Review paper

Cancer vaccines

LG Durrant
CRC Department of Clinical Oncology, University of Nottingham, Laboratory of Molecular Oncology, City Hospital, Hucknall Road, Nottingham NG5 1PB, UK. Tel: (+44) 115 962-8033; Fax: (+44) 115 962-7923.

A better understanding of immune recognition of cells has led to identification of potential new targets on tumor cells. Noticeable successes in melanoma have been immunization with the GM2 ganglioside vaccine, and the identification of novel antigens such as MAGE, BAGE and GAGE recognized by T cells cloned from cancer patients with regressing disease. However, the unexpected finding that other antigens recognized by these T cells were overexpressed normal differentiation antigens such as tyrosinase. Pmel 17 and Melan A has led to vaccines developed against differentiation antigens expressed in other solid tumors. Monoclonal antibody, anti-idiotype and antigen based vaccines for colorectal target antigens 17-1A, CEA and 791Tgp72 are all in clinical development. Similarly HER2/*neu* and mucin overexpression in breast cancer represent promising targets. Mutations in tumor oncogenes or suppressor genes which lead to malignant transformation can also present tumor-specific antigens. The most effective vaccines against infectious disease are live viruses. The development of DNA vaccines which act like viruses in entering cells and show continuous production of antigens offers great potential for the future.

Key words: Cancer, vaccine.

Introduction

A better understanding of the molecular recognition and stimulation of immune responses has led to the development of many new and exciting approaches for the immunotherapy of cancer. The immune response can be crudely divided into either antibody responses or T cell responses. Antibodies recognize and bind to conformational determinants on cell surface proteins. Once bound they can kill the cell by either complement-mediated lysis or antibody-dependant cellular cytotoxicity. In contrast, T cells do not recognize native protein but see small peptides which result from proteolytic digestion of proteins presented on the cell surface on specialized molecules called major histocompatibility (MHC) antigens. T cell activation requires a second signal or a co-stimulatory signal which is usually present on the surface of specialized cells called antigen-presenting cells. There are two main types of MHC molecules, class I which stimulates cytotoxic T cells ($CD8^+$) which can directly lyse appropriate target cells, and class II MHC which stimulates helper ($CD4^+$) T cells which provide cytokines to allow cytotoxic T lymphocyte (CTL) proliferation but which can also migrate to the tumor site and set up an inflammatory response. Helper T cells release cytokines such as tumor necrosis factor and interferon-γ which can have direct effects on the tumor or they can recruit non-specific killer cells such as natural killer (NK) cells or tumoricidal macrophages.

Novel vaccines designed to stimulate both antibody and T cell responses against human tumors are currently being tested in the laboratory and many have proceeded into clinical trials. Peptides which bind to MHC and stimulate T cell responses can be used directly as imunogens or pulsed on the surface of antigen-presenting cells *ex vivo* and reinfused. Anti-idiotypic antibodies are able to mimic antigen and elicit T cell responses. DNA vaccines encode the tumor antigen while viral vectors provide an alternative way of altering the host immune response. Target antigens can either be overexpressed normal differentiation antigens or oncogene products. This review will describe some of the more successful approaches in melanoma, colorectal cancer and breast/ovarian cancer.

Melanoma

Cellular vaccines

Autologous or allogeneic tumor vaccines were used as immunogens. Although some successes have been achieved with this approach,[1] it suffers from many

Correspondence to LG Durrant

disadvantages, not least of which is immune tolerance. Most of the antigens expressed on tumor cells are normal differentiation antigens which are inappropriately overexpressed on tumor cells. The recognition of self antigens appears to be density dependant where high-affinity T cells that recognize dominant self antigens are deleted in the thymus. However, low-affinity T cells recognizing subdominant epitopes persist and can be stimulated to recognize targets expressing high levels of antigen in association with MHC molecules.[2] During immune responses to antigen, immunity is elicited to only the dominant epitopes and the subdominant epitopes are ignored. This could explain why tumors growing *in situ* elicit poor immune responses and would be equally applicable to whole cell vaccines. Stimulating T cells that recognize these subdominant epitopes may be achieved using truncated proteins or peptides.

