSA146 peptide (HLA-A2-specific PSA peptide)+GM-CSF	Positive DTH response, T-cell immunity developed	-nil-	[118]

Note: Due to limitations of space, only summarised data from articles that have been published in *Pubmed* in the year 2006 are summarised in the table. [‘DC’, dendritic cell; ‘PSA’, prostate-specific antigen; ‘DTH’, delayed-type hypersensitivity; ‘GM-CSF’, granulocyte macrophage colony stimulating factor; ‘IL’, interleukin; ‘HLA’, human leucocyte antigen; ‘E coli’, *Escherichia coli*; ‘IFN-α’, interferon alpha; ‘PSMA’, prostate-specific membrane antigen; ‘ HPV’, Human papillomavirus; ‘ KLH’, keyhole limpet hemocyanin; ‘ TNF-α’, tumour

of tumour cells and loss of TAAs expression. [32] Other tumour escape mechanisms are shown in *Fig. 2*.

The infiltration and priming efficiency of effector cells into the tumour micro-environment may not guarantee anti-tumour immunity.[33, 34] The presence of large numbers of transgenic or *in-vitro* expanded tumour antigen-specific T cells do not consistently cause regression of tumour either in animals or patients.[35, 36, 37] It is most likely that this phenomenon may be caused by the trafficking properties of the activated T cells as well as the tumour environment.[38] Subpopulations of activated T cells may lose expression of CD62L and CCR7 that enable them to adhere to the peripheral basement membrane[35] and trafficking toward the inflammatory chemokine such as CCL3 and CCL5.[39, 40]

The use of *ex-vivo* cultured effector cells and autologous tumour cells, which is the third immunotherapeutic approach as mentioned earlier, is highly labour intensive. In addition, these cells are not always successfully grown *in vitro*.[38] To generate effector cells for adoptive transfer, novel cell culture methodologies are needed to improve the frequency of specific T cells. Studies have shown that T cells isolated from different anatomical sites including peripheral blood, solid tumour, liquid tumour, and tumour involved or tumour free lymph nodes have different growth signals.[41] Tumour infiltrating lymphocytes (TILs) can grow more consistently and to high levels than those isolated from peripheral blood. [41, 42] These differences may be reflected by different maturation state, nature of antigenic stimulus, frequency of re-stimulation, growth factors and co-stimulation of the T cells at different sites including the expression of chemokine receptors.[43] As for adoptive transfer with autologous tumour cells, large quantities of cells are needed. This attempt aims to immunise the patient with all the antigens that are endogenously expressed by their tumour. [7] However, some patients may have small tumour size and this can limit the quantity of cells available for study. Thus, the procedure for expansion of cells will become too costly for the patients. [7] In cases where the tumour lesion is not accessible, this approach will not be possible.

The presence of immunosuppressive cytokines such as, TGF-β and IL-10 within the tumour (as shown in *Fig. 2*) may disturb the maturation of dendritic cells as well as effector T cells generation and function.[44] For example, injection of either apoptotic or non-apoptotic tumour cells may not necessarily cause dendritic cell maturation but may provoke tolerance of tumour immunity.[45, 46, 47] There are many other cells types, which may have immuno-stimulatory effects, upon contact with apoptotic cells. Other than dendritic cells, macrophages, which are more commonly present at the tumour site have the ability to engulf apoptotic cells to cause secretion of a wide range of anti-inflammatory cytokines, including interleukin (IL)-10 and TGF-β.[48, 49, 50] In contrast, macrophages also respond to non-apoptotic cells but to a lesser extent to produce pro-inflammatory cytokines such as TNF-α and IL-1β.[48, 49, 50] Occasionally, apoptotic cells that have not been efficiently phagocytosed may lead to secondary necrosis, which may cause autoimmunity. [51] A study showed that mice bearing mutations resulting in defective phagocytosis of apoptotic cells have a greater tendency to develop autoimmunity than the null type mice.[52] Therefore, strategies to block phagocyte function or the inhibitory effect of cytokines produced during phagocytosis have been carried out. These apoptotic cells are found to be significantly more immunogenic to the dendritic cells. [53]

