Melanoma and Immunosuppression: A Systematic Review

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1 Introduction

One of the deadliest forms of skin cancer, melanoma incidence has increased over the past three decades (Gray-Schopfer et al., 2007) with metastatic melanoma carrying a 5 year survival rate of about 5% (Balch et al., 2009; Rosenberg et al., 2011). In the United States, melanoma incidence rates in 2010 were 27.4 and 16.7 per 100,000 for males and females, respectively (Ji et al., 2013). Globally, the incidence of melanoma in Caucasians appears to increase with relative proximity to the equator, (de Vries and Coebergh, 2004; Little and Eide, 2012) with Australia exhibiting the greatest incidence worldwide (Little and Eide, 2012). This review focuses on the mechanisms by which melanoma induces immunosuppression, the behavior of melanoma in the setting of immunosuppression, and possible therapeutic targets in the treatment of malignant melanoma.

2 Melanoma-Induced Immunosuppression: 5 General Mechanisms

There are essentially five mechanisms by which melanoma appear to manipulate the host immune system. Often referenced in the literature as the “melanoma microenvironment,” this malignancy-favorable medium develops due to induction of tolerance, mutations, secretion of immunosuppressive agents, surface antigen down-regulation and altered costimulatory function (McCarter et al., 2007). The ability of melanoma to dampen immune function creates a unique barrier to the development of reliable therapies that target immune modulation.

2.1 Induction of Immune Tolerance

The capacity of melanoma cells to induce immune tolerance and immunosuppression is central to this malignancy’s ability to thrive and progress within its host. Melanoma cells cause immunosuppression via multiple different mechanisms, including increased expression of genes that promote regulatory T cell function, inhibition of cytotoxic T lymphocyte (CTL) activity against malignant cells and altered secretion of cytokines (McCarter et al., 2007). Under normal circumstances, T regulatory cells play a critical role in the suppression of autoimmune reactions by inducing tolerance of self-antigens. Without T regulatory cell activity, autoimmune disease would be rampant. However, in excessive numbers, these cells dampen the antitumor immune response, contributing to the progression of melanoma and other malignancies (Baumgartner et al., 2007). In their analysis of cell populations in healthy patients versus patients with stage I and stage IV melanoma, McCarter et al. (2007) demonstrated that the number of T regulatory cells was greater in the latter two groups, and that the CD4+CD25+ cell frequency was found to be twice as high in metastatic melanoma than in either the healthy subjects or the subjects with stage I melanoma. These findings reinforce the notion that forced immune tolerance is an important mechanism in establishing and maintaining the melanoma microenvironment.

Although clearly more abundant in the setting of melanoma, the precise role of T regulatory cells in promoting a pro-malignant environment is not entirely clear. However, a transcription factor called FOXP3 that is found in greater numbers in the setting of melanoma, is expressed most abundantly by CD4+CD25+ cells (a phenotype of T regulatory cells) (Baumgartner et al., 2007). Multiple studies have shown that greater expression of this transcription factor is correlated with various different malignancies, including melanoma. Not only does this transcription factor favor melanoma progression; it also has implications for the efficacy of melanoma therapy. Melanoma patients exhibiting greater expression of
CD4+CD25+FOXP3+ cells are particularly resistant to treatment with immunologic therapies (Baumgartner et al., 2007; Polak et al., 2007; Viguier et al., 2004).

At this time, the mechanism by which melanoma induces T regulatory cell activity has not yet been explained. It is important to note that the finding of elevated numbers of T regulatory cells and FOXP3 expression is not exclusive to melanoma, or even to malignancy for that matter, as these cell populations are also upregulated in the setting of various inflammatory and disease states (Baumgartner et al., 2007; Levings et al., 2006).

The activity of dendritic cells (DCs), whose roles include the T cell activation and promotion of T lymphocyte tolerance of self-antigens, is also important in the understanding of melanoma. Studies of murine models have shown that samples exhibiting greater numbers of DCs also have greater numbers of T regulatory cells (Choi et al., 2012). Although an underlying relationship between DC populations and melanoma progression is likely, investigators at this point have failed to demonstrate a direct influence of melanoma on DC induction and activity (Baumgartner et al., 2012).

### 2.2 Mutations

An association between malignancy and production of myeloid derived suppressor cells (MDSCs) has also been identified. MDSCs, which stimulate T regulatory cell activity in vivo and antagonize cytotoxic T cell activity in vitro accumulate under the influence of factors produced by the tumor. The action of MDSCs results in depression of innate and adaptive immunity and diminished antitumor immunogenicity (Lindenberg et al., 2013). The FDA-approved BRAF inhibitor, vemurafenib, has recently been shown to exhibit anti-MDSC tendencies, indicating a possible dual mechanism of action for this therapy in the treatment of melanoma in patients with the BRAF V600E mutation (Schilling et al., 2013). Furthermore, in their analysis of human metastatic melanoma samples treated with vemurafenib, Khalili et al. (2012) demonstrated that BRAF inhibition can be used to retransform the melanoma microenvironment via the suppression of IL-1 cytokine expression. The role of BRAF mutations in melanoma and resistance to treatment is further discussed later in this chapter.

### 2.3 Secretion of Immunosuppressive Agents

The signaling factors most implicated in clinical research on melanoma and immunosuppression include IL-10, TGF-β and indoleamine 2,3 dioxygenase. Previous studies have demonstrated that IL-10 has a suppressive effect on both DCs and T lymphocytes (Lindenberg et al., 2013). During their laboratory investigation of Wnt/β-catenin signaling in melanoma cells, Yaguchi et al. found that with increasing expression of IL-10 in melanoma cells, DCs’ activity was suppressed via β-catenin expression by malignant cells. Furthermore, as cutaneous melanoma evolves from a radial to vertical growth phase, IL-10 expression is found to be greater as well (Itakura et al., 2011).

A specific regulator of cytokine signaling, Socs-1 (suppressor of cytokine signaling-1), plays an important role in the inhibition of cytokines IL-10 and TGF-β (Fu et al., 2009; Hong et al., 2009) and has been shown to augment the immunogenicity of DCs. In their investigation of a possible therapeutic target in DC immune function, Song et al. (2012) used murine subjects injected with Socs-1 treated B-16 melanoma cells. The investigators found that Socs-1 silenced samples demonstrated improved antigen presentation by DCs.

An enzyme implicated in melanoma-induced immunosuppression is indoleamine 2,3 dioxygenase (IDO). In a study of CD4+ cells and IDO in patients with melanoma, breast cancer, or renal cell carcinoma, Danish investigators found that melanoma-expressed IDO inhibits antitumor activity by catalyzing
the conversion of tryptophan to toxic metabolites that in turn suppress T cell effector function and promote conversion of naïve T cells to T regulatory cells (Munir et al., 2012). In contrast, the presence of IDO-reactive CD8+ T cells, which target tumor cells and DCs expressing IDO, is associated with stronger anti-tumor activity (Munir et al., 2012). In addition, melanoma cells expressing IDO portend a worse prognosis (Curti et al., 2009; Munn et al., 2004). This improved understanding of IDO-induced immunosuppression may provide a promising therapeutic target in the treatment of melanoma as well as other malignancies.

2.4 Antigen Down-Regulation

Evasion of host immune system surveillance is another important mechanism of melanoma-induced immunosuppression. Melanoma cells are capable of “masking” themselves from the immune system by down-regulating surface major histocompatibility complex class I (MHC I) tumor cell antigens. Integral to the preparation of antigenic peptides for display on these MHC I molecules are the transporter-associated antigen processing (TAP) heterodimers. A decrease in the activity of both TAP1 and TAP2 has been observed in the setting of melanoma (Kirkwood et al., 2008; Zhang et al., 2007). The result is a diminished host immune response to melanoma antigens. In fact, in a comparison of TAP1 and TAP2 expression in malignant melanoma (MM), Tao et al. (2008) found that malignancies with the lowest frequency of TAP1 and TAP2 exhibited more invasive growth, greater Clark level and more tumor infiltrating lymphocytes (TILs).

2.5 Altered Co-Stimulatory Function

A co-inhibitory molecule called B7-H1 (also known as PD-L1), has recently earned appreciation for its role in immunosuppression in the setting of melanoma. Under normal circumstances, the programmed death-1 receptor (PD-1) is activated by B7-H1 when CD4+ and CD8+ cells are chronically stimulated by T lymphocytes. By inhibiting T cell receptor (TCR) production and cytokine secretion, B7-H1 impairs antitumor immune function through induction of T cell anergy and apoptosis (Ahmadzadeh et al., 2009; Dong et al., 2002; Taube et al., 2012). Although the exact mechanism by which melanoma manipulates this process has not yet been elucidated, a definite correlation between this malignancy and B7-H1/PD-1 activity exists. Interestingly, greater quantities of PD-1 have not only been observed in melanoma, but also in cases of heightened viral load in HIV and hepatitis C infections (Taube et al., 2012). Conversely, PD-1 deficient mice have been found to exhibit a greater frequency of autoimmune diseases (Postow et al., 2012). A number of antibodies to PD-1 (Postow et al., 2012) are currently undergoing clinical testing for the treatment of melanoma and are discussed later in this review.

