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Combination Immunotherapies Implementing Adoptive T Cell Transfer for Advanced-Stage Melanoma

Kendra C. Foley¹, Michael I. Nishimura¹, and Tamson V. Moore¹

¹Department of Surgery, Cardinal Bernardin Cancer Center, Loyola University Chicago, Maywood, IL, 60153

Abstract

Immunotherapy is a promising method of treatment for a number of cancers. Many of the curative results have been specifically seen in advanced stage melanoma. Despite this, single agent therapies are only successful in a small percentage of patients, and relapse is very common. As chemotherapy is becoming a thing of the past for treatment of melanoma, the combination of cellular therapies with immunotherapies appears to be on the rise in *in vivo* models and in clinical trials. These forms of therapies include TIL, TCR or CAR modified T cells, cytokines (IL-2, IL-15, IL-12, GM-CSF, TNF- α , IFN- α , IFN- γ), antibodies (α PD-1, α PD-L1, α TIM-3, α OX40, α CTLA-4, α LAG-3), dendritic cell-based vaccines, and chemokines (CXCR2). There are a substantial number of ongoing clinical trials using two or more of these combination therapies. Preliminary results indicate that these combination therapies are a promising area to focus on for cancer treatments, especially melanoma. The main challenges with the combination of cellular and immunotherapies are adverse events due to toxicities and autoimmunity. Identifying mechanisms for reducing or eliminating these adverse events remains a critical area of research. Many important questions still need to be elucidated in regards to combination cellular therapies and immunotherapies, but with the number of ongoing clinical trials, the future of curative melanoma therapies is promising.

Keywords

Metastatic melanoma; immunotherapy; adoptive transfer; T cell; CAR; checkpoint; targeted therapy; combination therapy; cytokine

Introduction

The incidence of melanoma has been rising steadily since the 1950s, with over 200,000 new cases diagnosed worldwide in 2012, of which half were in patients under 65 years of age [1]. Once metastasized, mortality from melanoma is high, leading to approximately 50,000 deaths every year [2]. Historically, treatment for unresectable metastatic melanoma has been chemotherapy, primarily with dacarbazine, to which only about 10–20% of patients

Correspondence should be addressed to K.C.F. (kfoley5@luc.edu), Kendra Foley, M.S., Ph.D. Candidate, Department of Surgery, Oncology Research Institute, Loyola University Chicago, 2160 S. 1st Ave, Bldg 112, Room 308, Maywood, IL 60153, Phone: 708-327-2340, Fax: 708-327-3158, kfoley5@luc.edu.

responded and which generated less complete responses in less than 5% of patients [3]. In the past three decades, multiple immunotherapy approaches have been developed to treat melanoma. Melanoma is particularly susceptible to immunotherapy approaches, probably due in part to a high mutational burden [4]. However, immunotherapies as single agents do not induce complete responses in the majority of patients. In heavily pretreated patients or patients with extensive disease, single agent immunotherapy does not induce even partial responses in most patients.

The presence of tumor-reactive T cells, pre- and post-therapy, has been associated with the success of certain immunotherapies, most notably checkpoint blockades [5–8]. Though studies have shown that many melanoma patients have pre-existing populations of tumor-reactive T cells [9], the size, diversity, and TCR affinity of this pool varies, and many of these T cells are inhibited, exhausted or anergic [10–12]. For example, studies have found pre-existing T cells reactive to tumor-specific mutated self-antigens (neoantigens) in 62.5–75% of patients, but these cells are not sufficient to mediate melanoma regression [13, 14]. When patients do not have functional melanoma-antigen-specific T cells bearing high-affinity TCRs, T cell therapies can deliver such T cell populations by either expanding pre-existing anti-tumor T cells in an immunostimulatory environment or by using gene-therapy to alter T cells to become melanoma-specific with a high-affinity TCR. However, these T cells, when returned to the patient, have at best a 55% response rate, with more typical response rates between 10–20%. Mechanisms such as tumor expression of T-cell-suppressive molecules and antigen or antigen expression loss from the tumor have been shown to contribute to the ineffectiveness of T cell-mediated therapies [15–17]. Alleviation of tumor-mediated suppression, and overcoming tumor evasion are goals of many immunotherapy trials. Immunotherapy can also enhance epitope spreading and the activation of bystander T cells reactive to different tumor antigens, thereby bypassing primary antigen loss by the tumor. Combining T cells that target the tumor with immunotherapies to overcome suppression thereby has the potential to substantially enhance the efficacy of both therapies.