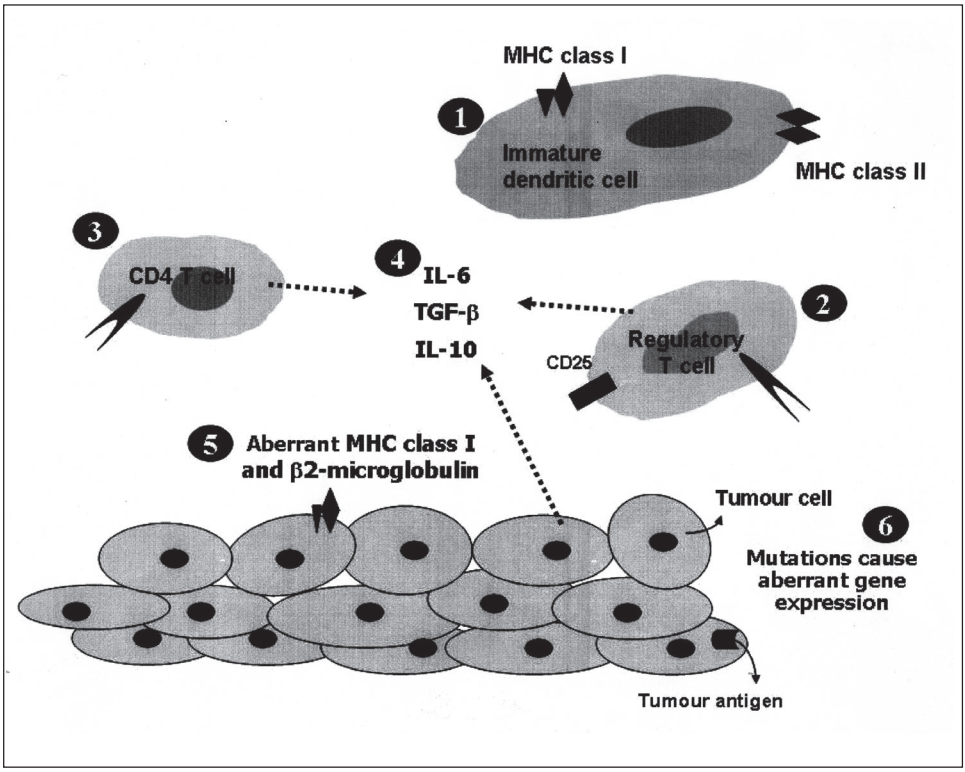

Figure 2. Proposed tumour escape mechanisms: (1) Local Dendritic cells (DCs) may be immature and unable to up-take, process, or present antigens. These DCs may be inhibited from migrating to regional lymph nodes or may actually induce tolerance, especially when presenting self-antigens. (2) Regulatory T cells which infiltrate the tumor site are able to mediate suppression of antigen-primed T cells. (3) The presence of helper CD4 T cell may skew the immune response toward a Th2 phenotype by secretion of Th2 cytokines such as IL-6, TGF-β and IL-10. This phenomenon inhibits the initiation of Th1 T cells and effective cellular immunity. (4) Tumor cells and the surrounding stromal may release a number of suppressive cytokines such as IL-6, IL-10, and TGF-â. This creates an environment that is not conducive to local immunity, which allows tumor cells to escape. (5) Tumor cells may express aberrant MHC class I molecules or â2-microglobulins, resulting in inadequate antigen presentation that prevent recognition of tumors by effector T cells. (6) Mutations may cause aberrant gene expression in components of signaling pathways as well as tumour antigen in tumour cells, resulting in abnormal cell behaviours and inadequate antigen presentation to activate the effector T cells.
(*Source*: Adapted from *www.innovitaresearch.org/news/04051401.html*)

STRATEGIES IN IMMUNOTHERAPY

Despite expression of a wide range of chemokines, cytokines with various immunomodulatory effects have also being found. One possible explanation is that counteractive cytokines such as TNF-α, IL-10, TGF-β are expressed simultaneously at the tumour site. Thus, activated effector cells are poorly attracted to the tumour site. To overcome this problem, steps have been taken to block the inhibitory effect of the cytokine and to genetically modify T cells to express a chemokine receptor for adoptive transfer has significantly enhanced the T cell trafficking to the tumour and anti-tumour efficiency. [54] In addition, modification of tumour cells to express CCL3 and CCL20 has generated effective anti-tumour immune response. [44, 55, 56, 57] Therefore, a strategy to generate T cells with appropriate trafficking properties as well as modification of the tumour cells may help to enhance the efficiency of immunotherapy.

