

# Immune correlates of melanoma survival in adoptive cell therapy

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**Abbreviations:** ACT, adoptive cell transfer; CCR, C-C chemokine receptor; CLA, cutaneous lymphocyte antigen; CTL, cytotoxic T lymphocyte; CTLA-4, cytotoxic T-lymphocyte antigen 4; DC, dendritic cell; IFN, interferon; IL, interleukin; MHC, major histocompatibility complex; PD-1, programmed cell death 1; T<sub>CM</sub>, central memory T; TCR, T cell receptor; T<sub>EM</sub>, effector memory T; T<sub>H</sub>, helper T; TIL, tumor infiltrating lymphocyte; TNF $\alpha$ , tumor necrosis factor  $\alpha$ ; Treg, regulatory T cell; T<sub>RM</sub>, resident memory T

The advent of immunotherapies for cancer has resulted in robust clinical responses and confirmed that the immune system can significantly inhibit tumor progression. The recent success of adoptive cell therapy against melanoma suggests that endogenous T-cell responses have the potential to control cancer. However, the lack of responses in some patients receiving such therapy indicates a need for a better understanding of the host immune response to solid tumors. In this review, we summarize the current knowledge on the characteristics of adoptively transferred T cells associated with successful anti-melanoma immune responses in humans.

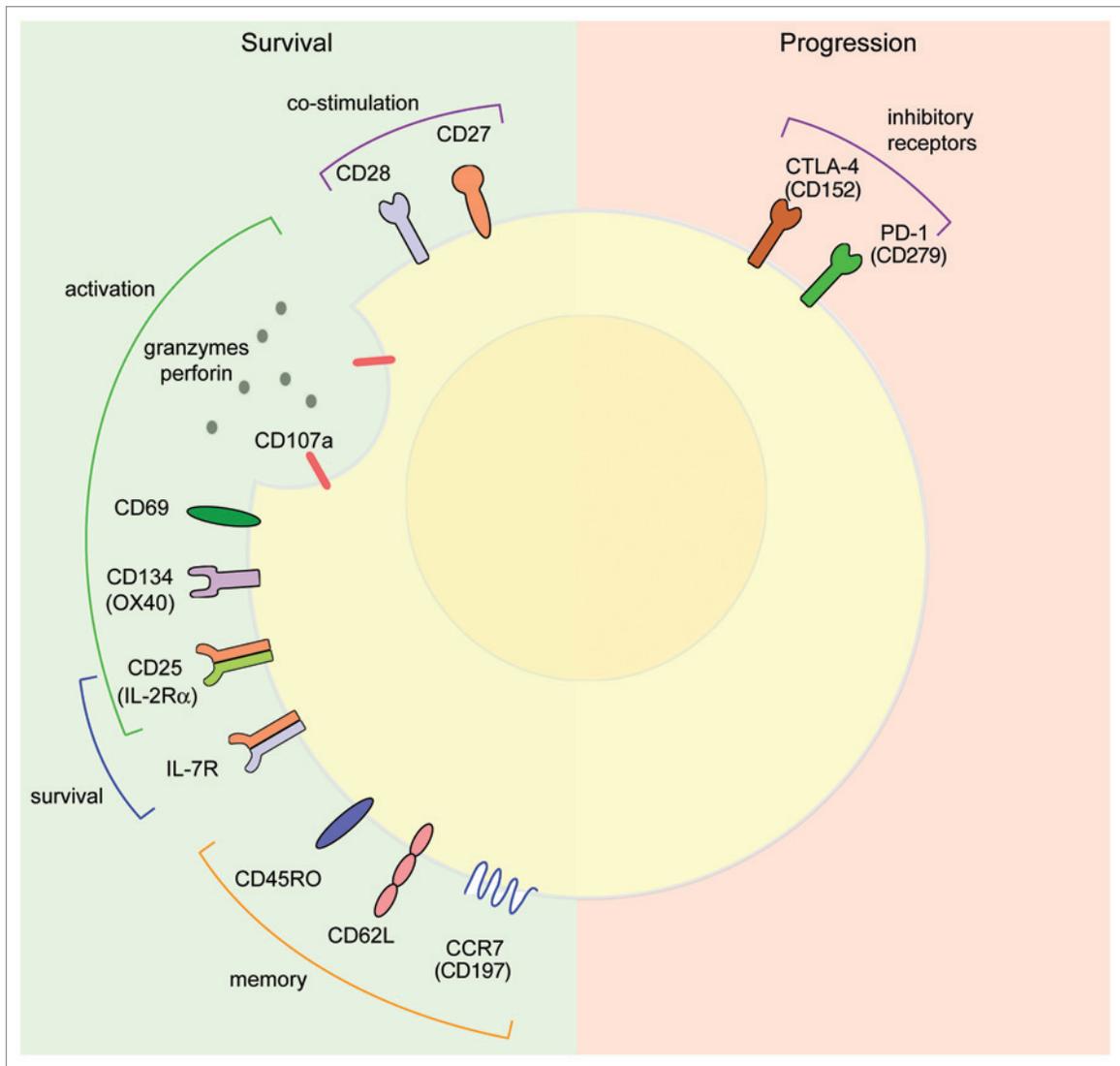
## Introduction

Melanoma is an aggressive skin cancer that leads to 48,000 deaths annually worldwide.<sup>1</sup> It is the most common cancer in young adults aged 20 to 30 years old and the leading cause of cancer death in women aged 25 to 30 years old.<sup>2</sup> Although the mortality rates of early stage melanoma are generally low, stage IV metastatic disease predicts poor disease outcome, with less than 10% survival rate at five years from diagnosis.<sup>3</sup> Depending on the stage of the disease, treatments for melanoma include surgical resection of tumor and draining lymph nodes as well as the amputation of affected body parts. Other treatment modalities, including chemotherapy, are generally not as effective in melanoma as in other types of cancer. In contrast, treatments targeted at improving immunity such as high dose interleukin (IL)-2, anti-CTLA-4 antibodies, and interferon (IFN) $\alpha$  are efficacious, at least in selected subsets of melanoma patients, indicating a prominent role for the immune system in control of melanoma.<sup>4-6</sup>

The concept of immunotherapy for melanoma stemmed from early observations of intratumoral lymphocytic infiltrates, suggesting the existence of a local antitumor immune response.<sup>7</sup> These tumor-infiltrating lymphocytes (TILs) included T cells, which could be expanded in culture with recombinant IL-2 and displayed cytolytic activity against autologous melanoma cells in vitro.<sup>8</sup> The presence of CD4<sup>+</sup> and CD8<sup>+</sup> T cells among TILs positively correlates with favorable disease outcome, further demonstrating an important role for T cells in antitumor immunity.<sup>9-13</sup> Since then, a broad spectrum of immunotherapies has been developed to boost protective T cell responses against melanoma (e.g., high-dose IL-2 and Type I IFN) or to unleash endogenous responses to tumor (e.g., anti-CTLA-4 and anti-PD-1 monoclonal antibodies).

