1 Introduction

1.1 Cutaneous Melanoma Is a Significant Public Health Problem

Melanoma, the most aggressive of skin tumours, is a significant public health problem. It is estimated that in 2000 circa 47,000 new cases and 7,700 deaths occurred in the United States alone [1].

Melanoma is a neoplasm with a propensity for distant metastases. While surgical excision is an effective measure for early stages of the disease, there is currently no standard treatment for metastatic melanoma. Patients with disseminated disease such as distant skin, nodal, visceral, skeletal or central nervous system metastases face an extremely poor prognosis with an average survival of only 4 to 6 months [2, 3]. The lack of efficient treatment for late stage melanoma patients necessitates the development of novel forms of therapy.

1.2 Melanoma Is Immunogenic and an Important General Model For Cancer Immunology

Cutaneous melanoma has become a general model for cancer immunology. Several practical considerations contributed to this. Firstly, compared with other solid cancers melanoma cell lines can be relatively easily established allowing extensive analysis in a laboratory environment. Secondly, due to the nature of the disease the clinical course can often be straightforwardly monitored by examining skin lesions. Importantly, a large body of evidence has accumulated that the human immune system can recognise the tumour. This has importance beyond melanoma itself since understanding of the immune response against this specific tumour will almost certainly lead to insight into how to therapeutically modulate the response against other tumours as well.

Evidence of host immune responses is provided by border irregularities in radial growth phase lesions and by depigmentation and partial tumour

destruction caused by dermal lymphocytes [4-6]. While the spontaneous and complete regression of disseminated melanoma is a very rare phenomenon, partial regression can be seen in up to 25% of primary melanoma [7]. Regressing primary melanomas display higher numbers of infiltrating $CD3^+$ cells than non-regressing melanomas [8].

Blood-derived lymphocytes of melanoma patients which are stimulated *in vitro* with autologous tumour cells result in proliferation of T cells that exert cytolytic activity [9-12]. Interestingly, the now widely used expression “tumour infiltrating lymphocytes” (TILs) had been initially coined by the pathologist Wallace H. Clark Jr. in 1967 to describe a patient’s immune reactivity against melanoma [13]. A patient’s metastasis which had earlier developed a halo of hypopigmentation and had diminished in size, exhibited focal tumour necrosis with a permeation of lymphocytes into the tumour and evidence of lymphocyte-tumour apposition. Subsequent systematic analyses revealed that in primary cutaneous melanoma a host cellular response consisting predominantly of lymphocytes is associated with the vertical growth phase of the tumour in a number of cases [14, 15]. Most importantly, these studies have shown that the occurrence of TILs is an independent prognostic factor and positively correlated with the disease-free time and survival. Moreover, brisk T cell infiltrates in regional lymph node melanoma metastases are also an indicator of prolonged survival [16].

Since the initial observations the significance of intratumoral T cells as a positive prognostic factor has been established in other malignancies such as esophageal, ovarian and colorectal cancer [17-21]. These findings exemplify the model character of melanoma and indicate it is of interest to develop new methods of anti-cancer treatment that elicit TIL responses.

1.3 Melanoma Associated Antigens Are Targets for the Immune Response

The observation that melanomas can be immunogenic led directly to the question of the identity of the recognised tumour antigens. The existence of antigenic determinants that allow the immune system to distinguish between normal and tumour cells had been intensely debated in the past. Basis for the doubts that tumour associated antigens (TAAs) exist, was the realisation that naturally occurring murine tumours are predominantly nonimmunogenic [22]. In a groundbreaking effort van Pel and Boon demonstrated the existence of specific antigens on cancer cells [23-25]. Their initial studies revealed that injections of several chemically mutated malignant teratocarcinoma cell lines were rejected by their murine hosts which was not the case with the parental tumour cell line. The injection of these mutated cancer cell lines provided partial protection against each other and the nonimmunogenic nonmutated parental teratocarcinoma line. These experiments presented evidence that “tumour-specific transplantation antigens” were expressed in the mutated as well as the original teratocarcinoma line. Moreover, the results showed that it was possible to make otherwise nonimmunogenic tumours visible to the immune system through vaccination.

Over the last 20 years a growing number of TAAs have been identified in melanoma with different approaches (reviewed in [26-29]). This body of work affirmed the hypothesis that TAAs are a general feature of cancer [30].

