Chapter VII
Melanoma vaccines
– the problems of local immunosuppression

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Sumitted
Abstract

The incidence of cutaneous melanoma in Europe is rising, and the disease is incurable once metastases occur. Since melanoma expresses antigens which can be specifically recognised by the immune system, and occasionally undergoes spontaneous regression mediated by anti-tumour immunity, a number of different melanoma vaccines have been developed and tested clinically. While most such vaccines show efficacy in vitro and an ability to stimulate anti-melanoma immune responses in blood, they have proven disappointing in clinical practice. It has become increasingly clear that the interaction between melanoma and the immune system is determined locally, within the tumour or draining lymph nodes. It is now clear that melanoma cells have the ability to anergise the immune system by inducing an immunosuppressive microenvironment which may explain the inability of systemic vaccines to alter patient outcome. This subversion of the immune system involves alteration of dendritic cell function by tumour-derived cytokines leading to the generation of suppressive and regulatory T lymphocytes. Successful melanoma vaccination probably requires therapeutic neutralisation of the immunosuppressive microenvironment, which will require greater understanding of the molecular mechanisms used by the tumour to promote immunosuppression. Nevertheless if these problems can be overcome, it seems likely that the efficacy of melanoma vaccines could be greatly enhanced.
Cutaneous melanoma affects around 60,000 patients each year in Europe, resulting in around 14,400 deaths, and the incidence is still rising. The prognosis for the patient with a melanoma depends upon its extent: while the 5-year survival is close to 90% for localized malignancies, it is less than 20% once the patient has distant metastases [1].

Metastatic melanoma is largely chemoresistant [2] and there is an urgent need for new treatments. This malignancy has long been recognized to be an immunogenic tumour, and the first attempts of immunotherapy against this malignancy date back as far as the 19th century; observations on the relationship between acute immune responses and melanoma regression are even older [3, 4]. Reported long-term complete remissions of cutaneous melanoma metastases [5-8] correlate with specific anti-tumour immune responses [9], expression of pro-inflammatory cytokines [10] and the presence of intra-tumoral T lymphocytic infiltrates [11, 12]. Melanomas express antigens, such as Melan A/MART-1 [13] that can be recognized by cytotoxic T lymphocytes (CTL) in association with MHC class I [14], [15]. However, unlike in viral infections, natural CTL in cancer patients are few and functionally inefficient [16]. Similarly, the presence of natural active anti-tumour immune responses and partial or complete regressions of primary melanomas do not ensure a favourable prognosis and often occur in the presence of metastasis [17]. Cutaneous melanoma usually spreads first via the lymphatic system, and it has developed numerous mechanisms to avoid recognition by the immune system, for example, a decrease in HLA Class I expression in the primary tumour [18]. Surprisingly, higher in situ expression of HLA-DR is correlated with a poor prognosis [19, 20].

Why melanoma avoids recognition - current theories

The complex interactions between the immune system and developing malignancies have been studied by many. The fact that immunogenic tumours, such as melanoma, develop in otherwise immuno-competent patients and the coexistence of tumour-specific immunity with a progressing tumour remain among the major paradoxes of tumour immunology [21, 22]. Among several theories which have been constructed to explain this paradox, those most commonly quoted are Immune Surveillance [23], recently updated to Imunoediting [24], Immune Ignorance [25, 26] and the Danger Model [27]. The first hypothesis presumes that cancer cells constantly develop in the
body, but that the majority of them are specifically detected and eliminated by constant surveillance of the immune system, on the basis of tumour cell expression of tumour-specific antigens or molecules induced by cellular stress [23, 28]. This theory was recently developed into the concept of immunoediting, which regards immunosurveillance as a phase of a more complex process which leads to the development of immuno-resistant tumour variants, which then escape immune recognition [24, 29]. Since this recognition is based on antigen presentation, loss of HLA molecules and impaired antigen presentation are the most obvious mechanisms of escape from destruction by cytotoxic T lymphocytes (CTL). Alterations in HLA expression are ubiquitous among tumours and include complete loss of any HLA allele, significant down-regulation of one or more alleles, expression of altered HLA alleles or immunosuppressive HLA alleles, and altered responsiveness to activation signals such as type I interferons [30]. Loss of HLA class I leads to impaired HLA peptide loading, and therefore inefficient cell surface antigen presentation [31]. Additionally, antigen recognition and a successful immune reaction against the tumour can be impeded by heterogeneity of surface protein expression, even within the same tumour [32].