Combination Therapies

T Cell Therapies

Tumor infiltrating lymphocytes (TIL) were detected in melanoma lesions over forty years ago, but their significance was not determined until IL-2 was discovered and found to permit long-term culture and restore lytic anti-tumor function of T cells [18–20]. Adoptive T cell transfer (ACT) of autologous TIL with high-dose IL-2 was the first clinical trial to show that tumor-reactive T cells could mediate melanoma regression, with an objective response rate (ORR) of 55% (11/20 patients) [21]. TIL cannot be isolated from all patients, therefore researchers have also utilized expanded tumor-reactive T cells from peripheral blood lymphocytes (PBL) in clinical trials with ORRs of 0–30% (Supplemental Table 1) [22, 23]. Not all patients have functional anti-melanoma T cells, and further studies isolated melanoma-reactive T cells from which they cloned T cell receptors (TCRs) for therapeutic use [24]. With the cloning of tumor-specific TCRs and the subsequent generation of chimeric antigen receptors (CARs, hybrid receptors with antibody-derived binding domains

linked to TCR-and costimulatory receptor-based signaling domains), CAR and TCR gene-modified T cells made their way into the clinic with efficacies up to 55% [25–27] and ORRs of 57% for a CD19-targeting CAR in refractory B cell leukemia [28, 29]. There exist limitations for the effectiveness of CAR T cells in solid tumors such as melanoma. Some of these limitations include trafficking to the tumor, tumor heterogeneity, and the immunosuppressive tumor microenvironment [30, 31]. One mouse model reported an anti-tumor response and increased T cell infiltrate into a B16 melanoma using T cells expressing an anti-VEGFR2 CAR [32]. In a clinical trial GD-2 specific CAR T cells failed to persist in patients resulting in no anti melanoma effect [33, 34]. Because suppression and exhaustion of T cells can result in failed CAR T cell persistence, combination with of CAR T cells with antibody mediated therapies or oncolytic viruses to deliver cytokines, chemokines, or co-stimulatory molecules could be beneficial to create a more favorable tumor microenvironment [30, 35]. Studies demonstrate that ACT using TIL or TCR gene-modified T cells can mediate strong anti-tumor responses but does not mediate tumor regression in most metastatic melanoma patients.

Cytokine and T cell combinatorial therapies

Various cytokines, including IFN- α , IFN- γ , IL-2, IL-7, IL-12, IL-15, IL-21, and TNF- α have been investigated for treatment of melanoma, both alone (Table 1) [36–47] and in conjunction with T cells (Supplemental Table 1) [23, 48–52]. Preclinical trials in mice demonstrated that human T cells and IL-2 could mediate regression of human melanoma xenografts [53]. Further studies found that treatment of melanoma with tumor-specific T cells was enhanced by co-delivery of IL-12, IFN- α , IFN- γ , and TNF- α [54–60]. In addition to pro-inflammatory cytokines, experiments have also targeted inhibitory cytokines: TGF- β is an immunosuppressive cytokine produced by some melanomas and engineering T cells to be resistant to TGF- β signaling augmented T cell mediated melanoma regression in a murine model [61]. After murine studies demonstrated anti-melanoma efficacy of ACT in conjunction with these cytokine-based therapies, clinical trials were performed or are being performed to determine the efficacy of these combinations in patients.