Initial serological studies in the laboratories of Old and Morton identified gangliosides as surface tumour antigens on melanoma and other cancers of neuroectodermal origin in the early eighties. G_{D3} was characterised with murine monoclonal antibodies [31-33], G_{D2} was identified by autologous and allogeneic sera [34, 35] and G_{M2} was found to be reactive with human sera and monoclonal antibodies [36].

The Boon group at the Ludwig Institute for Cancer Research in Brussels pioneered the field of characterising a large number of T cell-recognised TAAs. Genomic DNA or cDNA libraries are transfected into cells expressing the appropriate MHC molecule. Transfectants are screened with patient-derived

tumour T cells with specific antitumour reactivity. Candidate antigens are identified by determining T cell-mediated cytokine secretion or lysis of the transfectants [37, 38].

Another approach to aid identification of antigens displayed by T cells relies upon acid-based elution of the peptides bound to MHC class I. Subsequently, the peptides are fractionated by reverse phase high pressure liquid chromatography (HPLC) and tested with CTLs. After several rounds, positive peptides are sequenced by Edman degradation or mass spectrometry [39, 40].

Sahin *et al.* introduced the serological analysis of recombinant cDNA expression libraries (SEREX) for identifying TAAs [41]. A tumour cDNA-based phage expression library is screened with diluted patients' sera. Since the secretion of IgG antibodies depends on helper T cell-B cell interaction, this system is conceived to identify antigens that are detected by the humoral as well as the cellular part of the immune system.

The group of Rosenberg at the National Cancer Institute recently developed a method in order to characterise antigens presented by MHC class II [42, 43]. In a first step transformed human embryonic kidney (HEK) 293 cells were genetically engineered to express all the molecules necessary for processing and presentation of MHC class II-restricted antigens. In a second step these cells were transfected with invariant chain-cDNA fusion libraries. In this system fusion proteins are targeted to MHC class II compartments, undergo proteosomal degradation and are loaded onto MHC class II molecules. After coculturing HEK 293 presenting cells and T lymphocytes, candidate antigens are identified by GM-CSF secretion.

Numerous melanoma TAAs identified by these experimental approaches have been found to be of importance in other types of cancer as well (reviewed in [26]). Similarly, TAAs characterised in malignancies other than melanoma were subsequently found to be also expressed in this type of skin cancer. This insight stresses the character of melanoma as a general model for cancer immunology.

T cell-recognised TAAs in melanoma can be broadly divided into the following categories: One group of antigens consists of proteins whose expression pattern in normal tissue is limited to testis and, occasionally, placenta. This group is called cancer-testes (CT) antigens (reviewed in [44]) and members include the MAGE [45, 46], GAGE [47] and BAGE [48] gene families and also NY-ESO-1 [49]. It is now known that a large proportion of CT antigens map to chromosome X while the biologic function of most CT antigens remains elusive. Exceptions are SCP-1 [50], OY-TES-1 and CT15/Fertilin β [51-53] whose functions are known. There is also evidence that certain MAGE family members are involved in cell cycle control and apoptosis [54-57]. Due to the restricted expression pattern CT antigen-reactive T cells are not removed in the thymus. Since cells in the immunologically privileged testis do not express class I HLA [58], autoimmune reactions do not occur under normal circumstances. Therefore the immune system can potentially recognise and eliminate CT antigen-bearing cancer cells without triggering autoimmunity.

A second group of melanoma antigens comprises melanocyte differentiation antigens. Proteins of this antigen group are expressed in normal melanocytes and are associated with pigment production. MART-1/Melan-A [38, 59], tyrosinase [38, 60], gp100 [61], tyrosinase related protein (trp1) [62] and tyrosinase related protein 2 (trp2) [63] belong to this group. T cells recognising this class of antigens are unlikely to be centrally deleted in the thymus due to the restricted expression of the differentiation antigens. Instead, these potentially autoreactive T cells are kept in check by peripheral tolerance mechanisms. (It should be mentioned, however, that this model has been recently challenged by the finding of promiscus gene expression of peripheral antigens in medullary thymic epithelilal cells [64].) It has been demonstrated, however, that immunologic tolerance against melanocyte differentiation antigens can be broken, resulting in simultaneous anti-tumour activity and autoimmune reactions [65, 66].

