Melanoma vaccines

Bruce Elliott, Angus Dalgleish

Melanoma vaccines offer new hope to patients with metastatic melanoma, although convincing survival advantages have yet to be reported. This review outlines the progress made in this exciting field of research and looks ahead to the future.

Since the 1970s the incidence of melanoma in the UK has risen faster than any other major malignancy. Surgical management of early disease has lead to excellent improvements in prognosis. However, the impact of conventional therapy for metastatic disease is minimal, with best response rates nearing only 30% and cure rates well below 10%. There has never been a greater need to find new therapeutic targets for metastatic melanoma than at the start of the 21st century.

Whereas melanoma is considered relatively resistant to radiotherapy and chemotherapy, it does respond to different immunotherapies. Vaccination against infectious disease is a widely accepted practice, however, vaccination as a treatment for melanoma is less well known. In fact melanoma is one of the most immunogenic of all solid tumours and so lends itself extremely well to the concept of cancer vaccine therapy. For this reason most major advances in tumour immunotherapy have come from studies recruiting patients with melanoma. The various types of immunotherapy available for melanoma are outlined in *Table 1*. Melanoma vaccines have a major advantage over most conventional treatments as they cause only minor localized toxicity.

Unfortunately it became clear early in development that vaccination against tumour antigens (predominantly 'self') was considerably less efficient than vaccination against pathogens (not 'self'). Antigens from established tumours are often poorly immunogenic, with a proven track record for evading the immune system. However, recent advances provide strong grounds for optimism with the development of a better understanding of melanoma antigens and how to induce stronger immune responses against them.

HISTORICAL PERSPECTIVES

The earliest stirrings of cancer immunology are to be found in recordings dating back to antiquity. Ancient eastern and western medical writings described treatment of tumours by injection of purulent materials from infected wounds, leading initially to infection followed by tumour regression.

Scientific research did not commence until 1893, when a general surgeon from New York named William Coley began to inject live bacterial cultures around sarcomas. He quickly switched to filtered culture supernatant to avoid problems with sepsis, and found that his Coley's toxins were effective in causing regression of metastatic disease, albeit in a minority of his patients. The pathological term used for this process was tumour haemorrhagic necrosis.

Coley's toxins eventually lost ground to chemotherapy, but their legacy was the discovery of a soluble factor which induced tumour haemorrhagic necrosis called tumour necrosis factor. This has been shown to have potent anti-melanoma activity but unfortunately it causes significant toxicity in humans and therapeutic use is now confined to isolated limb therapy.

Interest in passive immunotherapy as a single agent continued with bacille Calmette–Guérin (BCG). Early studies reported induction of

TABLE 1.
Types of immunotherapy

Immunotherapy		Examples
Active	Whole cell vaccines	Allogeneic cell lines, autologous whole cell
	Antigen-based vaccines	Peptides, gangliosides
	Novel vaccines	Dendritic cells, heat shock proteins, DNA
Passive	Adoptive immunotherapy	NK cells, T cell clones
	Cytokines	GM-CSF, IL-2

GM-CSF = granulocyte macrophage colony stimulating factor; IL-2 = interleukin-2

Mr Bruce Elliott is Clinical Research Fellow and Registrar in Plastic Surgery and **Professor Angus Dalgleish** is Professor of Oncology, Division of Oncology, St George's Hospital Medical School, London SW17 0RE

Correspondence to: Mr B Elliott

antimelanoma antibodies and lymphocytic infiltration in regressed tumours. However, it was subsequently shown to have no impact on survival (Czarnetzki, 1993) and its use is now confined to adjuvant roles for augmenting active vaccination.

Over the last two decades, non-specific approaches to immunization have been superseded by attempts to induce specific immunity to tumours antigens. Just 20 years ago, the ability of the immune system to recognize cancer was hotly debated. Advances in molecular biology have since shown that antigens expressed by cancer cells can be recognized by autologous antibodies and T cells from the same patient.