The efficiency of immunotherapy depends greatly on the recognition of antigen bearing tumour cells by the antigen-specific effector population. Non-specific approaches to stimulate the immune system have been carried out by using either autologous or allogenic tumour cells expressing shared antigens, purified defined tumour antigens and epitopes have shown some promising results in small trials. [58, 59, 60] Non-specific immunostimulatory agents, including cytokines (IFN-γ and TNF-α), bacterial toxin (BCG) [3, 4] and immunostimulatory mAbs [9] have been used to up-regulate the immunity of the patients. High-dose IFN-γ has been used to treat patients with high-risk melanoma. [61] The tumoricidal ability of genetically modified tumour cells and cytotoxic T-cells has been tested out recently in several clinical trials. [62, 63] Randomised clinical trials of autologous GM-CSF –producing prostate cancer cells, APC8015 (Provenge) has demonstrated improved survival in patients with hormone-refractory prostate cancer. [64]

In addition, several strategies have been formulated to overcome the toxic effect of cytokine-based immunotherapy. O'Brien and colleagues have co-administered the rIL-2 with tauolidine in stage IV melanoma patients to reduce the toxicity without diminishing the therapeutic effect. [65] In addition, ReGel, an aqueous-based polymer has been used as an IL-2 delivery tool for cancer therapy. [66] The outcome of the study is very encouraging with sustained IL-2 tumoricidal effect and decreased IL-2 toxicity. [66] In addition, selection of alternative cytokines that have similar anti-tumour effect but lesser toxicity has been tested. Oniki and colleagues have recently used a combination of two IL-12-related cytokines, IL-23 and IL-27 to treat mice with poorly immunogenic B16F10 melanoma. Their study showed that systemic administration of these cytokines induced a protective anti-tumour activity which involved both the $CD8^+$ T-cell and natural killer (NK) cells with minimal toxicity as compared to IL-12 administration. [67] Furthermore, a novel cytokine molecule, which combines desired cytokine activities to render toxic effect, has been developed by Acres & colleagues. [68] An IL-2/IL-18 recombinant molecule which has a novel lymphocyte-stimulating activity with reduced IL-2 associated toxicities in murine models has been tested. [68]

Lastly, aberrant expression of tumour-associated antigen in tumour cells has directed the development of mAbs against cell-surface receptors of the immune system. [9] This strategy helps to overcome the problem of loss or low antigen expression in tumour cells and to boost or block the effects of immunosuppressive cytokines present in the tumour

environment. [69] These immunostimulating antibodies are designed against a number of molecules controlling the positive and negative signals in T cell activation. [70, 71] In animal models, antibodies against CD40, [72] 4-1BB, [73] CTLA-4 [72, 74] and CD25 [75] have been shown to provoke powerful tumour specific CTL responses capable of eradicating established tumour mass.

CURRENT TRENDS IN IMMUNOTHERAPY

New combinational immunotherapies are currently being tested to improve the efficacy of traditional treatment modalities. Combination of intratumoural dendritic cells with systemic cytotoxic therapies, such as chemotherapy and radiotherapy, have been reported in several studies. [76, 77, 78] In these models, the tumour cells were first killed by the chemotherapeutic drugs which was followed by the administration of dendritic cells to react with the apoptotic tumour cells to provoke anti-tumour immunity. [76, 77, 78] The combinational therapy was significantly more effective than either agent alone. [76, 77, 78, 79] Using combinational therapy, lower dose treatment regimens can be prescribed to the patients. [80] This innovative approach helps to avoid the higher medication level that causes undesirable side-effects when a single treatment modality is prescribed. [Please refer to Table 3 for the list of the clinical trials involve combinational immunotherapy]

The issues of tumour immunosuppression and lack of identified tumour-associated antigens (TAAs) are critical determining factors for successful immunotherapy. [81] High amounts of immunoregulatory lymphocytes (Treg), including $CD4^+CD25^+$ and $CD4^+CD25^+Foxp3^+$ subpopulations, IL-10 producing TR1 lymphocytes and tumour necrosis factor beta (TGF-β) producing TH3 lymphocytes have been detected at the tumour microenvironment which can inhibit the immune response and promote tumour outgrowth. [82] In addition, high numbers of monocytes, macrophages and neutrophils have been associated with very poor survival in patients with metastatic renal cell carcinoma. [83] Several groups of researchers have developed therapeutic approaches based on the concept of combinational immunotherapy to tackle these problems. For example, in patients with Burkitt-type lymphoma (BL) or lymphoblastic leukemia (B-ALL), the use of hyper-fractionated cyclophosphamide, vincristine, doxorubicin and dexamethasome (hyper-CVAD) together with rituximab (anti- CD20 monoclonal antibody) helped to achieve complete remission in 86% (24/28) of the evaluated patients. [84] Chemotherapeutic drugs not only eradicate tumour cell but they can also help to control Treg accumulation at the tumour microenvironment. [85] At the same time, histamine, which can help to protect NK and T cells against oxidative damage by inhibiting the formation and release of the phagocyte-derived reactive oxygen species, can also be used for immunotherapy. [83] Co-administration of histamine and IL-2 help to increase intratumoural CD56 NK cells ($p= 0.008$) and $CD8^+$ T cells ($p= 0.019$) with manageable toxicity compared to the baseline as well as treatment with IL-2 alone. [83] In an *in vivo* model of B16/BL6 melanoma, prescription of anti-CTLA-4 and a GM-CSF- transduced tumour cell vaccine (Gvax) helped to change the intratumour balance of Treg and effector T-cells to one which favors tumour rejection. [86] In this model, anti-CTLA-4 prevents release of inhibitory substances on T cell activation and proliferation, whereas,