The third class of melanoma antigens are encoded following somatic mutations and alternative/atypical transcripts such as LB33Mel.B/MUM-1 [67],

CDK4 [68], β-catenin [69] and myosin/m [70]. While antigens of all other groups are often shared among cancer patients, mutation- or atypical transcript-derived TAAs are usually unique due to their genesis. These antigens can be recognised by the patient's immune system because they contain neoepitopes that are normally not expressed.

Finally, a group of TAAs exists whose members are overexpressed self antigens. In melanoma this group includes P15 [71]. The most prominent TAA of this group is the growth factor receptor and oncogene HER2/neu [72] which is well known for its role in breast cancer [73, 74]. The immunogenicity of overexpressed self antigens is based on reaching a certain threshold that activates the immune system.

1.4 Monitoring Tumour Antigen-Specific T Cells *In Vivo*

The identification of specific antigenic targets on tumour cells and the knowledge that cancer patients have T cells that can recognise these antigenic determinants (although often at low frequencies) fueled efforts to examine TAA-specific T cell populations without extensive *in vitro* manipulation. The advent of new techniques such as soluble fluorescent class I MHC-peptide complexes (commonly referred to as tetramers) and ELISPOT assays [75] along with intracellular cytokine staining [76] allowed structure-based and function-based monitoring of antigen-specific T cells in a quantitative fashion. Most efforts so far have been focussed on the analysis of T cells in melanoma patients where a large number of TAAs have been characterised.

Ex vivo staining of melanoma patients' peripheral blood-derived T cells with tetramers revealed that only for few of the potential TAA epitopes specific T cell populations could be detected. *In vitro* stimulation, however, allowed the detection of additional epitope specific $CD8^+$ T cell populations [77]. T cells recognising Melan-A/Mart-1 derived epitope A26-35 have been detected in a significant number of melanoma patients [78, 79]. Surprisingly, tetramer studies

of peripheral blood samples revealed that not only cancer patients have high frequencies of Melan-A/MART-1-specific $CD8^+$ T cells, but also healthy individuals [80]. Subsequent analysis for differences between the two groups showed that there is a difference in phenotype [81-83]. The change in phenotype gives reason to believe that these T cells are antigen-experienced, however, futher studies are necessary to clearly correlate T cell phenotype and functional T cell subpopulations.

Despite some technical limitations of tetramer studies such as being restricted to one peptide in a certain MHC molecule and detection limits that are currently often too high to capture the small number of TAA-specific T cells present, this valuable approach allowed for the first time to study antigen-specific T cell populations with a minimum of *ex vivo* manipulation.

1.5 Concerted Cellular and Humoral Responses Against Tumour-Associated Antigens Are A Rare Phenomenon

Despite extensive analysis, examples of TAAs inducing responses from the humoral as well as the cellular arm of the immune system are rare. A notable exception is the CT antigen NY-ESO-1. Studies showed a clear correlation between cellular and humoral immune responses directed against this antigen on one side and NY-ESO-1-positive tumour burden in patients on the other [84, 85]. In another study NY-ESO-1-specific $CD8^+$ T cells could be detected in more than 90% of antibody-positive patients while antibody-negative patients displayed no detectable NY-ESO-1-directed T cell responses [86]. Furthermore, Jäger *et al.* demonstrated that certain NY-ESO-1 epitopes can be recognised by $CD4^+$ T cells in melanoma patients who have NY-ESO-1 positive tumours [86]. A later study by the same group established that the naturally occurring $CD4^+$ T cell responses were correlated with NY-ESO-1-specific antibodies [87]. 11 out of 13 cancer patients who tested seropositive, had polyclonal $CD4^+$ T cell reponses

against NY-ESO-1 epitopes, while no evidence for $CD4^+$ T cells was found in 18 seronegative patients.