In patients, ACT with autologous TIL and high-dose IL-2 had ORRs of 34–55% [21, 37]. High-dose IL-2 treatment induces moderate to severe toxicities in patients and promotes T_{reg} proliferation [48, 62]. To reduce toxicities from IL-2, ACT of TIL with reduced doses of IL-2 has been tested and found to induce clinical responses with less toxicity than high-dose IL-2 [50, 51]. To enhance T cell persistence post-transfer, non-myeloablative lymphodepletion was used to precondition patients prior to T cell transfer. Lymphodepletion with cyclophosphamide and fludarabine enhanced the ORR of ACT from 34% to 49% (Supplemental Table 1), possibly due to reduced T_{reg} cells [63] and higher levels of free IL-7 and IL-15 [64]. Adding total body irradiation (TBI) to the chemotherapeutic lymphodepletion increased the response rate to 72% (Supplemental Table 1) in one study but a later study of a larger cohort found comparable complete response rates with and without TBI [65]. As lymphodepletion can lead to life-threatening infections, IL-15 was tested for support of TIL cells, but the trial was canceled due to autoimmune toxicity (NCT01369888). Substantial toxicity of IL-15 was also noted in a clinical trial treating melanoma patients with i.v. IL-15 alone [41]. Currently, there are several open clinical trials treating melanoma

patients with IL-15 treatment subcutaneously or in altered formats (NCT01727076, NCT02452268, NCT01946789) but no trials with ACT. If the toxicity of IL-15 can be reduced to a manageable level, then it is likely to be tested with ACT, but until then, IL-2 remains the standard cytokine delivered with any ACT.

The pro-survival effects of IL-2 and IL-15 on T cells have been extensively studied. However, these are not the only cytokines that can enhance T cell-mediated tumor regression. IFN- α has been used alone as an effective adjuvant therapy to prevent relapse of resected melanoma [66]. IFN- α may enhance T cell responses through upregulation of MHC class I and tumor antigen expression [67] and by reducing the number of circulating T_{reg} cells [68]. In combination with melanoma-stimulated PBL-derived T cells, IFN- α has been utilized to treat patients with a 20% ORR (1/10 complete response (CR), 1/10 partial response (PR)), without lymphodepletion or IL-2 [23]. As well as IFN- α , IFN- γ has been tested with ACT. An adenovirus expressing IFN- γ was delivered to melanoma patients alongside TIL and IL-2 without lymphodepletion, with an overall response rate of 38.5% (3/13 CR, 2/13 PR) and no grade 4 adverse events [49]. These trials provide evidence that delivering T cells in combination with IFN- α or IFN- γ instead of IL-2 or lymphodepletion may promote anti-tumor responses with reduced toxicities. IL-12 is a potent immunostimulatory cytokine that enhances T cell activation and cytolytic function. Unfortunately, two trials delivering tumor-reactive T cells engineered to produce IL-12 have been terminated, one of which cited unexpected toxicity (Supplemental Table 1). GM-CSF has been utilized with ACT of invariant natural killer T (iNKT) cells to treat patients with minimal residual melanoma disease [69]. GM-CSF co-treatment did not increase the percentage of progression-free patients after treatment with iNKT cells, but the study size was too small for significance to be determined. The transfer of iNKT with GM-CSF was tolerable and safe and functional iNKT persisted in patients. Future trials may provide more information.

Research is still ongoing on the roles of lymphodepletion and cytokine treatment in the efficacy of T cell therapies. 32 recruiting clinical trials use lymphodepletion with T cells and 28 trials utilize cytokines with T cells. All open trials of ACT with cytokines use IL-2; one trial adds pegylated interferon- α 2b and another uses T cells rendered insusceptible to TGF- β signaling (Table 3). Results from these trials will help identify how cytokine supplementation or resistance may enhance the efficacy of ACT in treatment of metastatic melanoma.