In contrast, the theory of Immune Ignorance states, that despite the existence of numerous tumour-specific antigens, they are not recognized by cytotoxic T lymphocytes because of the lack of a co-stimulatory signal [25, 26]. Antigen presentation without a correct second signal may subsequently result in anergy and eventual immune tolerance. For example, melanomas can also express HLA class II proteins, whose expression is generally restricted to APCs and activated T cells. This ability does not enhance tumour immune susceptibility, but instead interferes with normal T helper function due to the absence of co-stimulatory molecules such as B7 on the tumour [33-36].

In opposition to self-nonself discrimination (important for immune surveillance or ignorance), the Danger Model implies that unresponsiveness is a natural state of the immune system, which only will become activated upon receiving appropriate stimulation, i.e. the “danger signal”. Accordingly, tumours can safely arise in immuno-competent organisms, unless an additional situation occurs, which is considered dangerous, such as surgical trauma, or infection [27]. To enable successful tumour elimination, this danger signal must be delivered in a form of microbial
pattern receptors or heat shock proteins, which play a critical role in cross-priming, i.e. presentation of antigens produced by one cell on the surface of another cell, preferably professional APC. Furthermore, the activation of an immune response must be continuous, not allowing the cytotoxic T lymphocytes to rest [27].

However diverse these hypotheses may seem, they all acknowledge the importance of proper antigen presentation, activation of antigen-specific T lymphocytes and maintenance of the anti-tumour immune response. Generation of successful anti-tumour immune responses would greatly benefit patients with aggressive tumours, and hence a number of approaches have been taken to develop protective immunity. Since melanoma immune escape seems to be largely based on impaired antigen processing and presentation, many of these attempts exploit the function of dendritic cells, which are professional antigen-presenting cells and may act as potent immune response stimulators.

**Melanoma vaccines**

1. Strategies for evoking anti-melanoma immune response

Activation of the immune response requires recognition of an immunogenic molecule, and if the memory response is to be generated, it is necessary that the antigen is presented in the context of HLA class II molecules by an antigen-presenting cell to T lymphocytes, supported by co-stimulatory signals. To find suitable antigens for vaccine purposes, melanoma proteins have been screened for peptides with potent immunostimulatory characteristics, presented by both HLA class I and II, to activate both cytotoxic and helper T lymphocytes. Studies have both identified HLA class I binding peptides, as well as several peptides that are presented in the context of multiple HLA-DR alleles and are recognisable by CD4+ T cells [37-41]. Melanoma peptides have been used as vaccines with or without immune adjuvants, modified to enhance antigen presentation by fusing with TAP-targeting sequences to facilitate antigen processing [42] or with heat shock protein to assist antigen delivery into dendritic cells [43]. Such antigens have been loaded into DC, generated in vitro from blood or bone marrow precursors. Since a single epitope is seldom sufficient for the induction of a potent immune response, multivalent vaccines have been tested,
including multipeptide cocktails, often enhanced by additional immunogenic proteins, e.g. Tetanus Toxoid, and lysates of whole tumour cells. Similarly, multivalent vaccines have been loaded into autologous or allogeneic DC, or tumour-specific DC generated by hybridization with melanoma cells [44-46], [47]. To obtain endogenous expression of antigen by DCs, genetic transfer using viruses encoding melanoma proteins has been used, in order to achieve prolonged antigen presentation in both an HLA class I and a II context, resulting in an enhanced anti-melanoma immune response.

2. Vaccine outcome in vitro

Overall, the majority of the in vitro approaches described above have been successful at the laboratory level with regard to antigen incorporation/transfection rate, protein production and presentation, and T-lymphocyte activation. Experiments in vitro, where patients’ lymphocytes were stimulated with vaccines and tested, have proven that dendritic cells are able to process and present melanoma-specific antigens derived either from whole melanoma cells, synthesised or purified peptides, or after genetic cell modification to produce tumour antigens (As reviewed in [48]). In the vast majority of studies both proliferative and cytotoxic immune responses were reported. Lymphocytes co-cultured with genetically modified DC produced Th1 type cytokines and show multiple antigen-specific cytotoxic responses, against melanoma cell lines, HLA-matched B cell lines pulsed with peptide and, most importantly, autologous tumour [48]. The ability of in vitro expanded lymphocytes to recognize naturally processed and presented epitopes illustrates the potential use of dendritic cells for vaccination in human cancer.