The second major problem with whole cell vaccines is that tumor cells have poor expression of both MHC and co-stimulatory molecules, and although these can be replaced by transfection they are still a poor second to professional antigen-presenting cells. Recent trials with autologous tumor cells transfected with either interleukin (IL)-2 or granulocyte macrophage colony stimulating factor (GM-CSF) have shown active recruitment of professional antigen-presenting cells to the injection site;[3] however, the problem of immunodominance still persists.

Gangliosides

Cancer carbohydrates such as the gangliosides GD2 and GM2 are suitable targets for immunotherapy because they are overexpressed at the surface of melanoma cells.[4] Extracted and purified or synthetic carbohydrate antigens conjugated with a T cell carrier such as keyhole limpet hemacyanin (KLH) plus an immune adjuvant QS-21 is the optimal immunization approach, far more immunogenic than unconjugated antigen plus adjuvant or irradiated whole cell or cell lysate vaccines.[5] A GM2-KLH plus QS-21 vaccine has resulted in consistent high titer IgM antibodies capable of inducing complement-mediated cytotoxicity and IgG antibodies mediating complement-mediated and antibody-dependent cell-mediated cytotoxicity.[6] Randomized phase III adjuvant trials are in progress to evaluate the effect of vaccination with GM2-KLH plus QS-21 on disease-free and overall survival.

Novel T cell antigens

Pioneering studies by Boon and colleagues, using T cells cloned from melanoma patients, showing spontaneous regression to screen cDNA libraries generated from the patients autologous tumor, ultimately allowed this group and others to isolate three families of tumor-associated antigens.[7,8] The first group consists of novel antigens designated MAGE, BAGE or GAGE which are overexpressed on a variety of tumor cells but their normal expression is restricted to the testes. The second group consists of melanoma differentiation antigens such as tyrosinase, Melan A/MART-1 and gp100/Pmel 17 which are expressed by both normal melanocytes and melanomas. The final group consists of new targets caused by either point mutations of normal genes such as connexin or CDK4 or antigens in which an intronic sequences is expressed in melanoma leading to the production of an antigenic peptide which is entirely encoded by an intron sequence.[9]

An alternative approach was to purify MHC molecules, elute the peptides and then using a combination of HPLC and electrospray ionization mass spectrometry isolate individual peptides that are recognized by CTLs cloned from cancer patients.[10] These peptides can then be used directly as immunogens either to stimulate T cells *ex vivo* for reinfusion or as vaccines. A recent clinical trial in which peptides from tyrosinase, Pmel 17 and Melan A were immunized as a cocktail in the presence of the cytokine GM-CSF, which activates and recruits antigen-presenting cells, showed promising results. Although detailed analysis of only three patients was presented, they all showed both helper and cytotoxic T cell responses to one or more peptide, and had either partial or complete clinical responses.[11]

Colorectal cancer

17-1A

CO17-1A is a 37–49 kDa antigen present on over 90% of colorectal cancers. Passive immunotherapy with the monoclonal antibody 17-1A may prolong survival in patients with primary tumors.[12] These results are currently being tested in a large multicenter study (Panorex). This therapy may be effective as the monoclonal antibody binds directly to the tumor cell and stimulates antibody-dependent cellular cytotoxicity or it may stimulate an immune cascade of anti-idiotypic antibodies. The theory is that antibodies have

unique idiotypes created by their variable regions which bind to antigen; a second antibody which recognizes this idiotype (anti-idiotypic antibody) must therefore mimic antigen. The main advantage of anti-idiotypic antibodies is their ability to stimulate immune responses under conditions where the corresponding antigen is non-immunogenic.[13]

