Cancer immunotherapy: an embarrassment of riches?

Mike Whelan, Joseph Whelan, Nick Russell and Angus Dalgleish

There is clear evidence that certain forms of immunotherapy can be successful against certain cancers. However, it would appear that cancerous cells of various origin are exceptionally adept at subverting the immune response. Consequently, it is probable that the most efficacious therapy will be one in which multiple responses of the immune system are activated. There is currently an embarrassment of riches with regard to multiple vaccine strategies in the clinic, although no single method seems to hold the solution. Here, we draw together several of the humoral- and cellular-activating strategies currently under clinical investigation.

Angus Dalgleish
Dept of Oncology
Mike Whelan*
Joseph Whelan
Nick Russell
Onyvax
St George's Hospital
Medical School
Cranmer Terrace
London
UK SW17 0RE
*tel: +44 20 8682 9494
fax: +44 20 8682 9495
e-mail: mwhelan@onyvax.com

Using the immune system to combat cancer is not a novel concept and can be traced back to the late nineteenth century with Coley's toxins [1]. His use of bacterial products to treat cancers was perhaps the first real attempt to use non-specific immunotherapy. Results from sarcoma patients treated with attenuated strains of *Streptococcus pyogenes* and *Serratie marcescens* indicated that the induction of a strong immune response could be beneficial in combating cancer. The idea did not re-surface until the publication of Burnett's seminal work in the 1950s when he clearly demonstrated that the immune system was indeed capable of mounting an anti-tumour response [2,3]. Burnett suggested that transplantation antigens expressed on tumour cells could send a signal to the immune system leading to the generation of protective immunity. However, this idea also fell by the wayside and has only recently become fashionable again. The general resurgence of immunotherapy has been attributed to the tremendous advances made in the dissection of the precise molecular mechanisms defining antigen presentation and T-cell stimulation.

Immunological response and escape mechanisms

Classically, the immune system is divided into the innate and adaptive responses. The first line of defence is the innate response, which includes physical barriers, such as the skin, and antigen non-specific cells such as macrophages and natural killer cells. The adaptive response is then further divided into the cellular and humoral responses, generating specific T-cells and antibodies, respectively. Both responses have been used as useful immunotherapies, with antibodies used to find specific tumour markers, and T-cells designed to attack specific cancerous tissue.

The T-cell response, in particular, has been the focus of a significant amount of current research because much of its function has only recently been elucidated. The sequencing and crystallization of the members of the major histocompatibility complex (MHC) has shown that they comprise three separate groups [4]. The MHC I alleles encode molecules that present peptides of 8–9 amino acid in length – derived from endogenous (intracellular) sources – to CD8-restricted T-cells. The MHC II genes encode molecules that present slightly longer peptides of 12–15 amino acids – derived from exogenous (extracellular) sources – to CD4 T-cells. The third group of MHC genes encodes a variety of immunologically active products, including HSP70 and tumour necrosis factor-α.

A recent key finding in immunotherapy has been that the dissociation between MHC I- and MHC II-restricted peptides is not a rigid one. Hence, it is possible to add a protein or peptide exogenously to a cell and still gain CD8 T-cell activation. This is termed 'cross-priming', where peptides can enter the MHC I pathway via the more usual MHC II mechanisms [5]. Indeed, this mechanism is the basis for a several of the immunotherapy strategies discussed later in this review.

An interesting insight into the immune response to cancers has come from the recent

1359-6446/03/$ – see front matter PII: S1359-6446(03)02633-3

work of Schreiber [6], who showed that interferon-γ receptor knockout mice have an extremely high incidence of spontaneous cancers. Consequently, it was suggested that the immune system does combat cancers, possibly on a daily basis. However, this immunity is clearly not complete, and immune escape can and does occur. It can occur in several ways, including downregulation of MHC [7,8], immune selection of the tumour [6,9], and secretion of inhibitory cytokines, such as TGFβ and IL-4 [10,11].

Cancer antigen nomenclature

Cancers are, by definition, 'self' cells that have bypassed normal homeostatic regulation mechanisms. Consequently, it is a challenge for the immune system to differentiate malignant and non-malignant cells. Furthermore, immune tolerance is such that the immune system will not attack a self-molecule, therefore tolerance barriers must be overcome before many therapies will work. Finding a truly novel cancer-specific antigen is highly problematic. Fortunately, there are several markers that can be used to identify and thus attack tumour cells specifically. These can be roughly divided into four major classes.