While many of these strategies have been successful in inducing the presence of anti-tumour cytotoxic T lymphocytes (CTLs) in peripheral blood [49-51], inducing an immune response to control antigens in 88% patients [52, 53], inducing homing of antigen-specific T cells into the tumour 75% cases [54] and T cells able to kill autologous tumour when exposed in vitro [50, 55], none of these strategies have been clinically effective. None have found a direct effective translation to patient care, despite considerable ingenuity and expenditure.
3. Effects of anti-melanoma vaccination in patients

Between 2003 and 2007, the results of 57 clinical trials of melanoma vaccines were published, using single peptide and multi-epitope approaches, with immune adjuvants or with lysates of autologous tumour cells loaded into dendritic cells, or using DC-melanoma hybridomas (Table 1).

Despite the enrolment of 1,260 patients in peptide-related studies, only 3 complete responses and 21 partial responses were recorded. It is possible that single successful vaccinations are not an effect of the therapy, but may instead be related to a unique immune system response in a particular patient (Table 1), as spontaneous regressions of cutaneous melanoma can occur even in very advanced stages of the disease (Nathanson 1976, personal observation).

Strategies employing genetically modified tumour cells, tumour lysates and melanoma-DC hybridomas are visibly more successful, and show consistently higher rates of objective responses – 18 CR and 28 PR in 794 patients. The rate of complete responses is, however, still low, not exceeding 1.7% for lysates-based vaccines, even when the vaccines were genetically modified to secrete GMCSF, a cytokine strongly influencing DC maturation [56, 57]. A comparison between the outcomes of “natural” tumour lysates and peptide vaccines, however, demonstrates that the immune response to purified tumour epitopes is not equal to the response evoked by total tumour cell content, which is a far more potent immune stimulator (Table 1). Of note is the significantly higher (8.5%) percentage of complete responses in the studies where DC were used as a vehicle for tumour antigens (Table 1). Some of the tested vaccines resulted in an increase of median and overall survival, and a prolonged disease stabilisation period (up to 60+ months) [52, 57-59], but the results have not been confirmed in randomised clinical trials, and work on some of the vaccines has actually been halted. All of these vaccines used additional potent immune adjuvants, such as IL2, GM-CSF and BCG, none of which have shown any major benefit in conjunction with these vaccination strategies.

The main obstacles to successful vaccination remains the failure to create sufficiently high numbers of efficient tumour-specific CTL [60], impaired homing of melanoma-
specific CTL to the tumour and the lack of efficient cytotoxic activity even when tumour-specific CTL are found within the melanoma [61], [62]. The reasons for the failure of vaccine therapies to date in melanoma are not clear, but there is growing evidence that the microenvironment within the tumour may suppress the immune response at multiple levels.

Table 1. Summary of outcome of melanoma vaccines clinical trials outcome; 2003-2008

<table>
<thead>
<tr>
<th>Vaccine type</th>
<th>NoP</th>
<th>CR</th>
<th>PR</th>
<th>MR</th>
<th>SD</th>
<th>Best reported 5 years survival</th>
<th>Best reported Median Survival [mths]</th>
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<tr>
<td>Single peptide</td>
<td>209</td>
<td>0</td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>[84] [85] [86] [87] [88] [89]</td>
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<td>52</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>7</td>
<td>12 [90] [91] [92]</td>
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<tr>
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<td>0</td>
<td>2</td>
<td>1</td>
<td>17</td>
<td>49% vs 20%</td>
<td>37.6 [93] [94] [95] [59] [96] [97] [98] [99] [100] [101] [102]</td>
<td></td>
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<tr>
<td>Multipeptide DC</td>
<td>76</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>6</td>
<td>[103] [96] [104] [99]</td>
<td></td>
<td></td>
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<tr>
<td>TCL or modified TC</td>
<td>699</td>
<td>12</td>
<td>20</td>
<td>6</td>
<td>24</td>
<td>44%</td>
<td>38 [57] [58] [105] [106] [107] [108] [109] [110] [111]</td>
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<tr>
<td>ATL DC</td>
<td>49</td>
<td>4</td>
<td>6</td>
<td>3</td>
<td>5</td>
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<tr>
<td>Tumour-DC hybridomas</td>
<td>44</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>9</td>
<td>8</td>
<td>[116] [117] [48]</td>
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</tr>
<tr>
<td>Monoclonal antibodies</td>
<td>114</td>
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<td>7</td>
<td></td>
<td></td>
<td>16</td>
<td>[118] [119] [120]</td>
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<tr>
<td>Viruses encoding MAA</td>
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<td></td>
<td>20</td>
<td>[124] [125] [126] [127]</td>
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TCL-tumour cell lysates, ATL DC- autologous tumour cell lysates loaded DC, MAA-melanoma associated antigens, general adjuvants: HSP, M. Vaccae, CD40-ligand. CR-complete response, PR-partial response, MR-mixed response, SD-disease stabilization
The immunosuppressive microenvironment