Adoptive cell therapy (ACT), a procedure whereby T cells are isolated from patients, expanded in culture, and eventually delivered back into the patient, have improved cure rates in melanoma patients by 20–40%.<sup>14</sup> T cells used in ACT are derived from TILs of resected primary melanomas or, when TILs are not available, from peripheral blood T cells genetically modified to recognize tumor antigens.<sup>15,16</sup> Retrospective analyses of ACT-based clinical trials have provided some insights into the characteristics of T cells that generate an effective antitumor response for melanoma. Most studies have focused on CD8<sup>+</sup> T cell populations identified through the use of peptide-MHC tetramers or through the analysis of T-cell receptor (TCR) variable regions (TCRV) to examine antigen-specific T cells or T cell clonotypes, respectively.<sup>17</sup> These methods are commonly used to monitor the frequency of specific T cells in the peripheral blood or among TILs, and are correlated with patient responses to therapy. Determining whether melanoma patients are good candidates for ACT is often challenging due to the variability of clinical presentation. An overriding question therefore must be whether certain features of T cells can be identified, such as cell surface phenotype, specificity or cytokine secretion profile, which might be used to predict patient survival. This review summarizes the characteristics of adoptively transferred T cells that promote tumor regression.

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**Figure 1.** Cell surface phenotypes in melanoma. On the left (green): cell surface markers associated with melanoma survival. Co-stimulatory molecules: CD27 and CD28; activation markers: CD107a, CD69, CD134 (OX40) and CD25 (IL-2R $\alpha$ ); cytokine receptors that promote T-cell survival: CD25 (IL-2R $\alpha$ ) and IL-7R; memory T-cell markers: CD45RO, CD62L, and CCR7. On right (pink): cell surface markers associated with melanoma progression. Inhibitory receptors, CTLA-4 and PD-1.

## Phenotype

Cytotoxic CD8<sup>+</sup> T cells have long been considered the principal immune cells involved in antitumor responses, based on their potent ability to directly kill tumor cells. The mechanisms for CD8<sup>+</sup> T cell-mediated tumor cell killing are well established, and primarily involve the release of cytotoxic mediators such as granzymes and perforin upon the recognition of tumor antigens presented on MHC Class I molecules.<sup>18</sup> Identifying the T-cell populations that are most effective at promoting tumor regression is the first step in determining how to enhance the number and/or function of T cells to treat melanoma.