Tumour-specific antigens

Tumour-specific antigens (TSAs) are a relatively small group of antigens exemplified by the cancer-testis antigens. These genes are silent in normal tissue but are expressed by cancerous cells. They are highly specific markers of disease and include the MAGE (melanoma antigen gene) antigens found in melanoma.

Tumour-associated antigens

Tumour-associated antigens (TAAs) are usually differentiation antigens expressed by normal cells but massively overexpressed in cancerous tissue. Targets initially thought to be specific for a particular cancer are actually quite common in many tumours, such as the gangliosides and mucin antigens. Classical differentiation antigens include MART-1 (melanoma antigen recognized by T cells) [12] and gp 100 [13], both from melanoma.

Mutational antigens

Point mutations are common in many cancers, and often occur in a similar location, such as the common mutation of the P53 oncogene. *In vitro* induction of human cytotoxic T-lymphocyte (CTL) responses against peptides of mutant and wild-type p53 has been reported [14]. In a mouse model, mutant p53-pulsed dendritic cells were able to induce p53 specific CTL and inhibit the growth of established tumours [15].

Viral antigens

Certain viruses are oncogenic and gene products encoded by these viruses can elicit immune responses and thus serve as cancer antigens. An example is the E6 and E7 proteins from human papilloma virus type 16, which have been shown to induce cytotoxic T-lymphocyte responses *in vitro* [16].

Non-specific immunotherapy

In broad terms, it is possible to divide potential immunotherapies into specific and non-specific modalities. However, this is a gross over-simplification because it is probable that both innate and adaptive responses of the immune system function together rather than in isolation. From a historical perspective, however, it is probable that Coley's original work was a non-specific activation of the immune system caused by engaging what Janeway defined as 'pattern recognition' receptors [17]. This theory was later refined into the eponymous 'Danger theory', in which an immune reaction does not occur unless an additional insult occurs by engaging specific receptors, or in trauma [18].

Such non-specific stimuli are now in common use and are usually termed 'adjuvants' as they act primarily as catalysts for the inception of an immune response. It is interesting to note that many adjuvants function as delivery vehicles (e.g. alum), although it is important to stress that this is not the common meaning when discussing immunotherapies, despite the uses of stimulator and delivery system often being used interchangeably. One example where adjuvant alone has shown success is in bladder cancer, where BCG (Bacillus Calmette Guerin) is commonly used as a therapy [19].

The search for novel adjuvants has become a 'Holy Grail' for immunologists. In particular demand are compounds that can alter the balance of the immune system between T_H1 and T_H2 cytokine responses. Conventional wisdom dictates that a T_H1 type response, as exemplified by the production of IFN-γ, IL-2 and IL-12, are more beneficial in immunotherapy because they promote cytotoxic immunity, rather than T_H2 type responses, such as the production of IL-4, IL-5, IL-6 and IL-10, which tend to promote antibody production. Indeed, the use of cytokines themselves falls into the category of non-specific immunotherapy. The use of low-dose IL-2, either in isolation or in combination with other cytokines (most notably the interferons), is an established therapy throughout Europe [20–22]. Similarly, granulocyte-macrophage colony-stimulating factor (GM-CSF) has become the cytokine of choice owing to its dendritic-cell-maturing properties [23,24].

Table 1. Methods of loading antigen into dendritic cells

DC antigen	Clinical data
Protein	In the form of CEA, led to infiltration of the injection site [50]. Similarly, Provenge is a fusion protein of PAP and GM-CSF. When loaded into DC 38% of patients developed an anti-PAP response and demonstrated a drop in PSA [51].
Peptide	DC loaded with MART1 peptide led to complete melanoma remission in 1 in 16 trial patients, with partial remissions in 2 in 16 [52]. Peptide vaccination led to the generation of CEA specific responses and regression in 2 in 12 patients [53,54].
Lysate	A combination of a peptide cocktail or tumour lysate loaded DC led to 5 in 16 responses in a melanoma trial [55]. Similarly, Chang [56] showed the generation of IFNγ secreting cells after lysate stimulation.

Abbreviations: CEA, carcinoembryonic antigen; DC, dendritic cells; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; MART1, melanoma antigen recognized by T cells; PAP, prostatic acid phosphatase; PSA, prostate specific antigen.

Specific immunotherapy

The myriad of current immunotherapies in the clinic can be divided into several categories.