Cutaneous melanoma arises in the skin, which in itself constitutes an immunological barrier for pathogens, and provides a network of active immune system effectors. Moreover, despite being an immunogenic tumour, melanoma paradoxically spreads through the lymphatic system, where the chances of immune recognition are highest. As potentially immunogenic tumours, melanomas need to develop strategies to escape immune response, and there is now firm evidence that the formation of a locally immunosuppressive microenvironment is a critical step in melanoma progression. To achieve this, melanoma cells express and release FasL, which causes apoptosis of T lymphocytes, shed tumour antigens to abrogate anti-tumour cytotoxic lymphocyte function, and, above all, they secrete immunosuppressive cytokines, including TGFβ, IL10, IL6 and IL8 [63-69] and the immunosuppressive enzyme, IDO [70, 71].

Secretion of immunosuppressive cytokines is so important to melanoma development, that not only are IL10, TGFβ and IDO present in patients’ blood, primary melanomas and sentinel lymph nodes (SLN) [72-75], but cytokine levels correlate with tumour progression and invasiveness [72, 76]. Similarly, investigations on melanoma SLNs showed that draining lymph nodes are profoundly immunosuppressed, and that the level of stimulatory or suppressive capacity correlates with the distance of the node from the nearest deposit of primary or metastatic melanoma [77]. This distance-related lymph node immunosuppression has been found to be related to the presence of the cutaneous melanoma [75] and has been further confirmed in studies on T lymphocyte activity [78], DC infiltrate [79] and DC morphology [80].

The evidence presented suggests that in fact melanomas not only avoid recognition by the immune system, but also actively alter the local functions of the immune system. It is therefore important to investigate how the immunosuppressive environment created by melanoma influences local dendritic cells, the key regulators of immune responses. In peripheral tissues, DCs continuously sense the microenvironment and recognize potentially dangerous pathogens, initiating T cell immune reactions against them, while tolerizing T lymphocytes towards harmless antigens. Since DC act as environmental sensors guarding the homeostasis of the organism, they must be very sensitive to any changes within their vicinity. In order to execute their functions they
undergo a complex transformation, including changes in shape, size, motility, and also complete alterations of surface antigen expression, in reaction to exogenous factors. A pluri-potent immature DC can differentiate into an activating or a tolerizing phenotype, depending on the signals it received at the time of antigen uptake. In peripheral tissues, DC are prone to become tolerogenic, as too sudden immune activation is not favourable for the organism, while circulating DC are more easily activated. While investigating the influence of the melanoma microenvironment on local DCs, we showed that the function of DCs residing within, in close proximity to a melanoma, and in melanoma-associated lymph nodes is compromised by the immunosuppressive microenvironment created by the tumour.