The inhibition of natural killer (NK) cells’ stimulation and cytolytic activity is yet another mechanism through which melanoma cells evade immunosurveillance. These effects are mediated by the malignant cells’ disruption of NK cell ligands such as NKG2D ligand, Fas ligand and APO2 ligand/tumor necrosis factor-related apoptosis-associated ligand. In fact, IDO, the melanoma-expressed enzyme found to antagonize T cell effector function, has also been identified as an important player in this process of NK cell inhibition in melanoma pathogenesis (Martinez-Lorenzo et al., 2004; Pietra et al., 2012).
3 HLA and Melanoma Cell Antigenicity

Supporting the understanding of melanoma as a highly immunogenic malignancy is the research on peptide prediction algorithms and HLA binding by Bredenbeck et al. (2005) and Jarmalavicius et al. (2012). Cancer immunogenicity is in part determined by the interactions of T cells with HLA-bound peptides originating from malignant cells (Jarmalavicius et al., 2012). Although peptide prediction algorithms that determine the ability of peptides to bind HLA class 1 molecules have elucidated some tumor-associated epitopes, they sometimes fall short (Bredenbeck et al., 2005). Bredenbeck et al. sought to identify tumor antigens exhibiting poor HLA-binding capacity that would otherwise have been neglected by currently available peptide prediction algorithms. They suggested that the obstacles to the identification of tumor associated peptides with established algorithms are the assumption that such peptides are universally strong HLA-binders, as well as the focus on individual amino acids rather than on the sequences that they compose (Bredenbeck et al., 2005). Through their work with multiple melanoma cell lines, Bredenbeck
at al. found that, although a number of the identified epitopes were suboptimal HLA-binders, they were equally efficient stimulators of T cells from both healthy donors and from cancer patients (Bredenbeck et al., 2005). The investigators anticipate that these findings will have significant implications for the development of antitumor vaccines (Bredenbeck et al., 2005).

Antigenic heterogeneity and cross-immunogenicity of tumor cells are important concepts for anti-melanoma therapy. By assessing the antigenicity of metastatic tumor cells extracted from 4 different human melanoma cell lines, Jarmalavicius et al. (2012) found that approximately only 10% of the HLA-bound peptides were shared across the strains, indicating a high degree of heterogeneity between the 4 cell lines extracted from four unrelated patients. Furthermore, the unique HLA sequences of each of the lines was found to be immunogenic not only for those peripheral blood mononuclear cells (PBMCs) of the patients from whom the samples originated, but also when introduced to healthy donor PBMCs (Jarmalavicius et al., 2012). Perhaps not surprisingly, 43% of the HLA ligands identified had originated from nuclear proteins, where the processes of DNA replication, gene regulation, cell cycle control and tumor suppression occur (Jarmalavicius et al., 2012). The authors expect that the HLA and origin of their associated proteins may guide future efforts in the modifications of HLA peptide prediction algorithms.

4 Melanoma and Solid Organ Transplantation

There are three different scenarios in which melanoma may emerge after organ transplantation. Subsequent to transplantation, a patient may develop melanoma as a consequence of immunosuppressive agents for the prevention of organ rejection, (Penn, 2000) may acquire malignant cells directly via donor tissue, or may experience recurrence of formerly dormant melanoma (Dreno, 2003).

4.1 Post-Transplantation De Novo Melanoma

Post-transplantation skin cancer is a very common phenomenon and follows a more aggressive course than in non-transplant patients (Kovach and Stasko, 2009). Compared to melanoma, the development of non-melanoma skin cancer (NMSC) in organ transplant recipients (OTRs) is difficult to refute, given the abundance of evidence in support of this relationship (Fekes et al., 2010; Lindelöf et al., 2000; Stockfleth et al., 2002; Wisgerhof et al., 2010; Zavos et al., 2011). The task of establishing a similar connection between organ transplantation and de novo melanoma has proven more challenging. From their analysis of a population-based cohort of 5,356 OTRs, Lindelöf et al. (2000) concluded that, while there was a greatly increased risk of NMSC in this population, there was no significant evidence of increased risk of melanoma. In contrast, a review of Medicare billing claims for de novo cancer in post-renal transplant recipients (RTRs) demonstrated that the incidence of melanoma in RTRs was approximately 5-fold higher than in the general population. However, the authors concede that given the use of Medicare claims in gathering information, a selection bias was inherently present (Kasiske et al., 2004). A more recent analysis supports the assertion that melanoma does indeed develop more frequently in OTRs: Chatrath and associates determined a standardized incidence ratio (SIR) of 5.8 for melanoma post-liver transplant (95% CI: 4.7 to 7.0) (Chatrath et al., 2013).

4.2 Melanoma Transmitted from Donor Organs

The transmission rate of melanoma from graft to recipient is very low, particularly compared to the proportion of patients on immunosuppressive therapy who develop de novo skin cancer (Buell et al., 2004).
Still, evidence for this phenomenon is available, mostly in individual case studies where PCR has been employed to prove donor origin of malignancy (Bilal et al., 2013; Chen et al., 2012). Further complicating the picture is the fact that melanoma can remain dormant for decades, possibly only being identified after death of the donor. In fact, postmortem investigation of cause of death of organ donors who were later determined to have transmitted melanoma, reveals that several had suffered cerebral hemorrhages, and that these were retrospectively attributed to occult melanoma metastases (Zwald et al., 2010). In practice, it is prudent to carefully consider each patient’s case independently, as the risk of not receiving a life-saving organ transplant may outweigh the future risk of donor-derived skin cancer.

### 4.3 Melanoma Recurrence Post-Transplantation

Data on the recurrence of melanoma post-transplantation is limited, with most research yielding little evidence for increased risk of melanoma recurrence in the OTR population. In a review of the Cincinnati Transplant Tumor Registry (CTTR) from 1968 to 1995, Penn observed that 19% of the 31 patients who had melanoma before receiving donor organs developed recurrences after transplantation (Penn, 1996 (Penn, 1996) as cited in Colegio et al. (2009). Of note, all 6 of these patients were RTRs. However, Breslow depth was not disclosed for any of these 6 patients, making interpretation of these findings difficult. Certainly, not all melanoma is created equal, as Breslow depth and time elapsed from original melanoma diagnosis to transplantation appear to be critical criteria in the evaluation of melanoma recurrence risk in the OTR population (Colegio et al., 2009). Melanoma staging factors also considered for OTRs are the presence of ulceration, lactate dehydrogenase level and mitotic rate (Balch et al., 2009). Colegio et al. (2009) also address the utility of sentinel lymph node biopsy to determine relative risk of melanoma recurrence after transplant. A sentinel lymph node biopsy negative for micrometastases coupled with a period of at least 2 years between melanoma diagnosis and transplant portends a better prognosis. The authors conclude that the risk of recurrence as estimated by these criteria ought to be carefully weighed against the implications of withholding transplantation.

### 4.4 Prognosis of Malignant Melanoma in the Setting of Organ Transplantation

Multiple studies have demonstrated a more aggressive course for malignant melanoma in OTRs than is expected for the general population. In the same analysis of the CTTR, Penn observed more rapid tumor growth and development of metastases due to MM in OTRs, with median survival of 10.5 months after surgery (Penn, 1996). In their comparison of 95 patients from SCOPE (Skin Care in Organ Transplant Patients, Europe) versus age, sex, tumor thickness and ulceration-status matched controls (Matin et al., 2008) from AJCC (American Joint Committee on Cancer), Matin et al. (2008) demonstrated worse prognosis in the OTR group at melanoma stages T3 and T4, but no significant reduction in survival rates for T1 and T2. A more recent Mayo Clinic retrospective review of 638 patients with melanoma diagnosed post-transplant supports this trend: compared to expected survival according to the SEER (Surveillance, Epidemiology and End Results) Program, melanoma cause specific survival was significantly lower among immunosuppressed OTRs for lesions with Breslow thickness of 1.51 to 3.00 mm and Clark level III or IV. A similar trend was not observed in patients with more shallow Breslow measurements (≤ 1.50 mm), nor in Clark levels I and II, indicating that MM exhibits especially aggressive behavior in more advanced lesions in the setting of organ transplantation. However, overall survival rates were lower for the OTR group, regardless of lesion thickness (Brewer et al., 2011).
5 Melanoma in Lymphoproliferative Disease

Similar to the iatrogenic immunosuppression for OTRs, the impediment of immune function inherent in lymphoproliferative diseases, combined with iatrogenic immunomodulation, increases patients’ susceptibility to malignancies, including skin cancer. The most commonly addressed diseases in the literature are non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukemia (CLL), a particular form of NHL. Several studies have highlighted this potential link between melanoma and non-cutaneous malignancies like lymphoma. A recent review of a melanoma registry in Rome revealed that 14.8% of patients with melanoma developed lymphoma, the majority of which (72.7%) were non-Hodgkin lymphoma (NHL) (Bottoni et al., 2013).