Nucleic acid vaccines

In their purest form, nucleic acid vaccines are simply 'naked DNA', usually injected into the muscle (reviewed in [25]). Clinical trials have begun in melanoma [26] and prostate [27]. Both trials are in the early stages and no efficacy data exist, although Phase I safety has been proven. A refinement of this technology has been to transfect autologous dendritic cells with tumour-derived RNA. This approach has been used in colon cancer with the notable detection of antigen-specific T-cell responses capable of lysing primary tumour targets [28]. Although no clinical response was observed in prostate cancer, dendritic cells loaded with prostate specific antigen (PSA)-RNA have been shown to generate a response to PSA, and have led to an alteration in the rate of its release in six out of seven patients [29].

Recombinant protein vaccines

As described earlier, several TAAs and TSAs have been identified. It is therefore logical to examine their efficacy as vaccines. This approach is attractive because it enables the patient's own immune system to cleave and bind peptides, thus making it amenable to all tissue types. The only restriction of such therapies lies in the relative shortage of potential specific antigens. Clinical trials in colorectal cancer, using carcino-embryonic antigen (CEA) and GM-CSF as an adjuvant, showed promising responses, with both an antibody and cellular response to CEA detected in eight out of nine individuals [30].

Peptide vaccines

Peptide vaccines are attractive from a development perspective because they are relatively simple to produce to Good Manufacturing Practice. However, they have the disadvantage that they are human leukocyte antigen-specific and, consequently, will only function on a limited subset of patients. In addition, they have been unsuccessful when used on their own. Second-generation products use a combination of CD8 and CD4 epitopes as well as multiple antigens. The principle of peptide vaccination appears to have been validated by Jaeger [31], who showed that melanoma, tyrosinase and flu peptide administration led to the development of specific CTL. However, no tumour regression was observed, although disease stabilization was claimed in two out of 10 individuals. A follow-up study using peptide with GM-CSF was much more encouraging and demonstrated tumour regression in all patients [23]. When NY-ESO-1-derived peptides were administered, it was interesting that only antibody-negative patients developed specific CTLs, suggesting that humoral immunity might play a role in defining the efficacy of this approach, possibly owing to the removal of antigen by antibody [32]. Similar data using different adjuvants such as IL-2 [33] or IL-12 [34] suggest that peptide vaccines can generate significant responses when used with a strong immune-stimulating agent.

Dendritic cells

Dendritic cells are the single most potent immune-stimulating cell and are therefore a logical candidate for vaccination [24]. They have been used in several different ways as they have a unique ability to present antigens in several forms with great efficiency (see Table 1). However, it should be noted that dendritic cells can also generate profound tolerance and must be used carefully [35]. One major problem with this approach lies in the ability to store enough cells to make an effective vaccine, although a significant amount of work has been done in the development of freezing protocols [36,37].

Table 2. Viruses commonly used in immunotherapy

Virus	Description	Clinical data
Poxviridae (e.g. *variola*, *vaccinia*, *avipox*)	Linear double stranded DNA Enveloped 130–375 kilonucleotides	*Vaccinia* encoding CEA leads to a strong antibody response [57] A replication incompetent *avipox* encoding CEA lead to an increase in CTL precursors [58] *Vaccinia* or *avipox* CEA transfectants increase IFNγ secreting cell numbers and this is enhanced by GM-CSF and IL2 [59] CEA and B7.1 both transfected into *avipox* led to leukocyte infiltration and some disease stabilization [60,61]
Adenoviridae (e.g. *adenovirus*)	Linear double stranded DNA Non-enveloped 30–42 kilonucleotides	Oncolytic viruses work best in combination with chemotherapeutic agents [62,63] An *adenovirus* transfected with PSA led to a drop in PSA levels at high dose of virus [64]
Herpesviridae (e.g. *simplex*, *cytomegalovirus*, *EHV*)	Linear double stranded DNA Enveloped 120–220 kilonucleotides	Disabled Infectious Single Cycle (DISC) *herpesvirus* [65]

Abbreviations: CEA, carcinoembryonic antigen; CTL, cytotoxic T-lymphocyte; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; PSA, prostate specific antigen.