The activation phenotype of CD8<sup>+</sup> T-cell populations has been used to predict clinical outcomes among melanoma patients. Independent studies that examined T-cell phenotypes in primary cutaneous melanoma showed that the expression of

the T-cell activation markers CD69 within neoplastic lesions and CD25 (the  $\alpha$  chain of the IL-2R) or CD134 (OX40) in peritumoral regions correlated with improved patient survival.<sup>19,20</sup> Other T-cell activation markers associated with enhanced patient survival include CD107a (LAMP-1), a marker for T-cell degranulation, and CD45RO, a memory T cell marker.<sup>21,22</sup> Thus, surface activation markers of CD8<sup>+</sup> T cells may be used to screen for protective T-cell populations in melanoma (Fig. 1).

Memory T cells constitute a specialized subset of antigen-experienced T cells that persists in vivo and confer long-lasting immunity against microbial infection. Memory T cell subsets include central memory T (T<sub>CM</sub>) and effector memory T (T<sub>EM</sub>) cells, which are characterized by distinct functional properties and migratory patterns.<sup>23</sup> T<sub>CM</sub> cells constitutively express the lymph node homing receptors CCR7 and CD62L/L-selectin and are characterized by their ability to rapidly expand and to

differentiate into potent effector cells in response to secondary antigen exposure.<sup>23,24</sup> In contrast,  $T_{EM}$  cells lack CCR7 and express low levels of CD62L, which allows for their migration to peripheral tissues for immediate effector functions. Adoptively transferred  $CD8^+ T_{CM}$  cells exhibited an enhanced recall response *in vivo* and significantly prolonged survival in mice with established B16 melanoma as compared with  $T_{EM}$  cells, suggesting that  $T_{CM}$  cells are more effective than  $T_{EM}$  cells in mediating antitumor responses.<sup>25</sup> Similar findings in melanoma patients showed that adoptively transferred  $CD8^+$  T cells that acquired a  $T_{CM}$  phenotype correlate with complete tumor regression.<sup>26</sup>

Based on the ability of  $T_{CM}$  cells to exert robust antitumor immunity in animal models,  $T_{CM}$  cells were considered ideal candidates for ACT in clinical trials. Recently Wang and colleagues developed a method to screen for  $T_{CM}$  and  $T_{EM}$  cells in the peripheral blood upon antigen exposure, based on stoichiometric production of IL-2 and IFN $\gamma$ .<sup>27</sup> Melanoma-specific  $T_{CM}$  cells produced high levels of IL-2, lower levels of IFN $\gamma$ , and demonstrated superior proliferative capacity as compared with  $T_{EM}$  cells, suggesting the potential of  $T_{CM}$  cells to mediate durable cancer regression. Unfortunately, the adoptive transfer of  $T_{CM}$ -derived effector cells failed to induce tumor regression in patients with melanoma. As the small patient sample may have been a limitation of this attempt, more studies are required to determine whether  $T_{CM}$  cells can improve the therapeutic outcome of ACT in melanoma patients.

The failure of adoptively transferred  $T_{CM}$  cells to mediate tumor regression suggests that selecting T cells based on memory phenotype alone may not be a reliable method of identifying the optimal antitumor cells for immunotherapy. Additionally, both high and low avidity CTL clones have a similar cell surface phenotype, indicating that cell phenotype does not necessarily correlate with immune function.<sup>28</sup> Thus, cell surface phenotypes may be useful for characterizing activated T cells (Fig. 1), but functional studies may be more relevant to predict their efficacy in immunotherapies.

Another T-cell phenotype (whose relevance for melanoma has not yet been established) is constituted by the newly described skin resident memory T ( $T_{RM}$ ) cells. Unlike other memory T cells that recirculate between the blood and lymph nodes, skin  $T_{RM}$  cells compose a unique population of memory T cells that remain in the skin and confer long-term immunity against secondary antigen exposure. Skin  $T_{RM}$  cells express CD45RO and maintain high levels of skin homing receptors such as the cutaneous lymphocyte antigen (CLA), CCR4, and CCR6, which enable them to persist in skin for several months.<sup>29</sup>  $T_{RM}$  cells readily poised in skin respond to previously encountered pathogens faster than other memory T cells, which need to be recruited from circulation.<sup>30,31</sup> Furthermore, skin  $T_{RM}$  cells have been shown to populate the entire skin surface and to be superior to  $T_{CM}$  cells at eliminating pathogens at distant skin sites as well as at the site of infection.<sup>30</sup> The ability of these cells to mediate long-lasting cutaneous immunity indicates that  $T_{RM}$  cells may be more effective than  $T_{CM}$  cells in providing protection against melanoma. Interestingly,  $T_{RM}$  cells appeared to be unaffected by drugs that targeted circulating T cells, suggesting that conventional

immunotherapies designed to provide systemic immunity against melanoma may not affect  $T_{RM}$  cell functions.<sup>31</sup> Thus, it may be worthwhile to monitor  $T_{RM}$  cells in melanoma patients receiving conventional immunotherapies or consider targeting these cells when designing future T cell-based treatments for melanoma.