The first breakthrough came with the realization in the early 1980s that autologous antibodies in the sera of melanoma patients could recognize glycolipid antigens called gangliosides found on melanoma. Then in the early 1990s, T cells were shown to recognize the melanoma differentiation antigens tyrosinase-related protein (TRP) 1 and MAGE (melanoma antigen gene), and tyrosinase through human leukocyte antigen (HLA)-restricted peptide presentation. It is now apparent that there are a large number of genes encoding melanoma antigens with more potential targets being discovered every year. *Table 2* lists some of the melanoma antigens recognized by human T cells.

SELECTING AN ANTIGEN

An ideal antigen for targeting with cancer vaccines would be strongly expressed by tumour cells, but not expressed by normal cells. Unfortunately most tumour-associated antigens (TAAs) are also expressed by normal cells, making them less specific targets.

Unique TAAs do exist, but they are mostly brought about by mutation or aberrant transcription meaning that each patient has a unique pattern. Vaccines against unique TAAs must be manufactured individually for each patient, making them expensive and time consuming to produce.

The differentiation antigens are shared by melanoma and a minority of normal cells. The antigens associated with melanin synthesis, for example, are found on melanomas and melanocytes. Cytotoxic lymphocytes specific for tyrosinase provided some of the first evidence for specific anti-tumour immune responses in humans. Many more have since been identified such as Melan-A/MART-1, gp (glycoprotein)100, and tyrosinase-related proteins (TRP) 1 and 2.

Another group of antigens in this category are the cancer-testis antigens (CTAs). These are encoded in the germ line but transcriptionally silenced in all cells except testis and placenta. CTAs are expressed by many epithelial and neuroectodermal malignancies and include the antigens MAGE, GAGE, RAGE and NY-ESO-1 (New York esophageal antigen). The increasing number of differentiation antigens and CTAs has created a large reservoir of targets for new peptide-based vaccine strategies.

Finally gangliosides are of interest as targets because of their high expression on melanoma as compared to other cells, and the ability to induce antibodies against them. Ganglioside vaccines have been used in several trials and are discussed below.

MULTIVALENT ALLOGENEIC VACCINES

The first melanoma vaccine models extracted antigens from allogeneic melanoma cell lines using three methods: attenuating whole cells, whole cell lysates produced by viral or mechanical disruption, and shed antigens extracted from cell line supernatants. Such multivalent vaccines have obvious advantages. One is their ability to immunize against multiple TAAs. They can also be mass-produced relatively easily.

However, multivalent vaccines also present some difficulties. There is unknown potential for differential antigen expression between sequential batches during production. Furthermore, it is difficult to quantify the overall immune response except through specific individual antigens, such as TRP-1, TRP-2 and GM2 and GD3 gangliosides. This can add a layer of uncertainty to the results of phase II studies. In addition, they con-

TABLE 2.
Melanoma antigens recognized by human T cells

Unique (mutation) antigens	β-catenin
	CDK4
	MUM-1
	p53
Differentiation antigens	Tyrosinase
	TRP-1/gp75
	TRP-2
	MART-1/Melan-A
	Glycoprotein 100 (gp100/pMEL17)
Cancer-testis antigens	MAGE-1 (melanoma antigen gene)
	MAGE-3
	NY-ESO-1

MAGE = melanoma antigen gene; NY-ESO = New York esophageal antigen; TRP = tyrosinase-related peptide

tain other non-tumour antigens which are inherently non-specific for cancer targeting.

The literature on allogeneic vaccines is relatively mature compared to other vaccine types. Initial results using mouse models were highly successful, showing tumour-specific immune responses and the rejection of tumour challenges. Several phase II trials have shown a clinical benefit, but convincing survival advantages have not yet been demonstrated by larger randomized phase III trials.

Canvaxin® (CancerVax Inc, Carlsbad) is an irradiated live cell preparation of three melanoma cell lines specifically selected for their high content of TAAs. The first two treatments are given with BCG as an adjuvant. Phase II data showed induced regression and improved survival compared to historical controls in a minority of patients with American Joint Committee on Cancer (AJCC) stage III and IV melanoma (Morton et al, 1992), with survival found to correlate with delayed-type hypersensitivity and increases in serum TA90 immunoglobulin (Ig) M complexes in later analyses (Barth et al, 1994; Hsueh et al, 1998).