A polyclonal anti-idiotypic antibody to CO17-1A has been developed and 30 patients with advanced colorectal cancer immunized.[14] Humoral responses were seen and all showed evidence of Ab3 production. This antibody showed identical binding of tumor cells as that observed with Ab1. Six patients showed partial clinical remission and a further seven arrest of metastases following treatment. Of these 13 patients, nine also received chemotherapy, making conclusions about the efficacy of Ab2 more contentious. A follow-up trial by the same group used a different goat polyclonal antibody in 12 patients who had undergone resection of their primary. Six of these patients developed antibodies against the anti-idiotypic antibody and two had antigen-specific T cells, which proliferated in culture on stimulation with the CO17-1A antigen. In addition seven of the original 12 showed tumor remissions which lasted between 1.1 and 4.1 years post-immunization. In support of Herlyn's work, evidence of cellular immunity has been seen in a further one patient with advanced colorectal cancer following immunization with SCV106, a goat anti-idiotypic monoclonal antibody that also mimics the tumor-associated antigen 17-1A. The patient concerned had two lung metastases from a previously resected colonic carcinoma. These were removed after completion of the antibody course. Antibodies eluted from the resection specimen were confirmed to be against the tumor antigen in a conventional ELISA, and immunohistochemical analysis of tissue confirmed 'massive' infiltrate of T helper and cytotoxic T cells.

Recent work has shown how passive immunotherapy with unconjugated monoclonal antibodies may give rise to an idiotypic network response that correlates with response.[15] Twenty-four patients with metastatic colorectal cancer were treated with monoclonal antibody 17-1A. After completion of therapy, five of the patients had peripheral blood T cells specifically recognizing human anti-monoclonal antibody 17-1A idiotypic antibodies. These same five patients were the only ones in the study who had any objective tumor regression following monoclonal antibody therapy. The association between the presence of anti-idiotypic-reactive T cells and clinical response was statistically significant.

791Tgp72

A human monoclonal anti-idiotypic antibody, 105AD7, which mimics a colorectal tumor-associated antigen, 791Tgp72, has been developed.[16] 105AD7 induced delay-type hypersensitivity (DTH) responses to 791Tgp72-positive human tumor cells in mice and rats,[17] and was therefore a candidate immunogen for idiotypic immunotherapy of colorectal cancer patients. Phase I clinical studies in advanced colorectal cancer patients showed that immunization with 105AD7 was non-toxic. Immunized patients showed evidence of both helper and cytotoxic T cell responses.[18] Notably, 105AD7-immunized patients had increased survival when compared with a contemporary group of patients treated in the same center.[19] A double-blind randomized study designed to confirm these encouraging results in a similar cohort of patients is currently in progress under the auspices of the CRC phase I/II committee.

In the phase I clinical trial of 105AD7 in advanced colorectal cancer, although several patients showed stabilization of disease, tumor regression was not observed. As this may be related to the immunosuppressive effects of large tumor burden it was felt that 105AD7 could achieve a greater effect as an adjuvant treatment for minimal residual disease. A small pilot study of 50 patients immunized pre-operatively with 105AD7 is being undertaken to study autologous tumor immune responses.

Initial studies have clearly shown enhanced autologous anti-tumor killing which was unrelated to NK killing in three of four patients.[20] Significant increases in infiltration of CD4 and CD8 T cells and NK cells to the tumors of 105AD7-immunized tumors as compared to control tumors which were matched for stage, grade, differentiation and site has been observed. This increase in infiltration of NK cells within tumors of immunized patients is supported by the increase in NK killing following 105AD7 immunization in seven of 13 patients.

These encouraging immunological responses are mirrored by patient survival. A 12 month interim analysis of the adjuvant patients shows a significant improvement in disease-free relapse ($p > 0.05$) compared to a contemporary group of stage-matched patients treated at the same center.[21] These results offer real hope that following optimization of the vaccine a controlled study may show a similar result.

Carcinoembryonic antigen (CEA)

CEA is one of the best characterized tumor marker antigens in terms of its tissue distribution, biochem-

istry and molecular structure. It is extensively expressed in humans on the majority of colorectal, gastric and pancreatic carcinomas as well as on approximately 50% of breast cancers and 70% of non-small cell lung cancers. CEA is also expressed to some extent on normal colon epithelial and in some fetal tissues. At the amino acid level CEA shares approximately 70% homology with non-specific cross-reacting antigen (NCA) which is found on normal granulocytes. However, CEA is highly overexpressed on tumor cells and is therefore a potential target for immunotherapy. The first evidence that T cells from cancer patients could recognize and respond to CEA was demonstrated by *in vitro* immunization with an anti-idiotypic antibody which mimics CEA.[22] A phase I clinical trial with another anti-idiotypic antibody, 3H1 which mimics CEA, in advanced colorectal cancer demonstrated anti-CEA antibody responses in nine of 12 patients with four patients showed T cell proliferative responses to CEA. Toxicity was limited to local reaction with mild fever and chills.[23] Studies are now focusing on treating patients with minimal residual disease.