Antibodies

The conventional use of antibodies is to bind a target antigen and thus either lyse or opsonize the cell. By using antibodies directed against TAA or TSA to target the tumour, it is possible to use the complement lytic mechanism of the immune system to attack the target [38]. Examples include the humanized antibodies Rituxan [39], which is specific for CD20, and Herceptin [40], which binds to Her2/neu-expressing tumours. This is essentially the logic behind the 'magic bullet' hypothesis, in which a tumour-specific antibody is conjugated to a drug, prodrug, enzyme or even radionuclide, and then activated at the site of disease. This approach has recently demonstrated promise using CEA-expressing tumours and the prodrug ZD2767p [41].

Anti-idiotype antibodies

From conventional network theory, it follows that to any specific antibody, a mirror-image antibody will be generated. Consequently, this anti-idiotype will spatially resemble the original target antigen and can thus be used as a surrogate vaccine in place of the original target protein [42]. This has several advantages because many antigens are difficult to clone, particularly those that are heavily glycosylated, and because anti-idiotype antibodies are particularly effective in breaking immune tolerance. Clinical data using an anti-idiotype vaccine for CD55 have shown elevated T-cell responses and increased natural killer cell activity [43,44].

Viral targeting

Viral targeting uses a recombinant virus – usually replication incompetent – to destroy a tumour directly. In practice, at least one round of replication occurs before the virus is incapacitated. Hence, the tumour is lysed, which often leads to systemic immunization with resulting protection. Common viruses in clinical use are summarized in Table 2. This approach has been refined further using genetic modification to enhance the immune response. For example, the genetic insertion of a human GM-CSF gene into a herpes simplex virus type 2 vector has been used improve the efficacy of the vaccine [45].

Whole-cell vaccines

Returning to classical vaccinology, as a tumour is the target of immune attack, it follows that it must contain several immunogenic moieties. Therefore, a vaccine made from a tumour could prove effective because it contains all possible cancer antigens. Perhaps the simplest approach is to use a syngeneic method, in which the patient's own tumour is removed, cultured, inactivated and then injected back into the patient [46]. Although an attractive methodology, this syngeneic approach has several constraints, including the time it takes to perform the manipulations, and that it is only possible when a tumour is palpable, that is, when the cancer is advanced. A similar method can be used with allogeneic tumour cells and this clearly overcomes any problems regarding tissue source. However, the mechanism-of-action is likely to be quite different and

probably relies on the sharing of antigens between tumour types as many of the oncogenic variations seen in cancer are relatively conserved. The seminal work in this field was carried out by Morton *et al.* who observed regression of melanoma (nine out of 136 patients) following allogeneic whole-cell vaccination [47]. This has recently been tested in a Phase I trial for prostate cancer [48], yielding an excellent safety profile, and is currently under Phase II investigation in our own laboratories. A further refinement is the use of transfected whole-cells, in which immune-stimulating molecules, such as cytokines, are added to improve the efficacy of the vaccine [49]. This has been used most successfully in the syngeneic system, although preclinical trials in our laboratory suggest that it might not be appropriate for the allogeneic system.

Conclusions and future perspectives

Recent data suggest that the field of immunotherapy is a burgeoning one, with many quite different strategies in the clinic. It is probable that multiple immune-stimulating strategies will be necessary to avoid subversion of the immune response by the cancer. This might be achieved either by combining therapeutic strategies (e.g. proteins and DNA vaccines), or by using a vaccine capable of multiple stimulations (e.g. whole-cell vaccines). It is clear that simple monovalent approaches, such as peptide vaccination, can activate the immune system, but these are unlikely to be a complete therapy in isolation. Consequently, we envisage a future in which many of these therapies coalesce into a unified strategy where multiple responses of the immune system are stimulated sequentially or concurrently.

References

1 Wiemann, B. and Starnes, C.O. (1994) Coley's toxins, tumor necrosis factor and cancer research: a historical perspective. *Pharmacol. Ther.* 64, 529–564

2 Burnet, F.M. (1967) Immunological aspects of malignant disease. *Lancet* 1, 1171–1174

3 Burnet, F.M. (1971) Immunological surveillance in neoplasia. *Transplant. Rev.* 7, 3–25

4 Rhodes, D.A. and Trowsdale, J. (1999) Genetics and molecular genetics of the MHC. *Rev. Immunogenet.* 1, 21–31

5 Zinkernagel, R.M. (2002) On cross-priming of MHC class I-specific CTL: rule or exception? *Eur. J. Immunol.* 32, 2385–2392