T cell therapies with vaccines

Although there are individual cases where patients respond, vaccination strategies as single therapies for metastatic melanoma have had little clinical benefit compared to standard of care [70, 71]. These vaccination strategies often lead to increases in the frequency of melanoma-reactive T cells without changing patient survival times. Unfortunately, melanoma-reactive T cells in patients are often nonfunctional [11] and do not protect from tumor progression in patients with extensive disease [12]. In patients with minimal residual disease, two recently published studies provide evidence that vaccination strategies targeting neoantigens may induce the expansion of neoantigen-specific T cells and protect patients

from tumor recurrence [72, 73]. Notably, in one of these studies, when patients did have tumors grow after neoantigen-specific vaccination, PD-1 blockade was highly effective, causing complete remissions in both patients treated after progression, suggesting that tumor-specific T cells may have been suppressed by PD-1 signaling.

While vaccine strategies alone have not been effective treating metastatic melanoma, vaccines could augment the activity of ACT or TIL or gene-modified T cells. Combining ACT with vaccinations had promising results in preclinical studies; established tumors could be killed by combinations of gp100 specific T cells with a fowlpox-based viral vaccine [74, 75] and with a GM-CSF-producing tumor-based vaccine [76]. In the clinic, vaccination with tumor cells, dendritic cells loaded with tumor lysates or with peptides, vaccination with replication-incompetent viruses carrying tumor-specific antigens, and vaccinations with peptides in adjuvant have all been tested in conjunction with TCR gene-modified T cells with reported objective response rates up to 55% (4/20 CR, 7/20 PR) (Supplemental Table 1). However, these studies lack the statistical power to discriminate the effects of the vaccine from that of the T cells. Ongoing research, including four current clinical trials testing dendritic-cell-based vaccinations in conjunction with ACT, may provide more evidence on the role of vaccination in promoting T cell responses in patients.

Combining T cell therapies with migration-modifying therapies

In addition to becoming activated and expanding sufficiently, tumor-reactive T cells must also migrate into the melanoma lesions to mediate functional responses. The chemokine ligand CXCL1 (MGS1) is expressed by approximately half of metastatic melanomas [77] and is thought to contribute to melanoma growth and metastasis [78, 79]. As CXCL1 induces migration of CXCR2-expressing cells into melanoma lesions, investigators introduced CXCR2 into tumor-specific T cells and found that these cells migrate to tumors and mediate more tumor regression in a murine melanoma model [80, 81]. One open clinical trial is treating patients with TIL transduced with CXCR2 (NCT01740557). Results from this study will help determine the clinical efficacy of enhancing tumor-targeting with CXCR2.

Combining antibody-mediated therapies with T cell therapies

Antibodies, most notably those blocking immune checkpoints, are promising agents for immunotherapy of melanoma, with single-agent ORRs up to 40% (Table 2). In mice, types of antibodies that enhance T cell responses include agonist antibodies that activate stimulatory receptors on T cells such as CD40 [82] and OX40 [83] and antagonist antibodies that block inhibitory receptors on T cells (checkpoint blockade), such as PD-1 [84], PD-L1 [85], and CTLA-4 [76, 86, 87]. Some of these potential therapies are not yet tested in conjunction with ACT in the clinic, including CD40 and TIM-3. Others, including OX40 agonist antibody treatment and PD-1 blockade, are being tested in clinical trials but results have not yet been published (Table 3). Finally, some combinations of antibodies with ACT have been tested with published results, including LAG-3 blockade which had little clinical efficacy [88], and CTLA-4 blockade, on which several studies have been published and are discussed below.