5.1 Lymphoma and Melanoma: Molecular and Genetic Associations

Characterized by a monoclonal proliferation of B cells, CLL is associated with a greater risk of melanoma according to multiple studies. This augmented risk is attributed to a number of different mechanisms through which CLL causes immunosuppression. Through the down-regulation of CD154 (a CD40 ligand on T cells), malignant B cells interfere with T cell-APC interactions (Aslakson et al., 1999, as cited in Brewer et al.) (Aslakson et al., 1999; Brewer et al., 2011; Cantwell et al., 1997). Expressed on B cells and other potential APCs, the purpose of CD40 is to engage CD154 to induce costimulatory interactions between cells. Inhibition of CD154 expression by the B cells of CLL results in a failure to solicit antigen presentation by the malignant cells (Wierda et al., 2000). As a risk factor for the development of melanoma, immune dysfunction resulting from lymphoproliferative disease results from suboptimal antigen presentation by monoclonal B cells, disruption of MHC I and II expression, altered expression of TCR variable genes and lower levels of immunoglobulins and complement (Kipps, 2000; Wierda et al., 2000).

Childhood survivors of cancer are at increased risk of developing melanoma in adulthood. According to the Childhood Cancer Survivor Study (CCSS) composed of 14,358 patients, a 2.5-fold risk of melanoma was seen in patients diagnosed in childhood with various cancers, including soft tissue and bone sarcoma, leukemia and lymphoma (subsequent invasive melanoma SIR: 2.42, 95% CI 1.77-3.23; AER: 0.10, 95% CI 0.05-0.15) (Meadows et al., 2009; Pappo et al., 2013).

Overexpression of antiapoptotic proteins such as BCL-2, a proto-oncogene, inappropriately permits survival of cells that normally should be eliminated through programmed cell death. Consequently, overexpression of BCL-2 by malignant cells provides a survival advantage that is particularly apparent in melanoma and lymphoproliferative diseases (Anvekar et al., 2011). In fact, 95% of CLL and 90% of melanoma exhibit BCL-2 overexpression (Mikhail et al., 2005; Thomadaki and Scorilas, 2006). Also often disrupted in CLL is the chromosomal domain of TP53, the “guardian of the genome.” In CLL, deletions of 17p, within which TP53 is located, are associated with a worse prognosis and greater resistance to treatment. Although TP53 is rarely mutated in melanoma, disruption of apoptotic pathways is often present. Researchers have recently identified a TP53 relative, TP63, that is often mutated in melanoma, contributing to apoptotic evasion by malignant cells (Matin et al., 2013). Interestingly, p63 deficiency in mice results in immature, non-stratified skin without expression of differentiation markers as well as severe developmental anomalies of the limbs (Mills et al., 1999).

Similar to the correlation between melanoma and lymphoproliferative diseases, breast cancer and melanoma appear to be related in the context of certain genetic predispositions. Mutations in CDKN2A are known to contribute to the development of melanoma, (Goldstein et al., 2006; Hansson, 2010) while BRCA2 mutations are correlated with female breast cancer (Nasir et al., 2009). Evidence suggests that a
mutual relationship exists: patients with CDKN2A mutations are predisposed to developing breast cancer and those with BRCA2 mutations are at greater than average risk of developing melanoma (Goggins et al., 2004). In a review of 1,884 patients from the SEER database, investigators found a statistically significant (p = 0.0002) elevated risk of cutaneous melanoma among survivors of female breast cancer treated with surgery and irradiation, and that survivors of melanoma were similarly more susceptible to developing breast cancer. A history of radiation for breast cancer likely also contributed to the elevated risk of melanoma in this population, although the melanomas did not necessarily develop in the previously irradiated field (Galper et al., 2002). Notwithstanding, these findings raise the question of a relationship between the pathogenesis of melanoma and breast cancer (Goggins et al., 2004).

5.2 Lymphoma and Melanoma Epidemiology

First noted in 1973, the connection between lymphoma and melanoma has been a topic of considerable interest over the past three decades and yet, a distinct explanation for this correlation has not been described. However, it is clear that with melanoma, there is an increased risk of lymphoma, and vice versa. Studies demonstrate a greater melanoma risk in NHL patients ranging from 1.8 to 2.4 fold, (Adami et al., 1995; Brennan et al., 2005; Dong and Hemminki, 2001; Goggins et al., 2001; Hisada et al., 2001; McKenna et al., 2003; Travis et al., 1991; Travis et al., 1992; Tsimberidou et al., 2009) while others show an increased risk of NHL in melanoma patients ranging from 1.3 to 2.7,(Adami et al., 1995; Brennan et al., 2005; Crocetti and Carli, 2004; Goggins et al., 2001; McKenna et al., 2003; Riou et al., 1995; Spanogle et al., 2010) suggesting a mutual relationship between these two malignancies. In their analysis of 109,000 cases of NHL, Brennan et al. determined a SIR of 1.92 for melanoma as a second primary cancer developing within 10 years of the first primary NHL (Brennan et al., 2005). In a retrospective review (1995 to 2009) of 52 patients with both CLL and melanoma who were treated at the Mofitt Cancer Center, subjects with melanoma were at greater risk of CLL compared to other malignancies. In the context of melanoma, CLL was 10-fold more common than colorectal cancer, 8-fold more common than prostate cancer and 4-fold more common than breast cancer (Farma et al., 2013). A review of patients treated for CLL at MD Anderson also supports this correlation, demonstrating an 8% incidence of melanoma in this CLL population, with a ratio of observed to expected cases of 6.17 (Farra et al., 2013; Tsimberidou et al., 2009).

5.3 Clinical Course of Melanoma in Lymphoma Patients

Most research on the survival rates of patients with both melanoma and lymphoproliferative diseases are based on small sample populations. However, a recent relatively large SEER population-based study of 212,245 patients conducted by Brewer et al. demonstrated that the overall survival of patients with MM and a preceding history of either CLL or NHL was in fact worse than expected, as evidenced by standardized mortality ratios (SMR for CLL, 2.6; 95% CI 2.3 to 3.0; SMR for NHL 2.3; 95% CI, 2.1 to 2.6). Malignant melanoma cause-specific survival was also worse than expected for both CLL (SMR, 2.8; 95% CI, 2.2 to 3.4) and NHL (SMR, 1.9; 95% CI, 1.3 to 2.8) (Brewer et al., 2012). This supports the earlier finding by Brewer et al. that patients with a diagnosis of CLL before the development of melanoma had worse overall survival (60.9%) compared to those whose melanoma preceded their CLL (96.2%) (Brewer et al., 2010).
6 Melanoma and Iatrogenic Immunosuppression

The very medications used in the treatment of malignancy and prevention of organ rejection may have pro-malignant effects via their suppression of the immune system. The offenders most referenced in the literature include: cyclosporine A (CsA), tacrolimus, azathioprine, corticosteroids, methotrexate, mycophenolate mofetil, 5-fluorouracil, the biologic response modifiers (“biologics”) and various chemotherapies (Kubica and Brewer, 2012). For example, cyclophosphamide has been employed as a tumor-suppressing agent because of its ability to reduce T regulatory cell numbers. However, recent evidence suggests that this medication also promotes the production of MDSCs, which in turn stimulate T regulatory cell production and suppress CTL activity (Becker and Schrama, 2013; Lindenberg et al., 2013; Sevko et al., 2013).

The use of azathioprine for immune system attenuation in OTRs has been correlated with the development of dysplastic keratosis, BCC and SCC. However, these findings appear to be attributable to mechanisms independent of the immunosuppressive action of this medication. Conjecture for this phenomenon addresses the UV-sensitizing effects of azathioprine’s imidazole degradation product and the carcinogenic behavior of its active metabolite, 6-thioguanine (Hemmens & Moore 1986, Taylor & Shuster 1992, Lennard et al., 1985, as cited in Penn, 1996 (Penn, 1996)). A similar relationship between the use of azathioprine and melanoma is uncertain (Penn, 1996).

As of yet, there lacks a clear consensus as to whether or not immunosuppressive modalities as a class permit malignant changes. A portion of the research on medications used in OTRs has suggested that the specific immunosuppressive agent is less important as a contributor to tumorigenesis than is the overall effect of immunosuppression (Baron and Krol, 2005; Bouwes Bavinck et al., 1996). The challenge of accurately attributing pro-oncogenic effects of immunosuppressive therapies is significant considering the many other risk factors, comorbidities and polypharmacy in the OTR population. Of note, these studies addressed malignancy in general and were not specific to melanoma (Kubica and Brewer, 2012).