6 Dunn, G.P. *et al.* (2002) Cancer immunoediting: from immunosurveillance to tumor escape. *Nat. Immunol.* 3, 991–998

7 Neeley, Y.C. *et al.* (2002) Antigen-specific tumor vaccine efficacy *in vivo* against prostate cancer with low class I MHC requires competent class II MHC. *Prostate* 53, 183–191

8 Palmisano, G.L. *et al.* (2001) Investigation of HLA class I downregulation in breast cancer by RT-PCR. *Hum. Immunol.* 62, 133–139

9 Khong, H.T. and Restifo, N.P. (2002) Natural selection of tumor variants in the generation of 'tumor escape' phenotypes. *Nat. Immunol.* 3, 999–1005

10 Conrad, C.T. *et al.* (1999) Differential expression of transforming growth factor β1 and interleukin 10 in progressing and regressing areas of primary melanoma. *J. Exp. Clin. Cancer Res.* 18, 225–232

11 Neuner, A. *et al.* (2002) Prognostic significance of cytokine modulation in non-small cell lung cancer. *Int. J. Cancer* 101, 287–292

12 Kawakami, Y. *et al.* (1994) Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. *Proc. Natl. Acad. Sci. U. S. A.* 91, 3515–3519

13 Kawakami, Y. *et al.* (1994) Identification of a human melanoma antigen recognized by tumor-infiltrating lymphocytes associated with *in vivo* tumor rejection. *Proc. Natl. Acad. Sci. U. S. A.* 91, 6458–6462

14 Houbiers, J.G. *et al.* (1993) *In vitro* induction of human cytotoxic T lymphocyte responses against peptides of mutant and wild-type p53. *Eur. J. Immunol.* 23, 2072–2077

15 Gabrilovich, D.I. *et al.* (1996) Dendritic cells in antitumor immune responses. II. Dendritic cells grown from bone marrow precursors, but not mature DC from tumor-bearing mice, are effective antigen carriers in the therapy of established tumors. *Cell Immunol.* 170, 111–119

16 Nakagawa, M. *et al.* (1997) Cytotoxic T lymphocyte responses to E6 and E7 proteins of human papillomavirus type 16: relationship to cervical intraepithelial neoplasia. *J. Infect. Dis.* 175, 927–931

17 Medzhitov, R. and Janeway, C.A., Jr (2002) Decoding the patterns of self and nonself by the innate immune system. *Science* 296, 298–300

18 Matzinger, P. (2002) The danger model: a renewed sense of self. *Science* 296, 301–305

19 Alexandroff, A.B. *et al.* (1999) BCG immunotherapy of bladder cancer: 20 years on. *Lancet* 353, 1689–1694

20 Atzpodien, J. *et al.* (2002) Thirteen-year, long-term efficacy of interferon 2α and interleukin 2-based home therapy in patients with advanced renal cell carcinoma. *Cancer* 95, 1045–1050

21 Baselmans, A.H. *et al.* (2002) The mechanism of regression of solid SL2 lymphosarcoma after local IL-2 therapy. *Cancer Immunol. Immunother.* 51, 492–498

22 Lissoni, P. *et al.* (2002) Ten-year survival results in metastatic renal cell cancer patients treated with monoimmunotherapy with subcutaneous low-dose interleukin-2. *Anticancer Res.* 22, 1061–1064

23 Jager, E. *et al.* (1996) Granulocyte-macrophage colony-stimulating factor enhances immune responses to melanoma-associated peptides *in vivo*. *Int. J. Cancer* 67, 54–62

24 Steinman, R.M. and Pope, M. (2002) Exploiting dendritic cells to improve vaccine efficacy. *J. Clin. Invest* 109, 1519–1526

25 Haupt, K. *et al.* (2002) The potential of DNA vaccination against tumor-associated antigens for antitumor therapy. *Exp. Biol. Med.* 227, 227–237

26 Walsh, P. *et al.* (2000) A phase I study using direct combination DNA injections for the immunotherapy of metastatic melanoma. University of Colorado Cancer Center Clinical Trial. *Hum. Gene Ther.* 11, 1355–1368

27 Mincheff, M. *et al.* (2000) Naked DNA and adenoviral immunizations for immunotherapy of prostate cancer: a phase I/II clinical trial. *Eur. Urol.* 38, 208–217

28 Nair, S.K. *et al.* (2002) Induction of tumor-specific cytotoxic T lymphocytes in cancer patients by autologous tumor RNA-transfected dendritic cells. *Ann. Surg.* 235, 540–549