Instead of using anti-idiotypic antibodies as surrogate antigens, the CEA gene has been cloned in baculovirus and recombinant protein has been used as the immunogen. This may be advantageous as the patient's antigen-presenting cells can process, present and select the most appropriate T cell epitopes. It has, however, the disadvantage that immune responses may be generated to the region of CEA which is homologous with NCA and may therefore generate toxicity to granulocytes.

Two clinical studies have been carried out using recombinant CEA. Two of five breast cancer patients showed CEA-specific proliferative responses and one also had a CEA-specific DTH response.[24] In the second study, the addition of the cytokine GM-CSF to CEA enhanced the proliferative responses to CEA from two of six patients to six of six patients. No toxicity was observed.[15]

A novel approach to vaccination which has been very successful in infectious diseases is polynucleotide immunization. DNA or RNA can be immunized by intramuscular injection whereby the myocytes take up the DNA and express the gene product.[25] The released protein is taken up by antigen-presenting cells which migrate to the draining lymph nodes and present antigen to the T cells. An alternative route of immunization is by intradermal injection where it is presumed the DNA is taken up by Langerhan cells which are skin dendritic cells or antigen-presenting cells. This will lead to a continuous intracellular production of protein antigens that may be presented in association with class I MHC molecules, thus eliciting CTL responses. The advantages of DNA vaccines are numerous. They can be easily purified, coated on gold particles and given directly into tissues by gene gun (bolistics). DNA may also be combined with genes for cytokines such as IL-2, IL-6 or IL-7, or GM-CSF, in order to enhance the immune response generated.[26]

Work has shown that mice may be immunized with a plasmid encoding the full length of complementary DNA for CEA.[27] Evidence of humoral and cellular responses against the glycoprotein was seen in all of the five mice immunized and three generated CEA-specific memory T cells. In addition a further two had IL-2/IL-4 release in response to CEA. Clearly evidence exists supporting this approach as a potential vaccine strategy. Approval has been granted for a phase I trial in colorectal cancer.

Recombinant vaccinia viruses are being considered for use in the treatment of cancer because it has been shown, in animal models, that co-presentation of a weak immunogen with the highly immunogenic vaccinia proteins can elicit a strong immune response against the inserted gene product. Animals showed good antibody and cell-mediated immune responses to CEA when they were immunized with complete cDNA of CEA inserted into the vaccinia genome (rV-CEA). A phase I study of this construct in patients with metastatic carcinoma showed for the first time that it was possible to induce cytolytic T cell responses to CEA which killed tumor cells.[28] Unfortunately the immune response to the vaccinia inhibited replication of the administered recombinant virus at subsequent immunizations and therefore it was not possible to boost the primary immune response to CEA with the vaccinia construct. However, animal studies have suggested that priming with rV-CEA and then boosting with either recombinant CEA or specific CEA peptides is a very efficient immunization protocol. Clinical trials are being planned.

Oncogenes

Peptide vaccines can bind to MHC molecules and elicit immune responses. Generation of CTL would be further enhanced if the peptide was presented by an antigen-presenting cell, such as a dendritic cell (DC). A murine model has shown that antigen-specific CTLs may be generated following subcutaneous administration of irradiated bone-marrow-derived DC, pulsed with ovalbumin peptide *in vitro*.[29] These results have been confirmed in a separate study, where β-galactosidase acts as the tumor-associated antigen.[30]

Immunization of mice with mutant p53 peptide-pulsed DCs generated from stem cells of other tumor bearing mice can induce effective anti-tumor CTL responses and lead to significant anti-tumor effects.[31] If the T cell epitope is as yet undefined, as is the case for a number of cancers, then CTL can still be generated using unfractionated acid-eluted tumor peptides in conjunction with the method outlined above.[32]