Evidence from clinical trials suggests that tumor regression in response to CTLA-4 blockade depends on the T cell repertoire prior to treatment [89], thus studies have focused on treating patients who received T cells with CTLA-4 blockade. One study performed a retrospective analysis of metastatic melanoma patients given ipilimumab after progression following TIL treatment [90]. Ipilimumab-treated patients who had previously received and progressed after TIL therapy had a 16% response rate (3/19 patients), not dissimilar from patients who receive ipilimumab without prior TIL therapy [91]. However, all three responding patients had durable (>17 months) complete responses, which is unusual for ipilimumab. Ipilimumab has a complete response rate of about 1.5%. In patients who received ipilimumab then TIL, the response rate was 38% (5/13), comparable to the 40% ORR observed in the study as a whole. The frequency and type of toxicities of TIL therapy did not increase with prior ipilimumab treatment nor did the rate or type of toxicities of ipilimumab treatment with prior TIL treatment. In another study, patients were given MART-1-specific PBL T cells with an ORR of 11% (1/9 PR). Five patients who progressed after T cell therapy were given ipilimumab, with an unusually high ORR of 60% (3/5 PR) and stable disease in the other two patients [92]. In both studies, delivery of ipilimumab after a T cell therapy had higher than usual efficacy, suggesting that ACT prior to ipilimumab therapy may increase the efficacy of ipilimumab therapy. Further and larger clinical trials with appropriate controls will help determine whether this effect is reproducible.

Another published study of 10 melanoma patients given ACT with IL-21-treated MART-1-reactive PBL-derived T cells and ipilimumab found a 30% ORR (2/10 CR, 1/10 PR) [52, 93], higher than the ORRs of 0–20% observed in prior trials utilizing MART-1-reactive PBL-derived T cells without ipilimumab or IL-21-treatment [94, 95]. Epitope spreading was observed in responders, which might have contributed to the efficacy of the therapy. Although these studies incorporate relatively few patients, they suggest that treating patients with T cells and CTLA-4 blockade may enhance the response rate of either therapy alone without inducing treatment-limiting toxicities.

Although a growing body of clinical evidence supports a strong role for pre-existing T cell responses in the success of PD-1 blockade [6, 96–98], there are currently no publications describing the results of receiving PD-1 therapy concurrent with or after T cell therapy. Considering PD-1 treatment prior to T cell therapy, in one recent study, eleven melanoma patients receiving TIL had failed prior therapy with PD-1 blockade [65]. The ORR of these 11 patients was 44% (2/11 CR, 2/11 PR). In the same study, the ORR in all patients was 47% (24/99 CR, 23/99 PR), indicating that prior exposure to PD-1 blockade does not preclude responses to TIL therapy. Melanoma-reactive T cells express PD-1 post-TIL-treatment [99], and results from five open trials of ACT and PD-1 blockade will help determine whether treatment with PD-1 blockade enhances responses to T cell therapies.

Targeted therapies and ACT

Targeted therapies can block specific signaling pathways that are overexpressed or overly active in melanoma cells. These pathways drive cell proliferation and/or survival so blocking them in patients that overexpress these pathways often reduces melanoma growth or induces regression. Studies in mice have shown that combining checkpoint blockade of PD-L1 and

MEK inhibition resulted in reduced tumor growth associated with enhanced T cell infiltration of the tumor [100], suggesting that blocking some of these signaling pathways might result in enhanced T cell mediated tumor destruction. Further studies in mice demonstrated that blocking VEGF did enhance T-cell mediated melanoma regression, associated with enhanced T cell infiltration of the tumor [32, 101]. In melanoma patients, combining VEGF-blockade and ipilimumab resulted in increased T cell infiltration of tumors [102] as did combined BRAF and MEK blockades [103]. In one pilot trial, treating melanoma patients with BRAF inhibitor (vemurafenib) and autologous TIL therapy demonstrated that the combination was effective, with an ORR of 64%, comparable to TIL therapy alone [104]. This combination had manageable toxicities comparable to those of TIL therapy and vemurafenib as single agents [105]. Taken together, these trials suggest that targeted therapies may enhance T cell infiltration of the tumor but their effect on transferred tumor-reactive T cells remains unclear. There are currently other targeted therapies that remain attractive for combination with immunotherapies. In murine models, combined inhibition of IDO and checkpoint blockade resulted in augmenting T cell resistance to anti-CTLA-4 and T cell dependent synergy [106]. Combination of pembrolizumab and the IDO inhibitor (epacadostat) are currently being tested in a phase III clinical trial in melanoma (NCT02752074) [107]. In order to enhance type I interferon immune responses, STING agonists have appeared to be promising in mouse models by reducing immune escape and enhancing the anti-tumor response in combination with CAR-T cells, and synergizing with checkpoint inhibitors [108–110]. In lung cancers, combination of tyrosine kinase inhibitors with checkpoint blockade are currently being tested in the clinic [105]. Canonical WNT signaling in melanoma can cause immune evasion and accumulation of T_{regs}. Combination of porcupine inhibitor WNT-C59 with anti CTLA-4 has shown synergistic effects in a pre-clinical model [105]. The combination of these targeted therapies with immunotherapies are expected to be examined in upcoming clinical trials.