1.6 Tumour Growth In the Presence of TAA-Specific T Cells

The important finding that TAA-bearing and MHC class I expressing tumours can survive and proliferate in the presence of significant numbers of TAA-specific T cells raises pivotal questions about anti-cancer immune responses. To date no clear answer has emerged but work in murine models has provided several clues about possible mechanisms. Experiments involving the spontaneous insulinoma model gave the insight that although an antitumour CTL response was induced it was short-lived, giving rise to the model of “effector exhaustion” [88]. Moreover, several laboratories contributed the insight that antigenic “ignorance” can stem from low antigen expression and ineffective crosspresentation, especially in the case of solid tumours [89, 90]. The presence of $CD25^+$ $CD4^+$ regulatory T cells has also been implicated in hampering anti-tumour immunity [91-93]. An induction of antigen-specific T cell anergy was encountered when naïve $CD4^+$ T cells specific for an antigen expressed by tumour cells were transferred into tumour-bearing mice [94]. Recent data emphasised that the mere presence of tumour-reactive $CD8^+$ T cells is insufficient to induce tumour regression [95]. In a transgenic (tg) mouse model with >95% of all $CD8^+$ T cells recognising the gp100 TAA, antigen-bearing tumours grew with the same rate as in the control mice. This was the case despite the fact that earlier *in vitro* experiments had shown that T cell receptor (TCR) tg T cells proliferated extensively, developed an effector phenotype and secreted IFN-γ upon encountering the TAA peptide.

1.7 Novel Therapeutic Methods Targeting Melanoma

The initial observation that melanoma cells are immunogenic, that they can be distinguished by their antigenic determinants from normal counterparts and that TAA-specific T cells are present in cancer patients (although predominantly in a functionally inactive state) were starting points for efforts developing immunotherapeutic regimen. Numerous clinical trials have been carried out within the last 20 years.

Therapeutic cancer vaccines can be broadly divided into two main categories, non-antigen- and antigen-based vaccines. Vaccines without a TAA component comprise monoclonal antibodies [96-98], biological response modifiers such as the cytokine IL-2 [99] and adoptive T cell transfer [100-102]. Among anti-cancer treatments that include administration of antigens can be distinguished between ones that include defined antigens and ones that are comprised of an unknown mixture of antigenic determinants. Peptides [103] and peptide-loaded DCs [104, 105] belong to the first group. The second group includes whole tumour cells/lysates [106], tumour-derived heat shock proteins [107] or tumour RNA transfected DCs [108, 109]. Antigen-based forms of treatment often include an adjuvant component such as bacterial antigens or cytokines like IL-2 and GM-CSF.

Antigen-based strategies that rely on the full repertoire of expressed proteins in tumour cells can potentially capture all possible – known and unknown – epitopes of the TAAs expressed. While therapeutic vaccines that employ defined antigens lack this advantage, they allow for close monitoring of the vaccine response to certain TAAs by T cell and antibody assays. Several groups, employing tetramer or ELISPOT assays, reported the occurrence or increase of the number of $CD8^+$ T cells specific for TAAs after vaccinating melanoma patients. Patients immunised with peptides derived from MAGE-3, NY-ESO-1, Melan-A/MART-1, tyrosinase and gp100 without adjuvants or DCs pulsed with CT peptides were generating peptide-specific $CD8^+$ T cells *in vivo* [105, 110-112]. Closer analysis revealed, however, that in most cases the

vaccine-elicited increased frequency or increased susceptibility to *in vitro* stimulation could not be correlated to tumour regression [113].

In some of the clinical melanoma trials a small number of patients showed immune responses. In most instances, these successes were anecdotal, the response limited to individual metastases or the patients eventually succumbed to the disease. Overall, it was not possible to demonstrate a vaccine-induced clinical improvement in significant proportion of the patients. It should also be mentioned that some of the current experimental forms of treatment are not without risk to the patient. In particular, the systemic administration of IL-2 can trigger severe and potentially lethal side effects [114].

Despite the limited success in clinical melanoma trials, however, it has been convincingly shown that cancer patients can benefit from immunotherapy. Patients with colorectal cancer who received injections of autologous irradiated tumour cells admixed with Bacillus Calmette-Guerin as an adjuvant displayed statistically significant longer recurrence-free periods and longer recurrence-free survival compared to a control group [115].

1.8 Development of a GM-CSF-Based Cellular Melanoma Vaccine

The idea that cytokines could be utilised for cancer therapy was put into practice by Forni *et al.* [116, 117]. Low doses of recombinant cytokine were peritumourally injected and positively influenced the patient's immune response. The availability of effective viral gene transfer systems that allowed the stable transduction of target cells created renewed interest in the therapeutic use of cytokines. Genetically manipulated tumour cells could be engineered to secrete cytokines to aid the host tumour response (for a review see [118]).