Conversely, numerous other studies have shown that specific classes of immunosuppressive agents are particularly tumorigenic. The class of calcineurin inhibitors, which includes cyclosporine A, exhibits pro-oncogenic properties through increased production of Bcl-2, fibronectin-guided migration of metastatic melanoma cells, (Juhász et al., 2009) disruption of DNA repair, (Dapprich et al., 2008; Kubica and Brewer, 2012) and promotion of angiogenesis via a TGF-β dependent mechanism (Koehl et al., 2004). For these reasons, the mTOR (mammalian target of rapamycin) inhibitors such as sirolimus and everolimus may be substituted for the calcineurin inhibitor class, as the latter family has even been associated with lower risk of malignancy (Kauffman et al., 2005). In fact, rapamycin (sirolimus) has been shown to oppose the oncogenic mechanisms of UV radiation, including the UV-induced down-regulation of Akt1, a protein kinase with tumor suppressing function. Sully et al. describe the finding of selective up-regulation of Akt1 after treatment with rapamycin in the setting of non-melanoma skin cancers such as squamous cell carcinoma (Sully et al., 2013). This observation is particularly intriguing, as it highlights a medication that appears to reverse a detrimental effect of UV radiation on the epidermis (Sully et al., 2013).

As the application of TNF-α inhibitors increases, and as the indications for these therapies expand, the concern over heightened risk of infection and malignancy similarly grows. The relationship between TNF-α and cancer is quite complicated because this cytokine has both pro- and anti-malignant properties (Balkwill, 2009). Analysis of melanoma’s association with TNF-α inhibitor therapy is limited, especially
compared to the data available on the biologics’ association with other malignancies including lymphoma. A review of 71 clinical trials to discuss the long-term safety of adalimumab (a TNF-α inhibitor) revealed an increased risk of melanoma in those treated for psoriasis (SIR 4.37, 95% CI, 1.89 to 8.61), but no greater incidence of the malignancy in the rheumatoid arthritis (RA) population (SIR 1.5, 95% CI, 0.84 to 2.47) (Burmester et al., 2013). Factors associated with psoriasis itself are potential contributors to this trend. For example, this patient population is more likely to have a history of psoralin-UV-A (PUVA) treatment than the RA population (Paul et al., 2003). Data for the relationship between TNF-α inhibitor treatment for RA and NMSC or melanoma risk is mixed, with the latter being more ambiguous (Askling et al., 2009; Chakravarty et al., 2005; Leombruno et al., 2009; Raaschou et al., 2013). With regard to outcomes in RA patients treated with TNF-α inhibitors, prognosis after melanoma diagnosis does not appear to be worse than compared to the TNF-α inhibitor-naïve population (Raaschou et al., 2011).

7 Melanoma and Autoimmunity

Because melanoma is a highly immunogenic cancer, vigorous immune surveillance and execution are critical to suppressing this malignancy. To that end, one might imagine that diseases in which immune activity is especially robust may provide protection against malignant melanoma. In fact, the epitome of super-vigilant immune function, autoimmunity, has been associated with better prognosis for melanoma (Satzger et al., 2007). In a randomized trial of 200 melanoma patients treated with high dose interferon-α, Gogas et al. compared the relapse free survival and overall survival in those who developed signs of autoimmunity compared to those who did not (Gogas et al., 2006). While relapse occurred in 73% (108 of 148) of patients without evidence of autoimmunity, only 13% (7 of 52) of patients with either autoantibodies or clinical signs of autoimmune disease experienced relapse of melanoma (Gogas et al., 2006). Supported by their univariate analysis of relapse-free survival demonstrating a positive relationship between autoimmunity and lack of melanoma recurrence (p<0.001), the investigators concluded that autoimmunity itself was a prognostic marker for greater relapse-free survival and overall survival (Gogas et al., 2006). A subsequent study by Satzger et al. provided the same conclusions for melanoma patients treated with low dose interferon alpha (Satzger et al., 2007).

8 Melanoma in Patients with HIV Infection and AIDS

The introduction of highly active antiretroviral therapy (HAART) in the 1990s represented a major turning point in the treatment of HIV infection and AIDS. Typically comprised of 3 medications with independent mechanisms of action, HAART has significantly decreased the number of AIDS-defining illnesses (Kubica and Brewer, 2012). As a result of its effect on the natural course of HIV/AIDS, HAART has allowed for greater survival with this chronic infection. However, with increased longevity emerged increased incidence of associated maladies such as lipodystrophy, osteopenia, metabolic syndrome, and non-AIDS defining cancers (NADCs) (Patel et al., 2008). Melanoma is among these various malignancies.

Patients with HIV infection are at 1.5 to 2-fold increased risk of developing malignancies compared to the general population (Burgi et al., 2005; Patel et al., 2008; Wilkins et al., 2006). In their 1996 to 2008 comparison of demographically matched HIV infected versus HIV non-infected patients from
California, Silverberg and coworkers determined a relative risk of 1.8 (95% CI, 1.3 to 2.6) for melanoma in the HIV+ population (p = 0.001) (Silverberg et al., 2011). Although they also correlated the increased melanoma incidence with CD4 count < 500 cells/µL, this finding was not statistically significant (p = 0.092) (Silverberg et al., 2011).

In an earlier study, melanoma was associated with a standardized rate ratio of 2.6 (95% CI, 1.9 to 3.6) in HIV+ patients (p value not provided) (Patel et al., 2008). In their prospective cohort analysis of 54,780 HIV+ patients from 1992 to 2003, Patel et al. (2008) found that melanoma was more prevalent in HIV+ patients and that the incidence of melanoma increased over time. In contrast, melanoma incidence remained unchanged for the same time periods in the general population. Although an attribute of this study was its large patient population, the inability to account for numerous confounding factors, including tobacco use, alcohol abuse, and infection with oncogenic viruses (Silverberg et al., 2011) presents a challenge in assessing the contribution of HIV-induced immunosuppression to the development of melanoma in this patient population (Kubica and Brewer, 2012; Patel et al., 2008).

### 8.1 Clinical Course in Patients with Melanoma and HIV Infection/AIDS

Considering the role of the immune system in suppressing malignant transformation, it stands to reason that the probability of developing cancer in the immunosuppressed state of HIV/AIDS is greater than that of the general population. One small study demonstrated that HIV+ patients’ disease-free and overall survival were lower than for patients in an HIV-negative control population (median OS: 2.8 years for HIV+, 6.4 years for HIV-; p = 0.045). This study illustrated that HIV+ patients experienced a more aggressive course of melanoma, highlighting the need for closer cancer surveillance in this patient population. Although not statistically significant, the same study also indicated shorter survival time for HIV+ melanoma patients as CD4 count declined (Rodrigues et al., 2002).

In the previously referenced study, Silverberg et al. (2011) also noticed a possible trend in the development of melanoma in HIV+ patients as CD4 counts declined. Once again, this relationship between HIV-induced immunosuppression and the appearance of melanoma was not statistically significant. Beyond these two studies, others have failed to illustrate a similar relationship between CD4 count and melanoma. With regard to the evolution of melanoma, the role of HIV-induced immunosuppression as well as the level of immune system compromise (indicated by CD4 count and HIV RNA viral load) is still unclear.

### 8.2 Immune Dysregulation in HIV Infection

Increased risk of melanoma in patients with HIV/AIDS results from the distortion of relative cytokine ratios, causing an imbalance of immune cell populations. Reuter et al.’s analysis of two HIV+ patient populations, the “elite controllers (EC)” and the “chronic progressors (CP),” has provided valuable insight into HIV-induced immune dysfunction. The elevated levels of IL-4 found in HIV infection lead to a shift of CD4 specialization away from Th1 and toward Th2 phenotype, resulting in greater levels of cytokines associated with the latter, including IL-4, IL-5, IL-6, IL-10 and IL-13 (Reuter et al., 2012; Wang et al., 1994). In turn, IL-4 induces B cells to produce immunoglobulins and provides positive feedback for further Th2 expansion. Conversely, IL-4 and IL-10 inhibit Th1 production (Reuter et al., 2012). This trend is consistent with the earlier findings by Clerici and Shearer, who recognized an HIV-associated Th1 to Th2 shift as levels of IL-4 and IL-10 rose and that HIV infection progressed to AIDS as IL-2 and IFN-γ declined. Effective immune control over HIV infection was correlated with more robust Th1 re-
responses to HIV antigens, further supporting the understanding that Th1 responses are critical in preventing HIV progression to AIDS (Clerici and Shearer, 1993).

9 Surgical Management of Melanoma

Although extensive research efforts have focused on pharmacologic strategies for malignant melanoma, wide surgical resection of the primary tumor remains the standard of care, with medical management serving an adjuvant role in MM (Dzwierzynski, 2013). Surgical excision that achieves deep margins down to the fascia and a specified safety margin is currently the only intervention that may offer a cure in malignant melanoma (Sladden et al., 2009). The factors considered during surgical planning of melanoma excision include anatomic location and size of the primary tumor, Breslow depth and histologic features. The National Comprehensive Cancer Network (NCCN) has established guidelines for adequate resection margins according to the size of the primary lesion (Wasif et al., 2013). For example, the recommended margin for lesions ≤ 1.0 mm is 1.0 cm, while a clinical margin of 1-2 cm is recommended for lesions measuring 1.01 to 2 mm (Wasif et al., 2013).