## T-Cell Persistence In Vivo

Two ACT-based Phase I clinical trials that failed to achieve objective clinical responses reported a rapid decline in adoptively transferred T-cell populations, which were virtually undetectable in the peripheral blood of patients two to three weeks following transfer.<sup>32,33</sup> These trials provided evidence for a relationship between T-cell persistence *in vivo* and tumor regression. The preconditioning of patients with non-myeloablative, but lymphodepleting chemotherapy to eliminate regulatory T cells and other tolerogenic mechanisms before the transfer of polyclonal TILs plus high-dose IL-2 significantly improved T-cell persistence in the peripheral blood to up to four months and led to objective clinical responses in 6 out of 13 patients.<sup>15</sup> A second study using a larger cohort of patients revealed that the levels of T-cell persistence in ACT responders were significantly higher than those of non-responders.<sup>34</sup> Together, these findings suggest that long-term T-cell persistence *in vivo* correlates with a favorable outcome in metastatic melanoma patients. Identifying long-term persistent T cell receptor (TCR) clones, or clonotypes, should hence make immunotherapies more effective.

Long-term persistent T cell clonotypes possess significantly longer telomeres, differentiate more slowly than less persistent clonotypes, and express higher levels of CD27 and CD28 (Fig. 1).<sup>35</sup> The amount of time that TILs passed in culture also influences *in vivo* T-cell persistence, so that minimally cultured TILs appear to persist for comparatively longer periods in patients, correlating with objective clinical responses.<sup>36</sup> Unlike TILs cultured for long time periods, minimally cultured TILs also retain CD27 and CD28 expression.<sup>37</sup> Recently, Li and colleagues have demonstrated that CD28, but not CD27, is required for maintaining T-cell survival *in vivo*. Indeed, the loss of CD28 after the expansion of TILs *in vitro* with IL-2 resulted in impaired T-cell proliferation in response to antigenic stimulation.<sup>38</sup> In contrast, CD28-expressing TILs retained expansion capability and exhibited improved survival *in vivo*.

T-cell persistence *in vivo* after ACT can be dramatically influenced by the cytokines that are used to expand T-cell populations from TILs *in vitro*. The traditional approach to expand TILs for ACT involves culturing tumor cells suspensions with recombinant IL-2. However, recent reports indicate that IL-15 and IL-21 may be more effective than IL-2 in generating long-term persistent T-cell clones.<sup>38</sup>  $CD8^+$  T cells cultured with IL-15 and IL-21 express higher levels of CD28 and divided more proficiently after tumor peptide stimulation than cells expanded with IL-2. Persistent T-cell clones commonly expressed IL-7R $\alpha$ , implying that IL-7 may also be important for long-term T-cell survival.<sup>39</sup> Interestingly, both IL-7 and IL-15 are implicated in the homeostatic proliferation of memory T cells and both cytokines signal through a common receptor  $\gamma$  chain, which is also shared by IL-2

and IL-21.<sup>40,41</sup> Whether ACT can be improved by identifying a cytokine cocktail that would enhance the persistence of T cells in vivo is currently unknown, but undoubtedly warrants further investigation.

### Specificity for Tumor Antigens

Identifying the antigen specificity of endogenous T cells and hence defining those proteins that might be used to select for tumor-specific T cells in ACT might dramatically enhance the therapeutic efficacy of this approach. The first successful ACT for the treatment of metastatic melanoma used highly reactive T-cell populations directed against MART-1/Melan A and gp100, highlighting the importance of antigen-specific T cells in effective cancer immunotherapy.<sup>15</sup> Several melanoma-associated antigens and their corresponding CD8<sup>+</sup> T cell epitopes have been identified through biochemical and molecular approaches, including mass spectrometry after peptide elution from HLA molecules, or by screening cDNA expression libraries with melanoma-reactive T cells.<sup>42,43</sup> Based on the expression patterns of parental proteins, T cell-relevant tumor antigens were categorized into four major classes: overexpressed antigens, melanocyte differentiation antigens (e.g., MART-1/Melan A and gp100), cancer/testis antigens (e.g., NY-ESO-1 and MAGE) and mutated antigens.<sup>42</sup> Many tumor-associated antigens are also expressed in normal tissues. Thus, selecting the appropriate antigen that preferentially targets tumor cells while avoiding toxicity to normal tissues is a major challenge in designing immunotherapies.