In the B16 murine melanoma model, irradiated, cytokine-secreting tumour cells were used as a vaccine to protect mice from a later challenge with wild type B16 cells [119]. Among the large number of cytokines tested, GM-CSF efficiently conferred immunity. The protective effect of GM-CSF-secreting tumour cells has

since been proven in many murine model systems [120-134]. The mechanisms that lead to the rejection of the tumour cells involve $CD4^+$ and $CD8^+$ T cells and antibodies [119, 135-137]. Among other properties, GM-CSF is known to modulate movement and maturation of dendritic cells [138, 139]. Indeed, closer examination of the underlying mechanism showed that vaccination with GM-CSF secreting tumour cells facilitates antigen uptake and presentation by antigen processing cells such as mature dendritic cells and macrophages. Several studies have shown the central role DCs play in anti-tumour immunity [140, 141].

Flt3-ligand (Flt3-L), like GM-CSF, induces the development of DCs [142-145] and has shown anti-tumour effects in several murine models [146-148]. Therefore our group decided to carry out a comparison of vaccination approaches based on these two cytokines in the B16 model. The results revealed modes of action by which GM-CSF causes anti-tumour immunity to a higher degree than Flt3-L [149]. GM-CSF stimulated DCs had a much higher ability to perform phagocytosis on particulate matter which is an important prerequisite for antigen presentation. Furthermore, GM-CSF-based vaccination led to a broad T cell cytokine response, while Flt3-L triggered only the secretion of Th1 type cytokines. With the GM-CSF-based vaccine high-level expression of the costimulatory molecule B7-1 and CD1d on the DCs along with a more myeloid phenotype (characterised by $CD11b^+$, $CD8\alpha^-$) was correlated with potent anti-tumour immunity.

The high levels of CD1d gave rise to the hypothesis that natural killer T (NKT) cells may contribute to the rejection of tumours. Our group showed that in immunisation experiments with the B16 GM-CSF model all wild type (wt) mice were protected while almost all CD1d and Jα281 deficient animals died after a B16 wt challenge due to their tumour burden [150]. Moreover, it was found that the CD1d and Jα281 deficient mice showed decreased production of Th2 type cytokines.

Cytotoxic T lymphocyte antigen-4 (CTLA-4) is a tightly regulated surface molecule on $CD4^+$ and $CD8^+$ T cells interacting with the B7 molecules that plays an important role in down-regulation of T cell responses [151-153]. This

important role was confirmed in CTLA-4-deficient mice that die due to extensive lymphoproliferation [154-156]. Recently, there has been interest in combining GM-CSF with CTLA-4-blocking antibodies in order to enhance T cell activation [157-160]. Injecting mice with GM-CSF-secreting irradiated tumour cells is not an effective treatment for established poorly immunogenic tumours such as B16. It has been shown that the GM-CSF-based approach along with the administration of anti-CTLA-4 antibody, however, has a synergistic and protective effect in a therapeutic setting [158]. Interestingly, this treatment also triggers autoimmunity; immune reactivity against normal melanocytes leads to depigmentation.

1.9 A Trial with GM-CSF-Based Vaccines Stimulates Potent and Long-Lasting Immune Responses in Late Stage Melanoma Patients

The aforementioned murine B16 model was the basis for a clinical phase I trial with autologous GM-CSF-based therapeutic vaccines in late stage melanoma patients by Soiffer et al. [161]. In brief, resected tumour tissue was processed to single cell suspension and short term tissue cultures were established. The melanoma cells were then infected with pseudotyped retroviral particles and frozen. Patients received half-intradermal, half-subcutaneous injections of the irradiated autologous cells.

The immunisation sites of all evaluable 21 patients who received the GVAX vaccine became densely infiltrated with T lymphocytes, DCs, macrophages and eosinophils. Interestingly, staining of biopsies showed high level expression of B7-1 on the DCs. While injections of irradiated autologous nontransfected melanoma cells failed to elicit any significant response prior to treatment, all patients reacted strongly after receiving several doses of the vaccine. Extensive erythrema and induration was observed along with dense infiltrates of T cells and degranulating eosinophils in delayed-type hypersensitivity (DTH) reactions.