To minimize selection bias caused by historical controls, a further matched pair analysis compared the vaccine with placebo on 107 patients with completely resected AJCC stage IV disease and found median survival times of 38 months in the vaccine cohort compared to 19 months with placebo (P=0.0009) (Hsueh et al, 2002). Matched analysis of 739 patients with resected AJCC stage III disease comparing the vaccine with placebo showed a consistent benefit for the vaccine cohort with a median overall survival of 55.3 months *vs* 31.6 months (P=0.0001) (Morton et al, 2002).

Based on these results, two multicentre phase III randomized trials were initiated in March 1998. The first trial initially compared Canvaxin plus BCG with interferon (IFN)-α2b for patients with resected AJCC stage III disease. However, the control arm was changed to placebo after the E1690 trial update suggested no survival benefit with IFN. The second trial compared Canvaxin plus BCG with placebo plus BCG after complete resection in AJCC stage IV disease. These trials have recruited from more than 50 centres worldwide and are expected to report during 2006.

The first randomized, multicentre phase III trial of a cancer vaccine (Wallack et al, 1998) used viral melanoma oncolysates (VMO) generated from four melanoma cell lines infected with vaccinia virus in 217 post-surgical stage III patients. This failed to show an improvement in disease-free interval or overall survival at a median final follow-up interval of 46 months.

The Sydney Melanoma Unit went on to conduct a prospective, randomized, multicentre phase III trial comparing adjuvant vaccinia cell lysate to surgery alone in 700 patients with stage IIB and III melanoma after a median follow up of 8 years (Hersey et al, 2002). Results again failed to show significant improvements in overall survival or relapse-free survival, although confidence intervals did not rule out important gains from the intervention.

Melacine® (Corixa Inc, Seattle) is a composite mechanical lysate vaccine made from two melanoma cell lines combined with the adjuvants monophosphoryl lipid A and Detox® (Corixa Inc, Seattle) (purified mycobacterial cell wall skeleton). In 1991 a randomized trial commenced to compare Melacine to a four-drug chemotherapy regimen in 140 melanoma patients. It reported low and statistically indistinguishable median survivals (7.2 *vs* 6.8 months respectively) (Mitchell and von Eschen, 1997). Canada accepted these data as sufficient to license its use on account of the differences in quality of life between the two regimens, and it has been available on prescription there since November 2000.

In a later randomized phase III trial of Melacine *vs* observation in 300 patients with stage II melanoma with 2 years follow up, no improvement in disease-free survival was found, although it was noted that the power to detect a small but clinically significant difference was low. There was, however, an interesting improvement in relapse-free survival shown in some of the HLA subsets (Sondak et al, 2002).

The work on shed antigen vaccines has mainly come from Bystryn's group. A randomized phase III trial using a shed antigen vaccine *vs* placebo demonstrated a delay in the time to recurrence (Bystryn et al, 2001). Interpretation is difficult, however, as only 38 patients were enrolled in this trial.

AUTOLOGOUS VACCINES

Autologous vaccines use the patient's own tumour as the source of antigen. This method takes advantage of unique antigens expressed by the tumour. However, metastatic tumour cells progressively downgrade antigen expression by a process of selection in the face of hostile immune responses. This means that autologous vaccines may not elicit potent anti-tumour activity. For this reason autologous vaccines have historically formed the basis for development of novel methods of increasing immunogenicity.

Furthermore, autologous vaccines require relatively large amounts of tumour tissue for production. Therefore studies tend to be skewed by patients with bulky disease and a worse prognosis. Autologous vaccines are also difficult to standardize and quality control.