We documented the presence of DC within the tumour tissue in 70% of lymph nodes with advanced melanoma metastases. These DCs within the tumour were immature, able to take up antigen and probably motile, as the distribution of DCs in tissue suggested that they actively concentrated in hotspots, possibly associated with the release of chemokines by tumour cells [81] and were often present at the border of the tumour, as previously described in breast cancer [82]. The DC in this situation expressed HLA-DR, therefore were able to present antigen, but did not express the co-stimulatory molecule, CD40, necessary for the correct dialogue with T lymphocytes. This indicates a profound immnosuppression of a lymph node invaded by melanoma. In experiments on DC isolated from lymph nodes with advanced melanoma metastases we further confirmed that intratumoural DC were HLA-DR positive, but did not express CD80 or CD83, and only low amounts of CD86 (Figure 1). After culture for 6 days with immune adjuvants (LPS, GM-CSF, Poly IC, or HSP70) intratumoural DC did not produce significant amounts of IL12p70 (Figure 2), IL15 or IL18, which are immune reaction-activating cytokines. However, melanoma-immune cell co-cultures secreted high concentrations of immunomodulatory IL10, in response to stimulation with Poly (I:C), a potent Th-1 type response stimulant (Figure 2). Strikingly, the secretion of IFN-γ showed reverse correlation with the concentration of Poly (I:C) (Figure 2). This may suggest that instead of stimulating potent T lymphocyte responses after activation with strong pro-inflammatory signals, DCs in an immunosuppressed tumour microenvironment modulate the T lymphocyte activity. In melanoma patients, such a reverse activation of intra-tumoral DCs would result in
local termination of any systemic anti-tumour immune responses which could be elicited after vaccination. This finding was further confirmed in a model culture system of immunosuppressed DC from tonsil, which contains DC that appear to use similar mechanisms to prevent immune responses [83].

Figure 1. Expression of antigen-presenting and co-stimulatory molecules on dendritic cells isolated from melanoma deposits in advanced lymph node metastases.

CD11c+/HLA-DR+ cells enriched from melanoma deposits by centrifugation on density gradient created between 0.7 and 3.5 % of low-density fraction. Examined cells expressed intermediate levels of HLA-DR (b), but no CD83 (c) and CD80 (d) and showed only low expression of CD86. All histograms show the antigen expression for CD11c+/HLA-DR+ cells, i.e. quadrant 2 (upper right a)

In a subsequent study [84], we showed that cutaneous melanoma at all stages of development indeed creates a highly immunosuppressive microenvironment, with regard to both the production of immunosuppressive cytokines and enzymes and to the presence of suppressor T lymphocytes and tolerizing DCs. The expression of all immunosuppressive factors except for TGFβ1 increased with the progression of melanoma, with a peak concentration in positive SLN. Most importantly, we also observed that even the negative SLN were already profoundly immunosuppressed, in comparison with both the primary tumour site, as well as the positive SLN, prior to any signs of melanoma dissemination. This confirms and extends findings from the
UCLA, Valencia and Amsterdam groups [75, 85-87]. We formed the hypothesis that DC tolerated by TGFβ2, produced by primary melanoma cells, may migrate to SLN, where they secrete IDO and TGFβ1, effectively creating an immuno-privileged site and thus pre-conditioning SLN for subsequent melanoma spread.

Figure 2. Expression of cytokines in co-cultures of cells isolated from advanced melanoma metastases to the lymph node.

Mixed cell population obtained by dissociation of lymph nodes with melanoma deposits were put in culture with immuno adjuvants for analysis of cytokine expression. After 6 days of culture cytokine levels were examined by high sensitivity luminescence immuno assay; the concentrations of each cytokine shown on y-axis in pg/ml. The concentration of released cytokines was proportional to concentration of Poly (I:C), a potent Th1-type response stimulant. Concentration of IFN-γ decreased with increased concentration of Poly (I:C) while concentration of IL12 p70 and IL10 increased with concentration of Poly (I:C). Only trace amount of IL12p70 was detected, while IL10 was the most ubiquitous cytokine in melanoma deposit co-cultures.

As the key function of peripheral DCs is discrimination between harmful and safe antigens, we postulate a mechanism for melanoma immune evasion, which includes modulation of DC antigen-presenting and co-stimulatory signals in the skin and SLN. Through secretion of immunosuppressive cytokines, melanoma cells appear able to inhibit DC maturation at these sites, turning the DCs into a tolerogenic subtype.
**Systemic consequences of melanoma-mediated immunosuppression**

The interactions between DC and T lymphocytes are the key event for immune-response induction. Once a dendritic cell presents antigen to a lymphocyte, it is stimulated in return, and the maturation process can be completed. On the contrary, antigen presentation by immature or tolerogenic DC leads to anergy, inactivation or suppression of tumour-sensitive lymphocytes [65, 67, 88, 89]. This may greatly support inhibition of anti-tumour immune responses *in situ*, but upon melanoma migration via lymphatics it would affect the very centre of the immune system, and provide a mechanism effectively similar to clonal deletion of tumour-specific lymphocytes. The local immunosuppressive microenvironment would therefore drastically modify systemic T lymphocyte responses, prevent T lymphocyte activation and proliferation, and trigger the creation of numerous suppressive and regulatory T lymphocyte clones.