![Figure 2: The Th1 to Th2 Shift in HIV Infection](image)

**Figure 2: The Th1 to Th2 Shift in HIV Infection.** Immune dysregulation in HIV infection. HIV-induced immune dysfunction is characterized by elevated IL-4, which preferentially stimulates helper T cells to differentiate into Th2 cells in greater ratios than into Th1 cells. Greater numbers of Th2 cells results in elevations of cytokines such as IL-4 and IL-10, which are responsible for further inhibiting the production of Th1 cells. This dampening of the Th1 response contributes to progression of HIV to AIDS (Clerici and Shearer, 1993; Reuter et al., 2012; Wang et al., 1994).

Interestingly, in their assessment of nation-wide observance of the guidelines for resection margins and sentinel lymph node biopsies, Wasif et al. show that there is significant room for improvement in the surgical care of melanoma patients in terms of compliance with these established protocols. In their review of 60,194 patients with MM from the SEER database from 2004 to 2008, Wasif et al. demonstrated...
that 66.2% of patients with T1 stage melanoma received clinical margins measuring < 1 cm (CI: 1.36 to 1.63, p < 0.001). They also found that only 53% of patients eligible for sentinel lymph node biopsy (stages Ib and II melanoma) received this intervention. However, interpretation of this latter finding is difficult due to the possibility that some sentinel lymph node biopsy candidates may have declined the procedure (Wasif et al., 2013).

The application of Mohs micrographic surgery to the treatment of melanoma remains a controversial subject. While Zitelli et al. argue that a benefit of Mohs surgery compared to traditional excision for melanoma is the ability to examine complete margins rather than simply representative sections, (Zitelli et al., 1997) artifact resulting from frozen specimen preparation may prevent accurate assessment for malignant cells (Dzwierzynski, 2013). Although Mohs for MM may be employed in investigational endeavors, there is not enough evidence to support its use for the general population at this time (Dzwierzynski, 2013). Dzwierzynski offers a detailed discussion of the surgical management of melanoma, addressing functionally and aesthetically sensitive anatomical concerns (Dzwierzynski, 2013).

10 Medical Management of Melanoma

<table>
<thead>
<tr>
<th>Category</th>
<th>Specific therapy</th>
<th>Actions</th>
</tr>
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<tbody>
<tr>
<td>Kinase inhibitors (Mackiewicz and Mackiewicz, 2012; Ngiow et al., 2013)</td>
<td>BRAF inhibitors: Vemurafenib Dabrafenib MEK 1/2 inhibitor: Trametinib</td>
<td>Mutations in components of the MAPK pathway, such as BRAF and MEK, favor melanoma cell expansion and survival (Mackiewicz and Mackiewicz, 2012; Ngiow et al., 2013) BRAF inhibitors target the most common location for BRAF mutations (BRAF&lt;sup&gt;V600E&lt;/sup&gt;) (Ngiow et al., 2013)</td>
</tr>
<tr>
<td>Immunotherapy (Den Otter et al., 2008; Rosenberg et al., 1985)</td>
<td>IL-2</td>
<td>Promotes proliferation and antitumor activity of CTLs Most promising for fast growing, highly vascularized tumors with pre-existing peritumoral leukocytic infiltrates (Den Otter et al., 2008; Rosenberg et al., 1985)</td>
</tr>
<tr>
<td>CTLA-4 inhibitors (Contardi et al., 2005; Ribas, 2007)</td>
<td>Ipilimumab Tremelimumab</td>
<td>CTLA-4 is a T cell regulator constitutively expressed on T regulatory cells (Ribas, 2007) and tumor cells (Contardi et al., 2005) CTLA-4 suppresses immune function by inhibiting production of IL-2 and IFN-γ and by inducing DC expression of IDO (Ribas, 2007)</td>
</tr>
<tr>
<td>Dendritic cell vaccines (Bocchia et al., 2000; Nestle et al., 1998; Schreibelt et al., 2013)</td>
<td>Nil</td>
<td>Mature monocyte-derived DCs loaded with laboratory-produced peptides → bind MHC class I molecules → tumor specific CTL responses (Schreibelt et al., 2013) May be especially useful in patients whose specific TAAs are unknown (Bocchia et al., 2000; Nestle et al., 1998)</td>
</tr>
<tr>
<td>ACT (Turcotte et al., 2013; Wu et al., 2012)</td>
<td>Nil</td>
<td>Antigen-specific T cells (usually TILs expanded by IL-2 ex vivo) are infused into the patient to augment the immune response to tumor cells (Turcotte et al., 2013; Wu et al., 2012)</td>
</tr>
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</table>

Table 1: Current medical therapies for melanoma.
10.1 Chemotherapy

There are two first line chemotherapy agents for metastatic melanoma: temozolomide (TMZ) and dacarbazine (DTIC). Both of these alkylating agents are converted to the active form of 5-(3-methyltriazen-1-yl)imidazole-4-carboximide (MTIC) and therefore have the same ultimate mechanism of action: DNA methylation resulting in double stranded breaks (Middleton et al., 2000). A significant difference between these two agents is TMZ’s capacity to penetrate the blood brain barrier, making it a useful therapy for CNS malignances such as astrocytoma, glioblastoma and brain metastases from MM. Unfortunately, outcomes of these individual chemotherapies in the treatment of MM have been disappointing with objective response rates of < 20% for DTIC (Quirt et al., 2007) and 21% for TMZ (Middleton et al., 2000). In reaction to these findings, subsequent studies have sought to investigate the impact of one chemotherapy agent in conjunction with immunotherapy. A 2007 analysis of 526 patients that examined chemoimmunotherapy (TMZ plus IFN-α) versus TMZ alone in metastatic MM showed no significant difference in survival time between the two populations (Larkin and Gore, 2008; Sasse et al., 2007). However, a smaller study of 73 patients conducted at the University of Pretoria, South Africa, demonstrated that DTIC combined with IFN-α compared to DTIC alone had a significantly greater overall response (20% for DTIC alone, 95% CI: 7 to 39; 50% for combination, 95% CI: 26 to 72; p = 0.007) (Falkson, 1995). Most studies currently in progress are investigating the combination of the main chemotherapy agents with other therapies in an effort to sensitize malignant cells to the chemotherapy regimens.

10.2 Kinase Inhibitors

The mitogen-activated protein kinase pathway (RAS/RAF/MAPK/ERK) favors melanoma cell expansion and survival. Mutations in one of this pathway’s components, the oncogenic serine-threonine protein kinase BRAF, are present in approximately 50% of human melanomas. BRAF mutations result in heightened tumor cell expression of IL-1α and IL-1β, ultimately augmenting PD-L1 expression and suppressing T cell antitumor activity (Khalili et al., 2012). Hence, therapies have been introduced to target the most common location for these mutations, occurring at V600 (BRAFV600E). These therapies are vemurafenib and dabrafenib, the BRAF inhibitors (Ngiow et al., 2013). Overall survival as demonstrated by the BRIM3 study (a phase III clinical trial) was greater with vemurafenib than with DTIC, with a median OS of 13.6 months versus 9.7 months (95% CI 0.57 to 0.87; p < 0.001). Similar findings were seen for progression free survival (PFS) in the BREAK-3 study comparing efficacies of dabrafenib and DTIC, although overall survival data is not yet available (Mackiewicz and Mackiewicz, 2012). Unfortunately, as with other anti-melanoma agents, resistance to these therapies has been observed, prompting consideration of other potential targets along the MAPK pathway, such as MEK. The METRIC study, a phase III trial comparing MEK1/2 inhibitor trametinib with paclitaxel/DTIC showed greater PFS in the trametinib arm (4.8 months) compared to the chemotherapy group (1.4 months) (95% CI: 0.31 to 0.64; p < 0.0001) (Mackiewicz and Mackiewicz, 2012). Investigators suspect that combination therapy to target multiple steps of this pathway simultaneously may help to circumvent melanoma’s resistance to individual therapies (Ngiow et al., 2013).

In their assessment of patients with BRAFV600E positive metastatic melanoma treated with either a BRAF inhibitor alone or with a combination of a BRAF inhibitor and a MEK inhibitor, Frederick et al. found that BRAF inhibition in general caused lower levels of the inhibitory cytokines IL-6 and IL-8 and higher levels of perforin and granzyme, markers of T cell cytotoxic activity (Frederick et al., 2013). Regarding the two comparison groups, melanoma treated solely with BRAF inhibitors yielded lower tumor
antigen expression and suppressed levels of CD8 cells, coinciding with the progression of melanoma. Interestingly, when one patient from this group was secondarily treated with the BRAF/MEK inhibitor combination therapy, both melanoma antigen expression and CD8 levels were reestablished (Frederick et al., 2013).