Conclusions & Future Directions

Benefits of combined ACT and immunotherapy

Combining T cell therapies with other immunotherapies that stimulate immune responses can have a number of benefits. First, the immunotherapy has the potential to enhance transferred T cell efficacy by ameliorating the known immunosuppression induced by the tumor. Immunotherapies can ameliorate immunosuppression either directly by inhibiting immunosuppressive mechanisms (checkpoint blockade, blocking immunosuppressive cytokines such as TGF- β), or indirectly by promoting immunostimulatory mechanisms (vaccination, stimulatory cytokines including IL-2, IFN- α) that override tumor-mediated immunosuppression. Second, the T cells may enhance the efficacy of the immunotherapy by producing inflammatory cytokines and reducing tumor burden by direct lysis. Third, the combination of T cell therapy and immunotherapy may enhance epitope spreading, in which novel T cells are recruited to attack the tumor. Epitope spreading may be enhanced as transferred T cells lyse tumor cells in an immunostimulatory environment provided by both the T cells and additional immunotherapy. Epitope spreading has already been observed after combined T cell therapy and CTLA-4-blockade [52]. Fourth, transferred T cells may be

genetically labeled or otherwise identifiable and thus provide a monitoring mechanism for the efficacy of the immunotherapy on tumor-reactive T cells.

Optimally, combining T cells and immunotherapy may help treat patients with widespread disease and large tumor burdens, which otherwise would likely escape a single therapy. In murine studies, it has been shown that while single therapies cannot induce regression of large established tumors, combinations of T cells and other therapies, including cytokines and vaccination, may mediate regression of large, established, poorly immunogenic tumors [111]. At least one recent clinical trial demonstrated success treating metastatic melanoma patients with tumors refractory to single therapies with a combination therapy [52]. It should also be pointed out that there are ongoing studies exploring the use of tumor exome analysis by next generation sequencing that will soon help determine what tumors may be susceptible to or resistant to, and these studies will help inform medical practitioners as to which combinations of therapies have the greatest potential for success.

Potential drawbacks of combining T cells with other immunotherapies

There are potential drawbacks to combining T cells and other immunotherapies. Combining therapies may not provide additional benefit. It is possible that when used together, one therapy may compromise the efficacy of the other. For example, T cell therapies utilize lymphodepletion prior to transfer to achieve clinical efficacy, which may deplete the endogenous anti-tumor T cells that mediate responses after checkpoint blockades. More clinical trials will be necessary to follow the fate of endogenous anti-tumor T cells upon lymphodepletion for adoptive transfer of T cells, the role of lymphodepletion, and the possibility of using cytokine therapies or checkpoint blockade instead of lymphodepletion. Another important concern is that combining therapies may cause additional toxicity that may make the treatment intolerable to many, if not all, patients. Both T cell-mediated and immunotherapy-mediated toxicities include general inflammation and T cell attack of healthy tissues, and these may be additive in the combination of these therapies. Three studies incorporating IL-12 and IL-15 with T cells were terminated, two of which cite toxicity (NCT01236573, NCT01369888, NCT01457131). IL-12 and IL-15 have both been shown to induce substantial toxicity as single agents. Conversely, a study of checkpoint blockade in combination with T cells did not find additional or intolerable toxicities [90]. Therefore, toxicity is not a foregone conclusion but must be investigated independently in each combination of immunotherapy and T cell transfer. Some of the toxicity will almost certainly be dependent on T cell specificity, as was the case with toxicities in the eye, ear and skin seen in clinical trials of autologous T cells gene-modified with melanoma/melanocyte-specific TCRs [112]. A third concern of combining therapies is expense, as both immunotherapies and adoptive transfer therapies are currently high-cost therapies due to the need to create a personalized product for each patient, derived from their own T cells. However, the price of these therapies will decrease with time and with more efficient production methods that are developed as the number of patients treated increases.