Table 3. List of recent clinical trials that are related to applications of combinational immunotherapy

Types	Disease	Agent used	Response	Toxicity	Reference
Radio- immunotherapy (Phase I)	Metastatic CEA-producing malignancies	(90)Y labeled cT84.66 anti-CEA conjugate to DTPA	Human anti-chimeric antibody (8/13)	Haematologic toxicity	[131]
Radio-immunotherapy (Phase I/II)	Hepatocellular carcinoma	^{131}I(iodine) labeled HAb 18G/CD147-specific mAb Fab' fragment	Improved survival rate	-nil-	[18]
Radio-immunotherapy	Medullary thyroid carcinoma (MTC)	Anti-CEA/anti-DTPA-indium BsMAb + ^{131}I-labeled bivalent hapten	Longer overall survival	Haematologic toxicityto bone related / bone marrow metastasis	[132]
Chemo-immunotherapy	Burkitt-type lymphoma (BL) & acute lympho-blastic leukemia (B-ALL)	Rituximab + hyper-CVAD (hyper-fractionated cyclo-phosphamide, vincristine, doxorubicin, dexamethasone)	Improved overall survival rate in adult BL and B-ALL	-nil-	[84]
Radio- immunotherapy	Advance lung cancer necrosis therapy	Iodine- 131-labeled tumour chimeric antibody (^{131}I-chTNT)	Promising results	-nil-	[133]
Chemo-immunotheray	Metastatic renal cell carcinoma, melanoma	Thalidomide + rIL-2	Durable and active response	Mild to moderate (somnolence, constipation, neuropathy, rash, flu-like symptoms, fluid retention, hypotension, hypothyroidism	[134, 65]
Chemo-immunotherapy	Extensive stage small cell lung cancer	DC transfected with full-length wild- type p53 gene in adenoviral	p53 specific T-cell response 57.1%), anti-adenovirus mAb, high-rate objective	-nil-	[104]

		vector + chemotherapy	chemotherapy response (61.7%)		
Radio-immunotherapy	Recurrent primary and metastatic malignant brain tumour (Phase II)	Iodine- 131-labeled murine antitenascin MAb 81C6 (^{131}I-m81C6)		Acute reversible/ irreversible neuro-toxicity, acute haematologic toxicity	[82]
External radiotherapy + Radio-immunotherapy	Non-small cell lung cancer (NSCLC)	^{111}In-F(ab')2 -HMFG1	Residence time and adsorbed dose in tumour are low	-nil-	[135]
Radio-immunotherapy (Phase II)	Relapsed or refractory indolent non -Hodgkin's lymphoma (NHL)	iodine-131 (^{131}I) -rituximab chimeric anti-CD20 antibody	Overall response (76%) with 53% complete response	Neutropenia, thrombocyto-penia (Grade IV)	[136]
Chemotherapy + Radio-immunotherapy (Phase II)	Advanced follicular lymphoma	Cyclophosp- hamide, doxorubicin, vincristine, and prednisone (CHOP)+ tositumomab/iodine I-131 tositumomab	Overall response rate (91%) with 69% complete remission	-nil-	[137]
Radio-immunotherapy (Phase I)	CEA-expressing tumors [nonmedullary thyroid carcinoma (non-MTC), medullary thyroid carcinoma (MTC)]	anti-carcino-embryonic antigen (CEA) hMN-14 x m734 bispecific antibody (BsmAb) + (131)I-di-diethylenetriamine pentaacetic acid (DTPA)- indium hapten	Better response in MTC group than in non-MTC group patients)	Hematologic toxicity (higher in MTC patients than in non-MTC	[138]
Chemo-immunotherapy (Phase I)	Chronic lymphocytic leukemia	Pentostatin, cyclophos-phamide and rituximab response	Response rate (91%) with 41% complete	Modest haematologic toxicity	[139]
Chemo-immunotherapy (randomised clinical trial)	Progressive metastatic renal cell carcinoma	Interleukin-2/interferon-alpha2a/13-retinoic acid	No survival advantage	-nil-	[140]
Chemo-immunotherapy (Phase II)	Metastatic renal cell carcinoma	Vinorelbine and IL-2 (low dose)	Improved overall survival rate	-nil-	[141]