10.3 Immunotherapy

Interleukin-2 (IL-2), a cytokine responsible for the proliferation and antitumor activity of CTLs, has been the subject of extensive research for years. In 1985, after promising results with IL-2 administration in murine models, Rosenberg et al. demonstrated objective cancer regression in 11 of 25 patients with various types of malignancies. In fact, one of these patients experienced complete regression of metastatic melanoma (Rosenberg et al., 1985). However, severe toxicity associated with systemic administration of recombinant IL-2 has limited its application in the treatment of human cancer. Den Otter et al. (2008) offer an extensive review of studies investigating local administration of IL-2 to avoid adverse events associated with systemic administration, such as vascular leak phenomenon. Although a comprehensive explanation of their findings is not included here, their work suggests that local IL-2 may be most appropriate for fast growing, highly vascularized tumors with pre-existing peritumoral leukocytic infiltrates.

10.4 The CTLA-4 Inhibitors

A therapeutic target of interest is the cytotoxic T lymphocyte-associated protein 4 (CTLA-4, also known as CD152), a key T cell regulator that is constitutively expressed on T regulatory cells (Ribas, 2007) and tumor cells. (Contardi et al., 2005). The functional analog of CTLA-4, CD28, competes for the same ligands (B7-1, B7-2), but serves the opposing role of providing the secondary activation signal for T cells (Wolchok and Saenger, 2008). Whereas CTLA-4 induces tolerance, CD28 augments antitumor immunity (Wolchok and Saenger, 2008). Upon receiving a costimulatory signal, CTLA-4 inhibits the production of IL-2 and IFN-γ and induces DC expression of IDO, making this negative regulator integral to the prevention of autoimmunity and lymphoproliferative disease as has been demonstrated in knock-out murine models (Ribas, 2007). In turn, inhibition of CTLA-4 has attracted much research interest, leading to the introduction of the CTLA-4 inhibitors, which are fully human monoclonal antibodies (Hodi et al., 2010).

In a clinical trial that ultimately led to the 2011 FDA approval of ipilimumab (a CTLA-4 inhibitor) for metastatic melanoma, greater overall survival was demonstrated with ipilimumab when compared to glycoprotein 100 (gp100), a melanosomal protein vaccine with immunogenic properties but questionable antitumor capabilities. All of the 676 subjects had unresectable stage III or IV melanoma and each was randomly assigned to one of 3 treatment arms: ipilimumab alone or in combination with a gp100 vaccine and the gp100 vaccine alone. Overall survival rates for the 3 groups were: 10.1 months, 10.0 months and 6.4 months respectively. The difference in OS between the ipilimumab treatment arm alone and the combination arm was not statistically significant (p = 0.76) (Hodi et al., 2010). A phase III trial for CTLA-4 inhibitor tremelimumab failed to demonstrate statistically significant increased overall survival (OS) compared to the first line chemotherapy treatments TMZ and DTIC, with median OS for tremelimumab at 12.6 months (95% CI, 10.8 to 14.3) and for chemotherapy at 10.7 months (95% CI, 9.36 to 11.96) (Ribas et al., 2013).

Small clinical trials involving the CTLA-4 inhibitors, ipilimumab and tremelimumab, with or without chemotherapy have shown mixed results. In a multicenter phase II trial of ipilimumab combined with DTIC versus ipilimumab alone, better response rates were observed in the combination arm (17% versus 5%), although also accompanied by greater toxicity (28% versus 18%) (Ribas, 2007). With over-
lapping confidence intervals, however, the significance of these findings is questionable. Further investigation in the form of a phase III trial of DTIC plus ipilimumab versus DTIC alone is currently ongoing (Fischkoff et al., as cited in Ribas 2007).

As with numerous other therapeutic strategies for melanoma, resistance to the CTLA-4 inhibitors has been observed. One proposed offender in the resistance to anti-CTLA-4 therapy is host-derived IDO (Holmggaard et al., 2013). Through the use of murine subjects, Holmggaard et al. (2013) sought to determine the inhibitory influence of tumor-derived and/or host-derived IDO on antitumor immune function in the setting of CTLA-4 inhibitor therapy. They found that IDO deficiency was associated with greater T cell infiltration of tumor after anti-CTLA-4 therapy, and that combination therapy of an IDO inhibitor called 1MT (1-methyl-tryptophan) with anti-CTLA-4 yielded greater ratios of T effector to T regulatory cells in the vicinity of the tumor.

Other investigators have examined the implications of certain CTLA-4 polymorphisms for treatment response to anti-CTLA-4 antibodies such as ipilimumab (Queirolo et al., 2013). In an attempt to elucidate predictive markers for positive response to anti-CTLA-4 therapy, Queirolo et al. (2013) assessed responsiveness among patients with variable SNPs (single nucleotide polymorphisms) of the CTLA-4 gene. Their work yielded greater overall survival in patients with 2 particular heterozygous CTLA-4 genotypes (p<0.001): -1577G/A and CT60G/A. Although a number of patients who succumbed to melanoma also exhibited these heterozygous mutations, all surviving patients carried the 2 CTLA-4 heterozygous genotypes (Queirolo et al., 2013). Because no therapy is devoid of risk, the availability of predictive markers for positive outcomes with anti-CTLA-4 therapy may guide clinicians to carefully select for the patients most likely to benefit from these medications (Queirolo et al., 2013).

A particularly interesting study by Hodi et al. (2003) examined the antitumor effects of MDX-CTLA-4 (a CTLA-4 inhibitor) in melanoma patients who had previously received irradiated tumor vaccines. With the intention of increasing antitumor immunity, vaccination stimulates the secretion of granulocyte-macrophage colony-stimulating factor (GM-CSF), generating greater numbers of activated DCs that in turn phagocytose tumor cells and present their antigens to lymphocytes within the lymph nodes. To the investigators’ surprise, pathologic analysis of MDX-CTLA-4 treated tissues demonstrated significant increases in neutrophils, possibly explaining the abundant tumor necrosis that was observed. Hodi et al. (2003) suggest that the substantial neutrophilic infiltrate may have been the result of T cell activation.

It has been demonstrated that CTLA-4 is also constitutively expressed in tumor cells and that, via recombinant CTLA-4 ligand stimulation, apoptosis of malignant cells can be induced. In their experimentation with various human tumor cell lines (including melanoma) expressing CTLA-4, Contardi et al. (2005) showed that recombinant CD80 and CD86, both ligands for CTLA-4, can be employed to induce apoptosis of the malignant cells expressing CTLA-4. Such findings suggest that, aside from CTLA-4 inhibition by monoclonal antibodies, CTLA-4 itself can be used as a target in the treatment of MM.

### 10.5 Dendritic Cell Vaccines

As antigen presenting cells intimately in tune with their surrounding environment by way of molecular signaling, DCs play an essential role in immunosurveillance and thus in anti-tumor immunity as well. Upon loading antigen, DCs degrade the proteins to peptides and display them in MHCs in order to present the antigens to B and T cells in lymphoid organs (Palucka and Banchereau, 2012). In a similar fashion, most clinically tested DC vaccines involve mature monocyte-derived DCs that are loaded with laboratory-produced peptides that bind MHC class I molecules, with the ultimate effect of producing tumor specific CTL responses (Schreibelt et al., 2013). In a trial with 16 advanced melanoma patients, Nestle et
al. (1998) examined the effects of DC vaccines pulsed with tumor lysate or with various different tumor associated antigens (TAAs) (depending on individual patients’ specific HLA haplotypes). They observed regression of metastases (skin, soft tissue, lung and pancreas) in 5 of the patients, 2 of whom experienced complete responses. Notably, 2 of the responding patients received the tumor lysate-pulsed DC vaccine without previous exposure to TAAs, indicating that the DC vaccine alone may be effective in tumors whose specific antigens are not known (Bocchia et al., 2000; Nestle et al., 1998). Since then, numerous other studies have employed DC vaccines in combination with other therapies and continue to show promising results, warranting continued investigation.

10.6 Adoptive Cell Transfer Therapy (ACT)

Adoptive cell transfer therapy (ACT) is the process by which antigen-specific T cells are infused into a patient to augment the immune response to tumor cells. Although various different types of ACT have been developed, the use of tumor infiltrating lymphocytes (TILs) that have been expanded with IL-2 ex vivo, is the most extensively studied and clinically successful of the methods, with response rates > 50% (Turcotte et al., 2013; Wu et al., 2012). In their clinical analysis of ACT with TILs for the treatment of GI adenocarcinoma or melanoma metastases, Turcotte et al. (2013) demonstrated that 43% (105 out of 246) of the melanoma cultures exhibited MHC-I mediated CD8+ reactivity. This is in comparison to 9% (17 out of 188) MHC-I mediated CD8+ reactivity in the GI metastases group (p < 0.001). The investigators speculated that the discrepancy between melanoma and GI tumor sensitivity to ACT was likely related to the former malignancy’s uniquely immunologic features compared to other cancers.