Phase II data are available on an irradiated autologous whole cell vaccine modified by the hapten dinitrophenol, licensed as M-Vax® (AVAX Technologies, Kansas City) (Berd et al, 1997). This trial investigated the use of M-Vax in 62 patients with bulky AJCC stage III melanoma in a postsurgical adjuvant setting. Patients were also given BCG and low-dose cyclophosphamide. After a median follow-up time of 55 months, projected 5-year overall survival rate was found to be 58 %, well in excess of historical controls. Phase III trials are ongoing.

GANGLIOSIDE ANTIGEN VACCINES

Antibody responses have identified the importance of the gangliosides, which comprise a series of glycolipids termed GM1, GM2, GM3, GD2 and GD3 according to their carbohydrate moiety. GM3 and GD3 are the most abundant on melanoma cells, although GM3 is also expressed on virtually all other cells.

GM2 has been shown to be the most immunogenic of the gangliosides through work with the GMK vaccine, a mixture of GM2 conjugated to keyhole limpet haemocyanin (KLH) and the adjuvant QS21. The landmark randomized multicentre E1694 trial compared the GMK vaccine with high dose IFN-α2b in 774 patients with stage IIB or III melanoma (Kirkwood et al, 2001). At a median 16 months follow-up period, the IFN patients had significantly improved relapse-free and overall survival compared to the GMK group. This was in spite of a strong trend towards increased relapse-free survival for patients who developed higher antibody titres to the GMK vaccine (P=0.06)

Although E1694 was closed at interim analysis, further follow up is warranted before final conclusions are drawn because of the known difference in timing of previously observed benefits from these two therapies. In the absence of further data, this trial forms the basis for the current position of high-dose IFN as the standard of care for patients with high-risk melanoma in the United States.

PEPTIDE ANTIGEN VACCINES

Advances in molecular biology have led to the discovery that CD8+ T cells are capable of recognizing proteins from melanoma cells if peptides from these proteins are presented by class I major histocompatibility complex (MHC) molecules on the cell surface. Furthermore, these T cells are capable of clonal expansion and lysis of melanoma cells expressing this peptide. The first antigen shown to be specifically recognized by CD8+ T cells in this way was MAGE-1, a CTA found in ~40 % of melanoma cell samples.

Tumour-derived peptide sequences identified in this way are obvious targets for vaccination and several clinical trials have reported their use. Because these sequences are HLA-restricted, recruitment is limited to patients who have MHC haplotypes which are able to bind the relevant peptide, typically HLA-A2. Most patients are found to launch a specific immune response, but only a minority undergo metastatic regression, usually in dermal, subcutaneous or lymphatic lesions.

Adjuvants are important in these trials, as pure peptide solutions are often poorly immunogenic or even tolerogenic to naïve T cells. Typically used substances are incomplete Freund's adjuvant, QS-21, interleukin (IL)-12 and granulocyte macrophage colony-stimulating factor (GM-CSF), the latter having the appeal of its known ability to recruit dendritic cells to the site of administration. One randomized peptide vaccine trial compared incomplete Freund's adjuvant, QS-21, and GM-CSF as immunogenic adjuvants (Schaed et al, 2000), and found that GM-CSF and QS-21 induced a greater cytotoxic lymphocyte response against tyrosinase and GP100 epitopes.

Clinical results from peptide vaccine trials have been disappointing, and a correlation between clinical and immune responses has yet to be shown. The fact that T cell clones generated by peptide vaccines usually remain CD45RA+, a marker associated with naïve phenotypes, may be indicative of an ineffective immune response. It may prove necessary to additionally utilize class II restricted peptide sequences, of the kind recently discovered within the tyrosinase, gp100, Melan-A/MART-1, and NY ESO-1 molecules, to sufficiently broaden the epitope repertoire for peptide vaccines.

HEAT SHOCK PROTEIN VACCINES

Heat shock proteins (HSP) are ubiquitous proteins produced by cells in response to physical, chemical or immunological stress. They function as intracellular peptide carriers and these HSP–peptide complexes theoretically represent the total set of processed peptides from that tumour cell. They are readily taken up by dendritic cells (DCs) for presentation to naïve T cells; they are also readily purified from individ-

ual tumours. HSP70 has been shown to activate specific antimelanoma T cells via MHC class I pathways (Castelli et al, 2001). There are to date no data available from clinical trials.