Antigen-specific T-cell populations from a single biological sample can be identified by a high throughput approach using dual color peptide MHC multimers.<sup>44</sup> A total of 90 T-cell responses were identified against melanoma antigens in TILs (63 TILs from 19 patients) using this approach.<sup>45</sup> T-cell reactivity against overexpressed antigens, which represent the largest class of antigens contributing to known T-cell epitopes relevant for melanoma, were rare and appeared to have no impact on clinical responses. Although TILs most frequently responded to melanoma differentiation and cancer/testis antigens, only reactivity against the latter correlated with patient sensitivity to TIL-based therapy.<sup>46</sup> These data suggest that the recognition of cancer/testis antigens by infused T-cell populations may be associated with favorable therapeutic outcomes.

Despite the prediction that T-cell responses to cancer/testis antigens would lead to favorable outcomes in melanoma, vaccination with the cancer/testis antigen MAGE-3.A1 peptide resulted in complete tumor regression only in 10–20% melanoma patients.<sup>47</sup> Unexpectedly, patients responsive to peptide vaccination had a very low frequency of MAGE-3.A1-specific CD8<sup>+</sup> cells in the peripheral blood, but a high frequency of CD8<sup>+</sup> T-cell precursors specific for other tumor antigens.<sup>48</sup> In some cases, antitumor T-cell clonotypes were observed in the blood before vaccination, while in other cases, antitumor T cells only emerged after vaccination.<sup>49</sup> The presence of antitumor T-cell precursors in the blood of non-vaccinated individuals suggested that spontaneous antitumor T cells that were quiescent or ineffective became active upon vaccination. In contrast, the appearance of new antitumor

clonotypes after vaccination demonstrated clonal spreading, or the emergence of new CTL clones against previously targeted antigens. Whether new antitumor T-cell clonotypes were a product of vaccination or naturally appeared during the course of disease has not been elucidated. New antitumor clonotypes specific for tumor antigens not targeted by vaccination may also arise from antigen spreading, or the development of CTL clones against previously ignored antigens.<sup>50</sup> Evidence for antigen spreading has previously been reported in one patient undergoing a complete regression that developed antitumor responses against a mutated antigen, for which no T cells could be detected prior to vaccination.<sup>50</sup> In a similar report, a patient who experienced tumor regression after receiving NY-ESO-1-specific TILs also demonstrated reactivity to MART-1 and MAGE-3 antigens.<sup>51</sup> It has been postulated that antigen spreading may result from new tumor antigens being released from killed tumor cells that are processed by antigen-presenting cells and used to activate new T cells. Collectively, these findings suggest that clonal or antigen spreading may increase the repertoire of tumor-reactive T cells to improve the effectiveness of immunotherapies. With this in mind, the use of several tumor-reactive T-cell clones (to target multiple melanoma antigens) rather than a single one may increase the therapeutic efficacy of ACT.

Although several immunotherapies target T cells that are specific for shared melanoma antigens, neo-antigens may also emerge from genetic mutations. For instance, approximately 50% of melanomas harbor a mutation in *BRAF*, a gene coding for a protein kinase involved in the RAS-RAF signaling pathway.<sup>52</sup> Although other mutated antigens in melanoma have also been identified, the magnitude of T-cell responses directed against mutated antigens for melanoma is very low.<sup>46</sup> Nevertheless, highly reactive T cells directed against mutated antigens have been reported to mediate complete regression in patients with metastatic melanoma.<sup>53,54</sup> In an attempt to identify genetic mutations in melanoma, Wei and colleagues performed whole exome sequencing of 14 matched normal and metastatic tumor DNA from untreated melanoma patients. This analysis allowed them to confirm previously identified mutations in *BRAF* and to identify new mutations in genes that had not previously been associated with melanoma, including *TRRAP* (coding for transformation/transcription domain-associated protein) and *GRIN2A* (coding for a glutamate receptor subunit).<sup>55</sup> Interestingly, most genetic mutations associated with melanoma encoded members of the glutamate signaling pathway. While this study helped to identify novel melanoma-specific mutations, the use of exome-sequencing to analyze potential target genes in all melanoma patients may be time consuming and costly. Thus, the development of more efficient methods to screen for patients with relevant mutations and neo-antigens is still required.

Regardless of the approach used to identify novel tumor antigens and the development of corresponding T-cell reactive clones, some patients that initially respond to ACT also experience relapse.<sup>56</sup> Recent evidence suggests that tumor resistance to therapy occurs when cancer cells lose melanoma antigens or undergo a process of “dedifferentiation,” enabling them to escape the recognition by antigen-specific T cells. Landsberg and colleagues

identified tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), a pro-inflammatory cytokine produced by T cells, as a critical factor responsible for dedifferentiation in melanoma cells.<sup>56</sup> Thus, future ACT approaches should consider targeting several tumor antigens to minimize or prevent the development of tumor resistance.