Mutations in codon 12 of K-*ras* are frequently found in pancreatic adenocarcinomas.[33] Mutant $p21^{ras}$ is therefore a tumor-specific antigen, that can be recognized by human T cells.[34] Synthetic *ras* peptides have been used in conjunction with antigen-presenting cells as a vaccine for pancreatic cancer, with encouraging results. This approach could also be applied to colorectal carcinomas, which also show mutations in codon 12 of K-*ras*. As an alternative to peptide vaccination it is possible to clone the peptide epitope as a minigene and use this DNA as the immunogen. Minigenes coding for a single epitope derived from mutant p53 have been shown to elicit CTL in a mouse model.[35]

Heat shock proteins (HSPs)

A completely different approach for the search for an effective cancer vaccine has been adopted with the use of HSPs. The premise is that HPSs derived from any cell type contain a wide variety of peptides (6–35 mers) non-covalently bound to or 'chaperoned' by the HSP. The binding of peptides to HSPs occurs during the normal physiological degradation of proteins.[36] One consequence of this phenomenon is that HSP preparations contain the entire repertoire of peptides generated in a cell. The repertoire consists of self-peptides and antigenic peptides, and thus HSPs derived from tumors are complexed with peptides derived from tumor antigens. Vaccination of animals with tumor-derived HSP–peptide complexes results in protective immunity. The main problem with this approach is that customized, patient-specific vaccines are required. However, in animal studies they are effective at low doses without the use of adjuvants.

Breast/ovarian

Mucins

The importance of mucins as tumor-associated antigens has recently been increased by the finding that CTLs from breast, pancreatic and ovarian cancer patients can recognize the MUC1 gene mucin. This mucin consists of a perfectly conserved, tandemly repeated 20 amino acid long peptide core which is heavily glycosylated in normal cells and under glycosylated in tumor cells. Using mucin-transfected B cells as antigen-presenting cells, over 200 T cell lines were generated from cancer patients. The majority of the T cell clones required inhibition of mucin glycosylation for target cell recognition and were therefore tumor specific. T cell recognition is not MHC restricted but experiments with synthetic peptides have shown that at least five tandem repeats are necessary for T cell activation via T cell receptor cross-linking. In a recent clinical trial in breast, pancreatic and colorectal cancer, patients were immunized with this peptide consisting of five tandem repeats of the MUC1 gene, and 37 of 60 patients showed strong recall responses to the 105 mer peptide.[37]

HER2/*neu*

Amplification and overexpression of the HER2/*neu* proto-oncogene have been demonstrated in a number of human tumors including breast, ovarian, uterus, lung and colon cancer.[38] HER2/*neu* protein overexpression is observed in approximately one-third of breast cancers and is associated with a worse clinical outcome. No mutation has been found in the HER2/*neu* gene and therefore malignant transformation is associated with overexpression of the gene. Immune responses to this self protein have been observed in cancer patients.[39] A high incidence of IgG antibodies which recognize HER2/*neu* has been observed in breast cancer patients. As the switch from an IgM to an IgG response requires T cell help, these results imply that HER2/*neu* contains a helper T cell epitope. Several groups have shown that lymphocytes can be stimulated *in vitro* to synthetic peptides based upon the sequence of HER2/*neu*. More recently HER2/*neu*-derived peptides have also been shown to stimulate CTLs which can recognize and kill either breast or ovarian tumor cells which overexpress the HER2/*neu* gene but not tumor cells with low expression. Supporting this evidence is the observation that breast patients which with a high density of local lymphocytic infiltration associated with HER2/*neu* overexpression have an improved prognosis. Preclinical studies have shown that when rats were immunized with HER2/*neu* peptides a substantial T cell and antibody immune response to HER2/*neu* protein were observed.[39] These preclinical studies provide the necessary basis for the development of human vaccine trials using HER2/*neu* peptides.

Conclusion

A better understanding of the molecular recognition of antigens by the immune systems has enabled the design of more specific cancer vaccines. Many of these approaches are currently being tested in clinical trials, and are providing a more profound understanding of the potential and limitations of these approaches. The clinical applications of vaccines are still in their infancy but the early results of no toxicity, stimulation of anti-tumor immunity and tumor control give great optimism that they will ultimately take their place alongside surgery and chemotherapy in the treatment of solid cancers.