29 Heiser, A. *et al.* (2002) Autologous dendritic cells transfected with prostate-specific antigen RNA stimulate CTL responses against metastatic prostate tumors. *J. Clin. Invest.* 109, 409–417

30 Samanci, A. *et al.* (1998) Pharmacological administration of granulocyte-macrophage-colony-stimulating factor is of significant importance for the induction of a strong humoral and cellular response in patients immunized with recombinant carcinoembryonic antigen. *Cancer Immunol. Immunother.* 47, 131–142

31 Jaeger, E. *et al.* (1996) Generation of cytotoxic T-cell responses with synthetic melanoma-associated peptides *in vivo*: implications for tumor vaccines with melanoma-associated antigens. *Int. J. Cancer* 66, 162–169

32 Jager, E. *et al.* (2000) Induction of primary NY-ESO-1 immunity: CD8+ T lymphocyte and antibody responses in peptide-vaccinated patients with NY-ESO-1+ cancers. *Proc. Natl. Acad. Sci. U. S. A.* 97, 12198–12203

33 Rosenberg, S.A. *et al.* (1998) Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. *Nat. Med.* 321–327

34 Lee, P. *et al.* (2001) Effects of interleukin-12 on the immune response to a multipeptide vaccine for resected metastatic melanoma. *J. Clin. Oncol.* 19, 3836–3847

35 Bonifaz, L. *et al.* (2002) Efficient targeting of protein antigen to the dendritic cell receptor DEC-205 in the steady state leads to antigen presentation on major histocompatibility complex class I products and peripheral CD8+ T cell tolerance. *J. Exp. Med.* 196, 1627–1638

36 Feuerstein, B. *et al.* (2000) A method for the production of cryopreserved aliquots of antigen-preloaded, mature dendritic cells ready for clinical use. *J. Immunol. Methods* 245, 15–29

37 Sai, T. *et al.* (2002) Freezing and thawing of bone marrow-derived murine dendritic cells with subsequent retention of immunophenotype and of antigen processing and presentation characteristics. *J. Immunol. Methods* 264, 153–162

38 Dillman, R.O. (2001) Monoclonal antibodies in the treatment of malignancy: basic concepts and recent developments. *Cancer Invest.* 19, 833–841

39 Coiffier, B. (2002) Rituximab in combination with CHOP improves survival in elderly patients with aggressive non-Hodgkin's lymphoma. *Semin. Oncol.* 29, 18–22

40 Ligibel, J.A. and Winer, E.P. (2002) Trastuzumab/chemotherapy combinations in metastatic breast cancer. *Semin. Oncol.* 29, 38–43

41 Francis, R.J. *et al.* (2002) A phase I trial of antibody directed enzyme prodrug therapy (ADEPT) in patients with advanced colorectal carcinoma or other CEA producing tumours. *Br. J. Cancer* 87, 600–607

42 Maruyama, H. *et al.* (2000) Cancer vaccines: single-epitope anti-idiotype vaccine versus multiple-epitope antigen vaccine. *Cancer Immunol. Immunother.* 49, 123–132

43 Durrant, L.G. *et al.* (2000) 105Ad7 cancer vaccine stimulates anti-tumour helper and cytotoxic T-cell responses in colorectal cancer patients but repeated immunisations are required to maintain these responses. *Int. J. Cancer* 85, 87–92

44 Durrant, L.G. *et al.* (2000) A neoadjuvant clinical trial in colorectal cancer patients of the human anti-idiotypic antibody 105AD7, which mimics CD55. *Clin. Cancer Res.* 6, 422–430

45 Loudon, P.T. *et al.* (2001) Preclinical safety testing of DISC-hGMCSF to support phase I clinical trials in cancer patients. *J. Gene Med.* 3, 458–467

46 Hanna, M.G., Jr *et al.* (2001) Adjuvant active specific immunotherapy of stage II and stage III colon cancer with an autologous tumor cell vaccine: first randomized phase III trials show promise. *Vaccine* 19, 2576–2582

47 Morton, D.L. *et al.* (1992) Prolongation of survival in metastatic melanoma after active specific immunotherapy with a new polyvalent melanoma vaccine. *Ann. Surg.* 216, 463–482

48 Eaton, J.D. *et al.* (2002) Allogeneic whole-cell vaccine: a phase I/II study in men with hormone- refractory prostate cancer. *BJU Int.* 89, 19–26