Thus far, the greatest limitation of ACT has been the failure to induce sustained antitumor activity via TILs. Possible explanations include persistence of T regulatory cells that dampen the antitumor activity of CTLs and an imbalance of T cell regulatory mechanisms (Rosenberg et al., 2011; Wrzesinski et al., 2010). As demonstrated by Wrzesinski et al. using murine models, lymphodepletion induced by total body irradiation (TBI) prior to the initiation of ACT improves the outcome of cell transfer therapy by abating T regulatory cell numbers and improving the T cell homeostatic regulation (Dudley et al., 2002). Their work illustrated a direct correlation between levels of fractionated TBI administered and the efficacy of subsequent ACT treatment. Also noteworthy, was the augmentation of the ratio of tumor reactive CD8+ T cells to CD4+ T cells, which was proportional to TPI dose (Wrzesinski et al., 2010). This data was supported by a subsequent clinical trial by Rosenberg et al. (2011) who studied 93 patients with metastatic melanoma, 95% of whose disease continued to progress despite intensive prior therapy. Objective response rates for the 3 trial groups with regimens of chemotherapy alone versus chemotherapy plus 2 or 12 Gy irradiation were 49%, 52% and 72%, respectively. As extensive exploration into the field continues, ACT offers hope in the search for therapies offering enduring metastatic melanoma regression. We refer the reader to more extensive reviews and research on the topic of ACT (Jensen et al., 2012; Turcotte et al., 2013; Wu et al., 2012).

11 Potential for New Therapies

The following is a brief introduction to newly identified targets and potential therapies for the management of melanoma. This is not intended to be a comprehensive review. Perhaps one of the most studied treatments for metastatic melanoma, IL-2 therapy has demonstrated consistent antitumor response in vitro and in vivo (Chou et al., 2013; Chu et al., 2013; Den Otter et al., 2008). However, the systemic toxicity of
IL-2, characterized by vascular leak syndrome and other phenomena, has limited the clinical utility of this immunotherapy (Chou et al., 2013). Therefore, recent research has focused on producing a therapy with the same mechanism of action but milder toxicity. Researchers Chou et al. (2013) have produced palmitate-derivatized (Ribas, 2007) IL-2 (pIL-2), a form of recombinant human IL-2 (rhIL-2) whose properties allow for localization of the drug to the tumor site, minimizing its systemic effects. In their investigations with murine subjects, Chou et al. (2013) demonstrated that, compared to rhIL-2, pIL-2 stimulated more CD8+ cells, promoted survival of antitumor immune cells, decreased incidence of lung metastasis and increased overall survival. These findings were applicable to both pIL-2 adoptive T cell transfer with CTLs and to intra-tumorally injected pIL-2 models. Although further investigation into pIL-2’s interactions with the immune system is necessary, the development of this modified form of IL-2 with theoretical lower toxicity is encouraging.

<table>
<thead>
<tr>
<th>Proposed therapy</th>
<th>Description</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>pIL-2 (Chou et al., 2013)</td>
<td>Palmitate-derivatized form of rhIL-2 that localizes to tumor site, minimizing systemic toxicity</td>
<td>Stimulation of CD8+ cells Improved survival of antitumor immune cells Decreased incidence of lung metastasis Increased overall survival</td>
</tr>
<tr>
<td>Nanoparticles (Zheng et al., 2013)</td>
<td>Liposomes conjugated with targeting molecules to a) label and b) expand ACT and endogenous T cells in vivo</td>
<td>Liposome-conjugated molecules: - Anti-Thy1.1-Lip: antibodies to ACT T cell antigens → successful labeling of transferred cells in vivo - IL-2-Fc-Lip: recombinant IL-2 → greater antitumor T cell expansion; more robust expansion of ACT T cells with each subsequent IL-2-Fc-Lip injection</td>
</tr>
<tr>
<td>LV-tSMAC (Emeagi et al., 2012)</td>
<td>SMAC-encoding lentiviral vectors</td>
<td>Greater antitumor immunity ↓ in vivo T regulatory cells Greater CTL activation</td>
</tr>
<tr>
<td>Oblimersen (Jain, 2001, 2005; Semenza, 2003; Shang et al., 2012; Stein et al., 2009)</td>
<td>Bcl-2 antisense</td>
<td>Sensitizes MM cells to subsequent chemotherapy - ↓ Bcl-2 synthesis - Potentiates FBF-2 activity to promote tumor angiogenesis, preventing hypoxia-induced MM upregulation</td>
</tr>
<tr>
<td>Mebendazole (Doudican et al., 2008; Doudican et al., 2013)</td>
<td>Antihelminthic</td>
<td>Promotes MM cell apoptosis - Interferes with interaction between Bcl-2 and Bax - ↓ XIAP, ↑ proteosomal degradation Inhibition of tubulin polymerization during cellular proliferation</td>
</tr>
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</table>

**Table 2**: New therapies for melanoma

The proposed use of nanoparticles as an adjunct to ACT may enable persistent in vivo T cell expansion without the systemic administration of highly toxic IL-2. Zheng et al. (2013) have shown that liposomes conjugated with targeting molecules can both specifically and nonspecifically augment ACT cells and endogenous T cell proliferation (respectively) to enhance antitumor activity. The liposome-conjugated targeting molecules used in these murine experiments were a) antibodies specific to ACT T cell antigens (anti-Thy1.1-Lip) and b) recombinant IL-2 (IL-2-Fc-Lip), a nonspecific T cell activator. Their work demonstrated successful in vivo labeling of transferred T cells via anti-Thy1.1-Lip and greater antitumor T cell expansion with liposome-bound IL-2 than with injection of equivalent free IL-2 doses, with each subsequent injection of IL-2-Fc-Lip inducing greater ACT cell expansion.
Considering its direct role in the interaction between CTLs and tumor cells, the programmed death (PD-1) receptor and its ligand (PD-L1) have garnered significant interest in the development of melanoma therapy. By suppressing the antitumor activity of CD4+ and CD8+ T cells, the PD-1/PD-L1 interaction may contribute to the progression of malignancy in PD-L1 expressing tumors (Brahmer et al., 2012). There are currently 3 anti-PD-1 antibodies undergoing clinical trials for the treatment of melanoma and other malignancies including renal cell carcinoma, non-small cell lung cancer and colorectal cancer (Postow et al., 2012). These PD-1 inhibitory antibodies are BMS-936558 (formerly MDX-1106), MK-3475 and CT-011 (Postow et al., 2012). In a phase I study of 296 patients with various advanced solid tumors (104 of whom had melanoma), Topalian et al. (2012) assessed the antitumor activity of a fully human monoclonal anti-PD-1 antibody, BMS-936558, the most thoroughly studied anti-PD-1 antibody (Postow et al., 2012). In this study, PD-L1 expression in tumors was assessed in 42 patients, 18 of whom had melanoma. Of those 42 patients, 25 demonstrated immunohistochemical positivity for PD-L1 expression and 9 of those 25 patients exhibited an objective response. The lack of objective response in all 17 patients who were negative for PD-L1 expression necessitates a thorough investigation into PD-L1 as a marker in the selection of patients for therapy with anti-PD-1 antibody (Topalian et al., 2012). The investigators also found that all doses tested showed antitumor activity and that objective responses were particularly notable in melanoma, non-small cell lung cancer, renal cell carcinoma and various sites of metastasis (Topalian et al., 2012). Phase II trials for the use of anti-PD-1 antibodies against PD-L1 expressing tumors are currently underway.

A particularly elegant cancer therapy is the oncolytic virus or OV. This therapeutic modality employs a live virus deprived of certain genes while enhanced with others in order to target cancer cells and disrupt the tumor microenvironment (Bartlett et al., 2013; Donnelly et al., 2013). Vulnerability to viral assault and replication is a feature inherently characteristic of malignant cells that distinguishes them from normal cells (Donnelly et al., 2013). Rampant viral replication within malignant cells essentially labels them for the immune system, while protecting normal cells whose antiviral mechanisms remain intact (Donnelly et al., 2013). T-VEC or Talimogene laherperevec (formerly OncoVex or JS1/ICP34.5-ICP47-CM-CSF) is an OV engineered from herpes simplex virus that does not express neurovirulent genes of the wild-type virus and instead, is equipped to express granulocyte-macrophage colony stimulating factor to enhance antitumor immunity (Bartlett et al., 2013). Intratumoral T-VEC injections have recently demonstrated promise in phase II clinical trials in metastatic melanoma. In a study of 50 patients with IIIc or IV metastatic melanoma, 74% of whom had received previous nonsurgical treatment for their disease, Senzer et al. (2009) demonstrated an overall response rate of 26% with most responses (92%) enduring for 7 to 31 months after treatment. Both un-injected and injected lesions exhibited regression, leading the authors to speculate that the effects on distant, un-injected lesions is probably immune mediated.