### T-Cell Cytokine Secretion Profile

T cells used for ACT are often selected from bulk TIL populations based on their ability to exert antitumor effector functions. Various *in vitro* assays have been used to assess the reactivity of effector CD8<sup>+</sup> T cells, including chromium release assays, which measure the killing capacity of T cells against chromium-labeled tumor cell targets, as well as assays that measure T-cell degranulation based on granzyme B release or CD107a expression.<sup>21,33</sup> T cell reactivity for tumor antigens may also be evaluated through the production of IFN $\gamma$  or TNF $\alpha$ .<sup>15,57</sup> These parameters for measuring T-cell functions define the quality of the T-cell response. However, simply measuring a single functional parameter may not accurately predict the magnitude of an antitumor T-cell response *in vivo*. For example, assays that measure T-cell degranulation lack the sensitivity for distinguishing high- and low-avidity CTL populations, which may account for differences in patient responses to ACT.<sup>28</sup> T-cells with a better quality, or those exhibiting a broader repertoire of effector functions, are able to elicit enhanced protection against microbial infection as compared with cells with a single functional profile.<sup>58</sup> In the context of tumor immunity, T cells exhibiting high avidity for peptide-MHC multimers, an elevated degranulation potential (based on CD107a expression), and producing IFN $\gamma$ , IL-2 and TNF $\alpha$ , demonstrated superior tumor recognition (Fig. 2).<sup>28</sup> Thus, selecting T cells based on multifunctional capability rather than a single functional parameter may dramatically improve the therapeutic outcome of ACT.

CD8<sup>+</sup> T-cell functions in cancer immunotherapies often overshadow functional responses by CD4<sup>+</sup> T cells, which make up to 19% of the total TIL population from primary melanomas.<sup>59</sup> Protective immune responses in melanoma may involve specific subsets of CD4<sup>+</sup> helper T (T<sub>H</sub>) cells that recognize tumor antigens presented on MHC Class II molecules. However, the characterization of protective CD4<sup>+</sup> populations in humans is poor. Although CD4<sup>+</sup> T cells are generally thought to provide help to enhance the cytolytic functions of CD8<sup>+</sup> T cells, CD4<sup>+</sup> T cells have also been shown to exert direct cytolytic activity against tumor cells.<sup>60–62</sup> Moreover, the adoptive transfer of both tumor-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell populations induced better clinical outcomes in melanoma patients than that of CD8<sup>+</sup> T cells alone, illustrating the importance of CD4<sup>+</sup> T cells in antitumor immunity.<sup>15</sup>

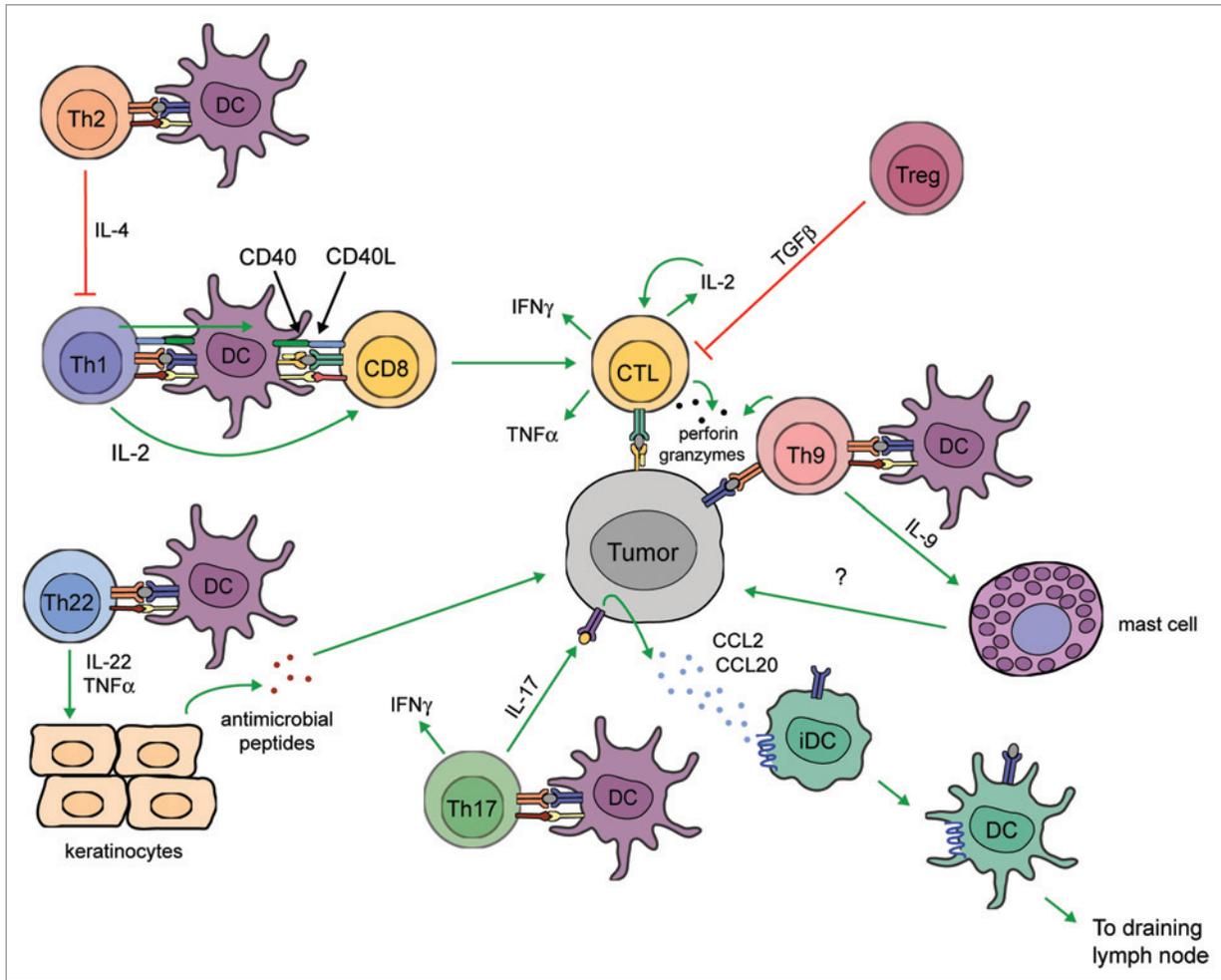
Although studies correlating CD4<sup>+</sup> T cells and cancer outcome have been performed in animal models, the characterization of CD4<sup>+</sup> cells in human melanoma is understudied and controversial. T<sub>H</sub>1 cells play a protective role in antitumor immunity in animal models by enhancing the cytolytic activity of CD8<sup>+</sup> cells, whereas—according to most reports—T<sub>H</sub>2 cells contribute to tumor progression.<sup>63,64</sup> Similar findings have been reported