References

1. Rosenberg SA. *Cell transfer therapy*. Philadelphia: Lipincott 1995.
2. Fairchild PJ, Wraith DC. Lowering the tone: mechanisms of immunodominance among epitopes with low affinity for MHC. *Immunol today* 1996; **17:** 80-4.
3. Baskar S. Gene-modified tumor cells as cellular vaccine. *Cancer Immunol Immunother* 1996; **43:** 165-73.
4. Hamilton WB, Helling F, Lloyd KO, *et al.* Ganglioside expression on human-malignant melanoma assessed by quantitative immune thin-layer chromatography. *Int J Cancer* 1993; **53:** 566-73.
5. Livingston PO. Approaches to augmenting the immunogenicity of melanoma gangliosides: from whole melanoma cells to ganglioside-KLH conjugate vaccines. *Immunol Rev* 1995; **145:** 147-65.
6. Kitamura K, Livingston PO, Fortunato SR, *et al.* Serological response patterns of melanoma patients immunized with a Gm2 ganglioside conjugate vaccine. *Proc Nat Acad Sci USA* 1995; **92:** 2805-9.
7. Boon T, Goayewski TF, Coulie PG. From defined antigens to effective immunisation. *Immunol Today* 1995; **16:** 334-6.
8. Rosenberg SA. The immunotherapy of solid cancers based on cloning the genes encoding tumor-rejection antigens. *Annu Rev Med* 1996; **47:** 481-91.
9. Van Pel A, van der Bruggen P, Coulie PG, *et al.* Genes coding for tumor antigens recognized by cytolytic T lymphocytes. *Immunol Rev* 1995; **145:** 229-50.
10. Tsomides TJ, Eisen HN. T-cell antigens in cancer. *Proc Natl Acad Sci USA* 1994; **91:** 3487-9.
11. Jager E, Ringhoffer M, Dienes HP, *et al.* Granulocyte-macrophage-colony-stimulating factor enhances immune-responses to melanoma-associated peptides *in-vivo*. *Int J Cancer* 1996; **67:** 54-62.
12. Riethmuller G, Scheidergadicke E, Schlimok G, *et al.* Randomized trial of monoclonal-antibody for adjuvant therapy of resected Dukes-C colorectal carcinoma. *Lancet* 1994; **343:** 1177-83.
13. Chattopadhyay P, Starkey J, Morrow WJW, *et al.* Murine monoclonal anti-idiotype antibody breaks unresponsiveness and induces a specific antibody response of human melanoma associated proteoglycan antigen in cynomolgus monkeys. *Proc Natl Acad Sci USA* 1992; **89:** 2684-8.
14. Herlyn D, Wettendorff M, Schmoll E, *et al.* Anti-idiotype immunization of cancer-patients—modulation of the immune-response. *Proc Natl Acad Sci USA* 1987; **84:** 8055-9.
15. Fagerberg J, Hjelm AL, Ragnhammar P, *et al.* Tumor-regression in monoclonal antibody-treated patients correlates with the presence of anti-idiotype-reactive T-lymphocytes. *Cancer Res* 1995; **55:** 1824-7.
16. Austin EB, Robins RA, Durrant LG, *et al.* Human monoclonal anti-idiotypic antibody to the tumour associated antibody 791T/36. *Immunology* 1989; **67:** 525-30.
17. Austin EB, Robins RA, Baldwin RW, *et al.* Induction of delayed-hypersensitivity to human tumor-cells with a human monoclonal antiidiotypic antibody. *J Natl Cancer Inst* 1991; **83:** 1245-8.
18. Robins RA, Denton GWL, Hardcastle JD, *et al.* Antitumor immune-response and interleukin-2 production induced in colorectal-cancer patients by immunization with human monoclonal antiidiotypic antibody. *Cancer Res* 1991; **51:** 5425-9.
19. Denton GWL, Durrant LG, Hardcastle JD, *et al.* Clinical outcome of colorectal-cancer patients treated with human monoclonal antiidiotypic antibody. *Int J Cancer* 1994; **57:** 10-4.
20. Durrant LG, Buckley TJD, Denton GWL, *et al.* Enhanced cell mediated tumour killing in patients immunised with human monoclonal anti-idiotypic antibody 105AD7. *Cancer Res* 1994; **54:** 4837-40.
21. Durrant LG, Buckley DTJ, Spendlove I, *et al.* Monoclonal antibodies as cancer vaccines. *Ann Oncol*, unpublished.
22. Durrant LG, Denton GWL, Jacobs E, *et al.* An idiotypic replica of carcinoembryonic antigen inducing cellular and humoral responses directed against human colorectal tumors. *Int J Cancer* 1992; **50:** 811-6.
23. Foon KA, Chakraborty M, John WJ, *et al.* Immune response to the carcinoembryonic antigen in patients treated with an anti-idiotype antibody vaccine. *J Clin Invest* 1995; **96:** 334-2.
24. Conry RM, LoBuglio AF, Curiel DT. Phase I a trial of a polynucleotide anti-tumor immunization to human carcinoembryonic antigen in patients with metastatic colorectal cancer. *Hum Gene Ther* 1996; **7:** 755.
25. Whalen RG, Davis HL. Short analytical review. DNA-mediated immunization and the energetic immune response to hepatitis B surface antigen. *Clin Immunol Immunopathol* 1995; **75:** 1-12.
26. Irvine KR, Rao JB, Rosenberg SA, *et al.* Cytokine enhancement of DNA immunization leads to effective treatment of established pulmonary metastases. *J Immunol* 1996; **156:** 238-45.
27. Conry RM, Lobuglio AF, Kantor J, *et al.* Immune-response to a carcinoembryonic antigen polynucleotide vaccine. *Cancer Res* 1994; **54:** 1164-8.
28. Tsang KY, Zaremba S, Nieroda CA, *et al.* Generation of human cytotoxic T-cells specific for human carcinoembryonic antigen epitopes from patients immunized with recombinant vaccinia-CEA vaccine. *J Natl Cancer Inst* 1995; **87:** 982-90.
29. Young JW, Inaba K. Dendritic cells as adjuvants for class I major histocompatibility complex restricted antitumour immunity. *J Exp Med* 1996; **183:** 7-11.
30. Paglia P, Chiodoni C, Rodolfo M, *et al.* Murine dendritic cells loaded *in vitro* with soluble protein prime cytotoxic T lymphocytes against tumor antigen *in vivo*. *J Exp Med*