49 Simons, J.W. *et al.* (1999) Induction of immunity to prostate cancer antigens: results of a clinical trial of vaccination with irradiated autologous prostate tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor using *ex vivo* gene transfer. *Cancer Res.* 59, 5160–5168

50 Morse, M.A. *et al.* (1999) A Phase I study of active immunotherapy with carcinoembryonic antigen peptide (CAP-1)-pulsed, autologous human cultured dendritic cells in patients with metastatic malignancies expressing carcinoembryonic antigen. *Clin. Cancer Res.* 5, 1331–1338

51 Small, E.J. *et al.* (2000) Immunotherapy of hormone-refractory prostate cancer with antigen-loaded dendritic cells. *J. Clin. Oncol.* 18, 3894–3903

52 Lau, R. *et al.* (2001) Phase I trial of intravenous peptide-pulsed dendritic cells in patients with metastatic melanoma. *J. Immunother.* 24, 66–78

53 Fong, L. *et al.* (2001) Altered peptide ligand vaccination with Flt3 ligand expanded dendritic cells for tumor immunotherapy. *Proc. Natl. Acad. Sci. U. S. A.* 98, 8809–8814

54 Fong, L. *et al.* (2001) Dendritic cell-based xenoantigen vaccination for prostate cancer immunotherapy. *J. Immunol.* 167, 7150–7156

55 Nestle, F.O. *et al.* (1998) Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. *Nat. Med.* 4, 328–332

56 Chang, A.E. *et al.* (2002) A phase I trial of tumor lysate-pulsed dendritic cells in the treatment of advanced cancer. *Clin. Cancer Res.* 8, 1021–1032

57 Conry, R.M. *et al.* (2000) Human autoantibodies to carcinoembryonic antigen (CEA) induced by a vaccinia-CEA vaccine. *Clin. Cancer Res.* 6, 34–41

58 Marshall, J.L. *et al.* (1999) Phase I study in cancer patients of a replication-defective avipox recombinant vaccine that expresses human carcinoembryonic antigen. *J. Clin. Oncol.* 17, 332–337

59 Marshall, J.L. *et al.* (2000) Phase I study in advanced cancer patients of a diversified prime-and-boost vaccination protocol using recombinant vaccinia virus and recombinant nonreplicating avipox virus to elicit anti-carcinoembryonic antigen immune responses. *J. Clin. Oncol.* 18, 3964–3973

60 von Mehren, M. *et al.* (2000) Pilot study of a dual gene recombinant avipox vaccine containing both carcinoembryonic antigen (CEA) and B7.1 transgenes in patients with recurrent CEA-expressing adenocarcinomas. *Clin. Cancer Res.* 6, 2219–2228

61 von Mehren, M. *et al.* (2001) The influence of granulocyte-macrophage colony-stimulating factor and prior chemotherapy on the immunological response to a vaccine (ALVAC-CEA B7.1) in patients with metastatic carcinoma. *Clin. Cancer Res.* 7, 1181–1191

62 Kruyt, F A. and Curiel, D.T. (2002) Toward a new generation of conditionally replicating adenoviruses: pairing tumor selectivity with maximal oncolysis. *Hum. Gene Ther.* 13, 485–495

63 Yoon, T.K. *et al.* (2001) Selectively replicating adenoviruses for oncolytic therapy. *Curr. Cancer Drug Targets* 1, 85–107

64 DeWeese, T.L. *et al.* (2001) A phase I trial of CV706, a replication-competent, PSA selective oncolytic adenovirus, for the treatment of locally recurrent prostate cancer following radiation therapy. *Cancer Res.* 61, 7464–7472

65 Ali, S.A. *et al.* (2002) Tumor regression induced by intratumor therapy with a disabled infectious single cycle (DISC) herpes simplex virus (HSV) vector, DISC/HSV/murine granulocyte-macrophage colony-stimulating factor, correlates with antigen-specific adaptive immunity. *J. Immunol.* 168, 3512–3519

Contributions to *Monitor*

All contributions, suggestions or queries relating to Monitor should be addressed to Dr Steve Carney, Editor, Drug Discovery Today, Elsevier, 84 Theobald's Road, London, UK WC1X 8RR.

tel: +44 (0) 20 7611 4132, fax: +44 (0) 7611 4485,

e-mail: DDT@drugdiscoverytoday.com