Note: Due to limitations of space, data summarised from articles that have been published in *Pubmed* in the year 2006 are summarised in the table. ['CEA', carcinoembryonic antigen; 'DTPA', Diethylenetriaminepentaacetate; 'mAb', monoclonal antibody]

Gvax primes the tumour-reactive effector T-cells which ultimately leads to T-cell activation and proliferation.[86]

Another innovative approach to tackle the problem with immunodeficiency and immunosuppression of patients is to up-regulate the immune system by using compounds or fragments from microorganisms or recombinant bacterial or viral vectors.[87] Local delivery of vaccinia virus expressing multiple co-stimulatory molecules including B7.1 (CD80), ICAM-1 and LFA-3 (rV-TRICOM) in patients with metastatic melanoma and breast cancer have been shown to enhance T-cell responses to TAA to a level far greater than any one or two applications of the co-stimulatory molecules in combination.[87, 88] Recently, immunotoxin-based therapy has been evaluated in several clinical trials in both haema-tological malignancies and solid tumours.[89] According to reports from several clinical trials, bacterial toxin such as Diphteria toxin (DT, 580 amino acid) or *Pseudomonas aeruginosa* exotoxin A (PE, 613 amino acid) that fused to the antibody Fv fragments has been shown to be a powerful antibody with selectivity to cell killing.[89] Immunotoxins with anti-CD25 fused to PE or DT was able to eliminate the Tregs population in the microenvironment, hence, preventing immune tolerance.[90, 91] According to results from meta-analysis of randomised trials, intravesical BCG administration effectively reduced the recurrence rate after transurethral resection of patients with superficial bladder cancers.[92]

Strategies have been made to encounter the technical problem of how to deliver molecules to the targeted destination. Dendritic cell transplantation has been widely used to overcome this problem. Autologous dendritic cells, which are extracted from the patients, are cultivated together with the tumour-specific antigen to be injected back into the patients. In order to further enhance the efficiency of this protocol, several groups have developed ordinary differential equation (ODE) systems by using mathematical models to predict the kinetics of the treatment, the influence of the treatment towards the normal physiologic function and the optimal schedule of injection of an immunotherapeutic agent against cancer.[80, 93] These system characteristics are useful to gain a better understanding of the dynamic of a tumour-specific system which can ultimately help to guide the development of combinational therapies for elimination of the entire tumour.[80]

In addition, the choices of clinical adjuvants used for delivery of the therapeutic agents to the target site are crucial. Due to the rapid degradation of the administrated immuno-dominant epitopes from tumour associated antigens (TAAs) *in vivo*, the efficacy and immunogenicity of the TAAs are low. Thus, this results in failure to mount an effective immune response to the target tumour cells.[94] In addition, improper use of an adjuvant may create undesirable secondary reactions that may increase the risk beneficial ratio for the patients.[95] The use of lipid- based adjuvants including liposome, virosome, [94] oil in water emulsion and bacterial immunostimulating compounds [95] have greatly enhanced the delivery as well as the therapeutic efficiency of the therapeutic agents including TAAs-specific peptides.[94]

CONCLUSION

Immunotherapy of cancer has re-emerged vigorously in the 1990s. More than 50 immunotherapeutic regimens are currently under clinical testing and more than 400 clinical

trials have been conducted.[95] Nevertheless, the clinical outcome and the response rate of the patients towards the testing agents are not sufficiently encouraging to prescribe it as a standard treatment regimen in a clinical setting. Through fundamental basic research in immunology, we will be able to figure out the 'bad guy' that is responsible for such a failure. Immune dominant tolerance by regulatory T-cells and immuno-suppressive role of Th2 cytokines are some examples of tumour escape mechanisms. Researchers have found numerous innovative approaches to overcome these barriers including the use of receptor-specific monoclonal antibodies to block the suppressive effect of Treg (Intrabodies) and the application of radio-labeled monoclonal antibodies to specifically guide the radioactive treatment to the target tumour cells. Recently, the role of innate immune response in tumour surveillance has been intensively studied. Clinical trials using natural killers against the solid tumour has been conducted.[96] The inherent complexity of the immune system as a network of multiple interactions and redundant control loops amongst a huge diversity of components may either create another barrier or open other options for immunotherapy. Re-evaluation of previous clinical trials has suggested that combinational immunotherapy with conventional cancer therapy have a better tumoricidal effect. A change in design and ultimate goals of clinical trials will be needed to ensure long term disease stabilisation and a better treatment outcome.

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