This mechanism of tumour-associated immunosuppression is capable of inhibiting the immune response to the tumour both locally, and with tumour progression, systemically. This may explain the difficulty of systemic immunisation of melanoma patients by anti-melanoma vaccines, and the discrepancy between their induction of systemic immunity and poor performance in the clinic.

The first complication arises when activation of local DC is attempted, by means of immuno-adjuvants or TLR dependent molecules. The great part of today’s understanding of the biology of dendritic cells was acquired by studies using monocyte or blood precursor-derived dendritic cells, generated and matured *in vitro*. Peripheral DCs, and tumour-residing DCs in particular, are profoundly immunosuppressed, and react adversely to stimulation with immune activating agents. The discrepancies between these two populations are remarkable and conclusions about the biology of tissue-residing DC should not only be based on studies of laboratory-generated dendritic cells.

Even when the problem of correct antigen presentation is resolved by loading autologous DC with tumour-specific peptides and effective *in vitro* activation, both injected DC and clones of activated tumour-specific T lymphocytes will encounter
profound, general and antigen-specific immunomodulation at the tumour site. Apparently, this local melanoma-associated immunosuppression is so potent, that even the most successful vaccines, able to achieve elimination of autologous tumour cells in vitro, fail in confrontation with this profound local immune suppression [90]. This hypothesis explains, why even when vaccination results in large numbers of circulating effector CTL, melanoma-specific CTL are not found in the tumour and the clinical impact is limited [61] [62], and why efficient anti-tumour CTL even when induced by vaccination have difficulties homing to the tumour and cannot kill it.

**Melanoma vaccines - a lesson from uveal melanoma**

Uveal melanoma is another aggressive malignancy originating from melanocytes. Since this tumour arises in an immuno-privileged site, it is interesting to analyze how this impacts upon uveal melanoma immunogenicity and whether immunotherapy will be feasible.

Thanks to its privileged localisation, uveal melanomas can safely express tumour associated antigens [91, 92], and HLA class I molecules [93], to which the host is not tolerated [94]: hence, it constituents a potent stimulus for anti-melanoma vaccines. Although only a few studies address the possibility of a vaccine for uveal melanoma, the results confirm the strong immuno-stimulatory potential of primary uveal melanoma cells [95], the immunogenicity of dendritic cells pulsed with apoptotic uveal melanoma [96], and the responsiveness of the immune system after vaccination or adjuvant therapy [97, 98]. Even though uveal melanoma resides in an immuno-privileged site until it metastasises, and actively adds to local immune-modulation by the secretion of cytokines such as TGFβ, a study in mice clearly showed that this does not make immunotherapy impossible, and that adoptive transfer of antigen-specific cytotoxic T lymphocytes was able to prevent the growth of an eye tumour in the absence of immunopathological damage [99]. Moreover, performing a study documenting the presence and maturity of dendritic cells in uveal melanoma, we found that, paradoxically, they are more mature than their counterparts in cutaneous melanoma and that uveal melanoma necrosis can be associated with expression of CD83 by local dendritic cells [81, 100]. This suggests, that immuno-privilege can be overcome, and that the right combination of stimuli might well render melanoma susceptible to immunotherapy.
Melanoma vaccines – future possibilities

Although some mechanisms for interaction between a melanoma and the immune system have been discovered, including alteration of dendritic cell function and maturation by tumour-derived cytokines leading to the generation of suppressive and regulatory T lymphocytes, the exact molecular pathways need to be investigated. Investigations of the local and systemic immune suppression within melanoma will allow the dissection of discriminating immune system-related key events in melanoma progression, and generate the necessary therapeutic targets.

There is no doubt that the therapeutic neutralisation of the immunosuppressive microenvironment will be challenging and that it will require greater understanding of the molecular mechanisms used by the tumour to promote immunosuppression. Nevertheless if these problems can be overcome, it seems likely that the efficacy of melanoma vaccines could be greatly enhanced.

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