in the human setting, in which CTL responses were enhanced by T<sub>H</sub>1 and T<sub>H</sub>0 cells, but not by T<sub>H</sub>2 cells.<sup>65</sup> Additionally, elevated serum levels of IL-4, IL-6 and IL-10 and lower levels of IFN $\gamma$  and IL-2 have been found in patients with malignant melanoma, suggesting that an imbalance between T<sub>H</sub>1 and T<sub>H</sub>2 cytokines contributes to melanoma development.<sup>64</sup> Interestingly, the prognostic value of IFN $\gamma$  levels failed to reach statistical significance in regressing melanomas, suggesting that IFN $\gamma$  may not be a reliable marker for favorable outcome.<sup>66</sup> Nevertheless, IFN $\gamma$  production is often used as a marker to select for tumor-reactive T-cell populations from TILs. At least two clinical cases of tumor regression in patients treated with melanoma-reactive CD4<sup>+</sup> TILs have been reported, with one case resulting in long-term complete remission.<sup>51,67</sup> In both studies CD4<sup>+</sup> TILs were expanded *in vitro* and selected for reactivity to melanoma antigens based on IFN $\gamma$  production, indicating that tumor-specific CD4<sup>+</sup> cells were predominantly T<sub>H</sub>1 cells.

Unlike T<sub>H</sub>1 cells, which are traditionally regarded as the CD4<sup>+</sup> helper/effector cells mainly involved in antitumor responses, the role of T<sub>H</sub>17 cells in tumor immunity is unclear. Tumor-specific T<sub>H</sub>17 cells eradicated transplanted B16 tumors in mice more efficiently than tumor-reactive T<sub>H</sub>1 cells, suggesting a superior protective role for T<sub>H</sub>17 cells in tumor immunity.<sup>68</sup> Surprisingly, the antitumor effects of T<sub>H</sub>17 cells were dependent on IFN $\gamma$  production, as the depletion of IFN $\gamma$  but not that of IL-17A or IL-23, inhibited tumor rejection. Furthermore, T<sub>H</sub>17 cells induced the production of CCL2 and CCL20 by tumor cells, leading to the recruitment of dendritic cells (DCs), which were able to take up tumor antigens and migrate to draining lymph nodes to activate tumor-specific CD8<sup>+</sup> T cells (Fig. 2).<sup>69</sup> In humans, T<sub>H</sub>17 cells have been shown to play a protective role in ovarian and prostate cancers but a pathogenic role in hormone-resistant prostate cancer.<sup>70–72</sup> Patients with hormone-resistant prostate cancer had high levels of circulating T<sub>H</sub>17 cells prior to immunotherapy, and this correlated with faster disease progression.<sup>72</sup> In other types of cancer, the prevalence of peripheral blood T<sub>H</sub>17 cells appears to be comparable to that of healthy individuals, suggesting that elevated levels of circulating T<sub>H</sub>17 cells may indicate an underlying infection.<sup>70</sup> Thus, the criteria for patient selection may account for conflicting results across different studies. This said, additional efforts to investigate the impact of T<sub>H</sub>17 cells on the survival of melanoma patients are warranted.