1996; **183:** 317-22.
31. Gabrilovitch D, Ciernic IF. Dendritic cells grown from bone marrow precursors but not mature dendritic cells from tumor bearing mice can be effective antigen carriers in the therapy of established tumours. *Proc Am Ass Cancer Res* 1996; **37:** 475.
32. Zitvogel L, Mayordomo JI, Tjandrawan T, *et al.* Therapy of murine tumours with tumor peptide pulsed dendritic cells: dependence on T cells, B7 costimulation and T helper cell 1-associated cytokines. *J Exp Med* 1996; **183:** 87-97.
33. Gjertsen MK, Bakka A, Breivik J, *et al.* Vaccination with mutant ras peptides and induction of T-cell responsiveness in pancreatic carcinoma patients carrying the corresponding RAS mutation. *Lancet* 1995; **346:** 1399-400.
34. Jung S, Schluesener HJ. Human T lymphocytes recognise a peptide of single point-mutated, oncogenic ras proteins. *J Exp Med* 1991; **173:** 273-6.
35. Ciernik IF, Berzofsky J, Carbone DP. Mutant oncopeptide immunisation induces CTL specifically lysing tumor-cells endogenously expressing the corresponding intact mutant P53. *Hybridoma* 1995; **14:** 139-42.
36. Blachere NE, Udono H, Janetzki S, *et al.* Heat-shock protein vaccines against cancer. *J Immunother* 1993; **14:** 352-6.
37. Finn OJ, Jerome KR, Henderson RA, *et al.* MUC-1 epithelial tumour mucin-based immunity and cancer vaccines. *Immunol Rev* 1995; **145:** 61-89.
38. Slamon DJ, Godolphin W, Jones LA, *et al.* Studies of the Her-2/*neu* proto-oncogene in human-breast and ovarian-cancer. *Science* 1989; **244:** 707-12.
39. Cheever MA, Disis ML, Bernhard H, *et al.* Immunity to oncogenic proteins. *Immunol Rev* 1995; **145:** 33-59.

(Received 9 July 1997; accepted 17 July 1997)