DNA VACCINES

Some of the problems of MHC restriction have been solved with DNA vaccine technology. Antigens can be incorporated into cDNA sequences and cloned into a bacterial expression plasmid with a eukaryotic promoter. The full-length cDNA can encode multiple antigenic epitopes while the bacterial plasmid DNA itself contains immunogenic CpG motifs to act as a potent adjuvant. Once injected into patients, the plasmid is taken up by DCs, before presentation to naïve T cells in the sentinel lymph node. DNA vaccines have been shown to have effective anti-tumour activity in murine models; clinical trials are currently underway.

DENDRITIC CELL VACCINES

DCs have been proposed as a more natural form of adjuvant to amplify specific anti-tumour responses. DCs are the most efficient antigen-presenting cells to both the cell-mediated and humoral arms of the immune system. DCs can be harvested from peripheral blood, cultured in vitro using cytokine separation techniques, loaded with peptides, tumour lysates or total tumour RNA, before being reinfused into the patient. Early clinical trials using DCs pulsed with melanoma peptides have demonstrated both immune and clinical responses (Nestle et al, 1998; Thurner et al, 1999), paving the way for phase II studies in the future.

Efforts have been also been made to recruit DCs to vaccination sites in vivo, to avoid the labour-intensive procedures required for ex-vivo expansion (typically to numbers in excess of 1×10^7). The cytokine GM-CSF is being used in one such strategy; studies have either injected GM-CSF as an adjuvant or incorporated it into DNA using gene therapy (Dranoff et al, 1993). A randomized phase II trial compared the use of GM-CSF against pulsed DCs as adjuvant or vehicle respectively for a multiple peptide vaccine in 26 patients and found significantly larger immune responses in the GM-CSF arm (Slingluff et al, 2003).

FUTURE DIRECTIONS

A major obstacle to further progress is overcoming immune tolerance. A better understanding of natural adjuvants, including the discovery of toll-like receptors as important receptors for the innate immune system, should lead to further improvements.

The encouraging results shown by Morton's matched pair analyses support the conviction that melanoma vaccines may be the most effective therapeutic option in patients with AJCC stage III and IV disease. In the short term this will be achieved by surgery, but melanoma vaccines may eventually target cells showing the very earliest malignant changes to prevent melanoma altogether and mirror successes with cervical cancer (Plummer and Franceschi, 2002).

If the multicentre phase III allogeneic melanoma vaccine trials reporting in the near future show a convincing survival advantage, melanoma vaccines may become standard adjuvant therapies for patients with metastatic melanoma. Regardless, it is likely that ongoing development with more specific vaccine modalities will lead to greater sophistication and improved efficacy in the future.

CONCLUSIONS

There are now several vaccine approaches under trial for treating malignant melanoma. Many such candidates have encouraging phase II results and the first vaccine to show positive phase III results will lead to widespread acceptance.

Rapid advances in molecular biology over the past two decades have allowed detailed study of tumour antigenicity. However, antigen vaccines have not been developed to the same extent as whole cell vaccines and phase II results have been disappointing to date. Optimal presentation of antigens through adjuvants, cytokines and non-MHC restricted modalities remains unclear. HM

Conflict of interest: Professor Dalgleish is a member of the scientific advisory board for Progenics, the manufacturer of the GMK vaccine.

KEY POINTS

- There is a clear need for new treatments for metastatic melanoma; melanoma vaccines are attractive candidates because they cause minimal toxicity.
- Melanoma vaccines appear to work best in patients with low disease burdens, making them best suited as surgical adjuvants, and raising the intriguing possibility of melanoma prophylaxis.
- Phase III allogeneic vaccine trials are beginning to report but have yet to show a convincing survival benefit.
- The optimal combinations of vaccine type and adjuvant have not yet been clarified.
- Dendritic cell, DNA and heat shock protein vaccines are novel methods of vaccination with encouraging early results.
- The biggest obstacle to progress may be overcoming the anergy caused by immune tolerance.

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