Naturally occurring regulatory T cells (Tregs), which express CD25 and the transcription factor FOXP3, are also present in the tumor microenvironment. Tregs suppress the proliferation and activation of effector T cells and their abundance correlate with poor prognosis in melanoma patients. Accordingly, treatments that overcome the suppressive effects of Tregs or reduce their frequency in the peripheral blood improve patient survival (Fig. 2).<sup>73–75</sup> Combination therapies that boost the responses of tumor-reactive T cells while removing the suppressive effects of Tregs have shown encouraging results in animal models, but these still need to be confirmed in humans.<sup>76</sup>

T-cell subsets implicated in skin immunity may further complicate the identification of protective T cells in melanoma (Fig. 2). In animal models, IL-9-producing T (T<sub>H</sub>9) cells



**Figure 2.** Functional T-cell populations in tumor immunity. Antitumor T-cell functions: Cytotoxic CD8<sup>+</sup> T lymphocytes (CTLs) directly kill tumor cells by releasing cytotoxic granules containing perforin and granzymes and secrete interleukin (IL)-2, interferon (IFN) $\gamma$ , and tumor necrosis factor (TNF) $\alpha$ , which contribute to antitumor functions in several ways. IL-2 promotes CD8<sup>+</sup> cell survival and proliferation. IFN $\gamma$  enhances CD4<sup>+</sup> T<sub>H</sub>1 cell differentiation, inhibits angiogenesis, and activates antitumor biochemical pathways in macrophages and dendritic cells (DCs) (not shown). TNF $\alpha$  directly induces tumor cell death and promotes CD8<sup>+</sup> cell proliferation and recruitment. CD4<sup>+</sup> T<sub>H</sub>1 cells promote antitumor responses by secreting IL-2 and IFN $\gamma$  and prime DCs to activate CD8<sup>+</sup> T cells through CD40-CD40 ligand (CD40L) interactions. T<sub>H</sub>17 cells secrete IFN $\gamma$  and IL-17, the latter of which induces tumor cells to release CCL2 and CCL20, which promote DC recruitment to tumor tissues. DCs bearing tumor antigens migrate to draining lymph nodes and activate tumor-specific CD8<sup>+</sup> T cells. In mice, T<sub>H</sub>9 cells directly kill tumor cells through the release of granzymes and secrete IL-9, which promotes antitumor responses through an unknown mechanism that involves mast cells. T<sub>H</sub>22 cells secrete IL-22 and TNF $\alpha$ , which activates keratinocytes to produce antimicrobial peptides that exhibit antitumor activity. Pro-tumor T-cell functions: T<sub>H</sub>2 cells facilitate tumor growth by inhibiting the functions of T<sub>H</sub>1 cells. Naturally occurring regulatory T cells expressing CD25 and FOXP3 also promote tumor cell growth by suppressing the proliferation and activation of effector T cells.

demonstrated cytolytic activity against B16F10 melanomas and recombinant IL-9 inhibited tumor growth in a mast cell-dependent manner, suggesting a role for T<sub>H</sub>9 cells and IL-9 in antitumor immunity.<sup>77</sup> IL-2 producing T (T<sub>H</sub>22) cells may also promote antitumor immune responses by activating keratinocytes to produce antimicrobial peptides, which can contribute to antitumor immunity.<sup>78,79</sup> Lastly, CD1-restricted T cells, in particular CD1a-restricted T cells, recognize lipid antigens presented by Langerhans cells to produce IL-22 and mediate epidermal immunity.<sup>80</sup> A role for CD1-restricted T cells has not been described in the context of melanoma, but given the role of these cells in maintaining epidermal homeostasis, further studies are warranted. The relative contribution of understudied T cells

to melanoma should be evaluated before considering the use of these cells for immunotherapy.

### Concluding Remarks

Metastatic melanoma continues to be associated with a high mortality rate despite the development of novel therapeutic strategies, and current therapies can result in significant morbidity. An overriding goal of these therapies is to liberate endogenous immunity with minimal collateral damage. Achieving this goal will result from identifying the most effective T-cell populations with regard to phenotype, persistence in vivo, antigen specificity and function, as all these parameters can influence tumor regression.

This said, the selection of T cells solely based on cell surface phenotype is an unreliable approach for predicting clinical outcomes and functional studies should always be performed to confirm the efficacy of T cells for immunotherapy. Multifunctional T cells, as determined by cytokine production and granule release, may constitute promising mediators of antitumor responses, as this has been previously shown for microbial infection. An approach that is optimal with regards to all these parameters and/or that simultaneously targets several melanoma antigens may generate

the host response that mediates tumor regression in the greatest number of patients.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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