While metastases surgically removed prior to enrollment were minimally infiltrated, dense infiltrates of T lymphocytes and plasma cells were observed in the metastatic lesions of 11 out of 16 patients examined after treatment began. Subsequent staining of paraffin-embedded tissue sections revealed $CD4^+$ and $CD8^+$ T cells, $B220^+$ B cells and IgG-secreting plasma cells. The infiltrates were associated with significant tumour destruction (at least 80%), fibrosis and oedema. TILs from metastatic lesions displayed specific cytotoxicity and cytokine production when incubated with autologous melanoma cells. Immunoblots and flow cytometry with post-vaccination serum demonstrated high titre antibodies against antigenic determinants on melanoma cells. Furthermore, we observed an immune response mediated by eosinophils, neutrophils and lymphocytes against tumour blood vessels in four patients. None of the patients showed significant side effects or signs of autoimmunity.

Since a phase I study is not designed to evaluate efficacy, it is not possible to assess prolonged survival of patients. Given the nature of the disease, however, it seems remarkable that two patients with disseminated melanoma, K006 and K008, are still alive in the autumn of 2003 more than seven years after enrolling in the trial. The laborious technical procedures and intricate safety tests that are required for studies with retroviral-mediated gene transfer limit this type of clinical trial to a small scale. In contrast to retroviral vectors that require short term tissue culture of the target cells, adenoviral vectors can be easily used to infect single cell suspensions of resting tumour cells. These practical considerations led to the development of two clinical phase I studies in which patients with metastatic melanoma or metastatic non-small-cell lung carcinoma received autologous irradiated tumour cells engineered by adenoviral-mediated gene transfer to secrete GM-CSF [162, 163].

Similar to the retroviral study, the patients in both studies showed reactivity at vaccination sites and the same type of DTH response when given injections of non-transfected autologous tumour cells. Moreover, as in the initial melanoma study, distant metastases became infiltrated with $CD4^+$, $CD8^+$ T lymphocytes, $B220^+$ B lymphocytes and IgG secreting plasma cells. Compared to

the retroviral melanoma trial there was a notable difference in overall anti-tumour immunity. Brisk infiltrates were found in a smaller group of patients (adenoviral melanoma GVAX: 6 out of 16; adenoviral lung GVAX: 3 out of 6) and the extent of tumour necrosis was smaller. Despite this difference, however, 10 out of 35 melanoma patients are still alive with a minimum follow-up of 36 months. Likewise, it is of interest to note that two adenoviral lung GVAX patients surgically rendered as having no disease at enrollment remained free of disease at 43 and 42 months and five patients showed stable disease durations between 33 and 3 months.

Three melanoma patients from the adenoviral GVAX trial were administered an additional infusion of the CTLA-4 blocking antibody MDX-CTLA-4 [160]. Remarkably, the antibody treatment elicited extensive tumour necrosis with granulocytic and lymphocytic infiltrates. In all three cases a year or more had passed between GVAX and MDX-CTLA-4 treatments. Interestingly, four melanoma patients in this trial who had earlier received autologous DCs engineered to express gp100 and MART-1 by adenoviral-mediated gene transfer (3 patients) [164] or modified gp100 peptide plus IL-2 (1 patient) did not show any of the potent immune reactions.

1.10 The GM-CSF-Based Cancer Vaccine is the Basis for the Identification of Tumour-Associated Antigens

Due to the striking findings in the clinical trials with GM-CSF secreting autologous tumour cells, we are interested in understanding the vaccine-induced anti-tumour immunity. Although numerous TAAs have been identified in melanoma, it is questionable whether a particular antigen can elicit a significant clinical response in patients, i. e. whether it can serve as a tumour rejection antigen. It is worthwhile pointing out that most technical procedures to identify TAAs were initially not designed to characterise the clinically most promising targets. The repeated cytokine-driven stimulation and proliferation of a very small number of

precursor CTLs or the elution and sequencing of MHC class I bound peptides may not identify the most potent tumour rejection antigens. Therefore it seems to be important to examine the antigenic targets in cancer patients with an encouraging clinical course, i. e. patients with spontaneous [61, 70, 165] or treatment-induced tumour regressions. Thus we want to identify the targeted TAAs in our patients and analyse the processes that lead to tumour regression and tumour rejection. We would like to identify suitable TAAs for future clinical studies. Our longterm goal is to replace our current autologous whole-cell based vaccine with a generic melanoma vaccine that consists of defined antigenic components and thus provide an alternative, more effective form of melanoma therapy. Moreover, there is currently very little known about the molecular basis for the transformation from a melanocyte to a melanoma cell. Furthermore, the basis for the propensity to metastasise and for the resistance to radio- and chemotherapy is not understood. The characterisation of overexpressed TAAs may lead to a better understanding of melanoma biology.