Evasion of apoptosis is a survival mechanism common to many malignancies and is particularly important in melanoma. Although there are two general pathways for apoptosis to proceed, they ultimately converge to the same end. There are many participants in this mechanism, one of which is called second mitochondria-derived activator of caspase (SMAC) or direct inhibitor of apoptosis-binding protein with low pI (DIABLO), (Emeagi et al., 2012) hereafter referred to as SMAC. SMAC functions to antagonize IAPs (inhibitors of apoptosis) whose role is regulation of capsases (Vucic et al., 2002). More specifically, SMAC and SMAC-like peptides have been shown to inhibit the function of various types of IAPs, including XIAPs (X-linked IAPs) and ML-IAPs (melanoma IAPs), both of which are expressed in human malignancies (Vucic et al., 2002). This interaction highlights a promising therapeutic target, one which
has recently been further explored by Emeagi et al. In their investigation using murine melanoma models, Emeagi et al. (2012) were able to harness the pro-apoptotic effects of SMAC through transduction of SMAC-encoding lentiviral vectors (LV-tSMAC). Though requiring more extensive exploration, the results are encouraging: LV-tSMAC bearing mice exhibited greater antitumor immunity, more activated CTLs and decreased in vivo T regulatory cells.

Although DTIC is a first-line therapy for metastatic melanoma, overall improvement in prognosis is marginal due to the chemotherapy resistant features of melanoma (Avril et al., 2004; Bedikian et al., 2006; Chiarion Sileni et al., 2001). Thus, a key component of anti-melanoma therapy is the administration of a sensitizing agent that combats the melanoma microenvironment of immune evasion. Recognizing that B-cell lymphoma 2 (Bcl-2) overexpression contributes to melanoma cells’ capacity to avoid apoptosis, researchers introduced oblimersen (G2139), a relatively new therapy currently in clinical trials. Oblimersen, also known as Bcl-2 antisense, would be used as an adjunct or pretreatment for patients to be treated subsequently with a medication such as DTIC, TMZ or albumin-bound paclitaxel (Ott et al., 2013). (Antisense refers to a factor that prohibits the translation of a certain protein’s mRNA) (Bedikian et al., 2006).

Oblimersen-induced sensitization of melanoma cells to chemotherapy may involve mechanisms beyond suppression of Bcl-2 synthesis. Stein et al. (2009) demonstrate that oblimersen promotes tumor angiogenesis in rat models by potentiating FGF-2 induced cellular mitogenesis. Although seemingly contradictory to tumor eradication principles, Jain et al. (2001, 2005) explain that antiangiogenic factors actually dampen chemotherapeutics’ efficacy through hypoxia, diminishing the amount of medication that can reach the target site. Evidence also suggests that antiangiogenic-induced tumor hypoxia results in more aggressive and metastatic cancer (Shang et al., 2012). As an example, in response to hypoxic conditions, Bcl-2 may promote angiogenesis by enhancing production of hypoxia-inducible factor-1α (HIF-1α) and ultimately vascular endothelial growth factor (VEGF) (Semenza, 2003).

In an experiment of drug repositioning, where a known therapy is implemented in a novel application, (Doudican et al., 2013). The authors demonstrated the pro-apoptotic features of the antihelminthie mebendazole (MBZ) and its relationship to XIAP. Mebendazole’s capacity to induce apoptosis in vitro and in vivo can be attributed to several mechanisms. Through phosphorylation, MBZ prevents Bcl-2 from interacting with pro-apoptotic factor Bcl-2 associated X protein (Bax), causing selectively higher rates of apoptosis of melanoma cells compared with melanocytes (Doudican et al., 2008). Mebendazole also promotes melanoma cell apoptosis via the interaction between XIAP and SMAC, which results in abatement of XIAP levels and proteosomal degradation. In addition, as an inhibitor of tubulin polymerization, MBZ can suppress cellular proliferation from a structural standpoint (Doudican et al., 2013). The investigators observed that the decline in XIAP levels was correlated with tumor cell susceptibility to MBZ’s cell cycle suppressive effects. Although MBZ impeded melanoma xenograft growth in vivo, its application did not result in xenograft tumor regression.

12 Recommendations

Although the scientific community’s understanding of melanoma continues to grow, establishing concrete protocols for the treatment of malignant melanoma in the immunosuppressed presents an ongoing challenge. Along with the modification of greater vigilance and a higher index of suspicion, it is recommended that melanoma screening and care of the immunosuppressed patient essentially parallel that of the im-
munocompetent population. Particularly in the settings of lymphoproliferative malignancy and organ transplantation, a multidisciplinary approach is growing more popular, integrating dermatologists, oncologists and surgeons, and offering transplant clinic dermatology visits for surveillance of these patients (Otley and Pittelkow, 2000). Considering both the increased incidence and more aggressive nature of MM in these patients, especially attentive regimented screening is prudent (Brewer et al., 2011; Rodrigues et al., 2002).

A significant challenge in the management of immunosuppressed OTR patients is balancing the prevention of donor organ rejection with the potential for de novo, recurrent, or (rarely) graft-transmitted melanoma. When appropriate, immunosuppressive therapy may be reduced to temporize this risk. Recommendations for adjusting immunosuppressive therapy dose according to melanoma stage and donor organ type are provided by SCOPE (Otley, 2006).

Lymph node assessment and evaluation for distant metastases are critical components in the determination of the appropriate level of immunomodulation. The multidisciplinary approach is especially key here, where cooperation between dermatologist, oncologist and surgeon provides expert input, consideration of adjuvant therapy and the option of lymphadenectomy if indicated (Berg and Otley, 2002). In the event of extensive regional or distant metastases, indefinite postponement of transplantation has been recommended (Dinh and Chong, 2007).

Recommendations regarding organ transplant candidates with a history of melanoma are limited, with Penn’s analysis of the CTTR providing the most information for this patient population (Penn, 1996). As of yet, there does not appear to be evidence for contraindication to organ transplant and associated immunosuppression in patients with a history of thin melanomas, but careful consideration of risk of recurrence versus implications of withholding organ transplantation is crucial in cases of advanced melanoma, requiring the cooperation of the entire medical team involved.

Specific recommendations for the management of melanoma in HIV/AIDS patients are sparse. Optimal medical control of this chronic infection with HAART is crucial, as the immune dysfunction making these patients more susceptible to malignancy is the target of antiretroviral therapy. Some authors also recommend the implementation of cancer surveillance programs within HIV clinics, although with an emphasis on non-melanoma cancer such as prostatic and anal carcinoma (Burgi et al., 2005). It is also wise to consider the potential for medication interactions between anti-neoplastic agents and members of the HAART regimen when managing this patient population (Spano et al., 2008). As with other immunosuppressed patients, HIV/AIDS status warrants more frequent surveillance and compliance with preventative measures (sun protection).

13 Conclusion

A substantial amount of research exists regarding melanoma’s immunogenic properties as well as its presence in various forms of immunosuppression. Yet, much is to be learned about the long-term implications of malignant melanoma in these settings, as well as the appropriate treatment modifications for the special patient populations of the iatrogenically and otherwise immunosuppressed. With continued advancements in our understanding of melanoma and identification of new therapeutic targets, treatment of this malignancy will likely become more personalized, as immunotherapies approach the forefront of our anti-melanoma arsenal. As we anticipate new developments and recommendations in the surveillance and
treatment of melanoma in said populations, vigilant screening and early intervention remain the standard of care.

**Appendix: Abbreviations and Acronyms**

CCSS = Childhood Cancer Survivor Study; SCOPE = Skin Care in Organ Transplant Patients, Europe; SEER = Surveillance, Epidemiology and End Results; AJCC = American Joint Committee on Cancer; IDO = indoleamine 2,3-dioxygenase; TNF = tumor necrosis factor; TGF = transforming growth factor; CI = confidence interval; SIR = standardized incidence ratio; SMR = standardized mortality ratio; PAF = phospholipid platelet-activating factor; PAF-R = phospholipid platelet-activating factor receptor; Treg = regulatory T cell; APC = antigen presenting cell; DC = dendritic cell; MDSC = myeloid derived suppressor cell; CLL = chronic lymphocytic leukemia; PUVA = psoralin-UV-A; HAART = highly active antiretroviral therapy; HIV = human immunodeficiency virus; AIDS = acquired immunodeficiency syndrome; IL = interleukin; NHL = non-Hodgkin lymphoma; OTR = solid organ transplant recipient; PBMC = peripheral blood mononuclear cell; TAP = transporter-associated antigen processing; TMZ = temozolomide; DTIC = dacarbazine; MTIC = 5-(3-methyltriazen-1-yl)imidazole-4-carboximide; mTOR = mammalian target of rapamycin.

**References**