Future directions

Current clinical trials are largely focused on activated $\alpha\beta$ T cells and prominent immunotherapies such as PD-1 and CTLA-4 blockade. However, researchers are

investigating other subsets of T cells and other mechanisms by which tumors suppress T cells. Numerous mechanisms have been identified that may prevent T cell therapies from eradicating cancer, even when utilizing T cells that demonstrate robust anti-tumor responses *in vitro*. The tumor microenvironment has been found to be highly immunosuppressive for a variety of reasons. Tumors produce and/or express a variety of immunosuppressive molecules including inhibitory receptors (e.g. PD-L1, [113]), cytokines (TGF- β , [114]), and small molecules such as indoleamine 2,3 dioxygenase (IDO) [115, 116]. Tumors recruit and maintain immunosuppressive cells, including myeloid-derived suppressor cells [117] and T_{reg} [118]. In addition, tumors may be hypoxic and have low glucose levels, further reducing T cell function [119]. Collectively, these factors combine to reduce T cell responses to melanoma tumors.

Metabolic modification and ACT

Solid tumors produce hypoxic low-glucose microenvironments that are thought to be immunosuppressive in part because the low levels of available oxygen and glucose may curtail T cell activation and proliferation. Interestingly, metastases in the lungs are in a high-oxygen environment and thus might be expected to be more immunogenic. However, the high levels of oxygen in the lungs were shown to activate oxygen-sensitive prolyl-hydroxylase (PHD) proteins which down-regulate HIF-1 α in T cells. Reduced HIF-1 α was shown to increase iT_{reg} differentiation, reduce Th1 differentiation, and reduce IFN- γ production by CD8⁺ T cells [120]. Increasing HIF-1 α expression in T cells was found to enhance T cell-mediated control of melanoma growth in two murine melanoma models [120, 121]. Higher HIF-1 α expression does not always enhance T cell responses. In another model, the hypoxic tumor microenvironment or *in vitro* culture in a hypoxic environment led to upregulation of HIF-1 α in T cells, which induced upregulation of LAG-3 (a suppressive receptor), reduced T cell polyfunctionality, and reduced Tbet expression [122]. Finally, in this model, a 30% down-regulation of HIF-1 α by transduction with shRNA led to increased anti-tumor responses as seen by a delay in tumor growth. Overall, there is more research to be done on how oxygen-sensing receptors and downstream signaling interact with T cell activation and T cell anti-tumor responses to identify how to best manipulate these pathways to augment anti-tumor immunity.

Reactive oxygen species (ROS), of which there may be high levels in melanoma tumors, have also been found to inhibit tumor-reactive T cells. T cells expressing more thiols, a marker of having higher antioxidant levels, have been found to tolerate lower glucose levels and be more effective at controlling melanoma growth in a murine model [123]. Treatments that reduce ROS during activation can enhance survival of both murine and human T cells, including TIL from melanoma patients [124]. These preclinical experiments provide substantial evidence that factors that regulate T cell metabolic status, particularly T cell susceptibility to hypoxia and/or hypoglycemia, can modulate T cell anti-tumor responses. In the future, T cells may be cultured in conditions and/or genetically modified in ways that support T cell survival in the tumor microenvironment.

Alternative T cell subsets for ACT

Different T cell subsets may have differential success targeting tumors in spite of tumor-mediated immunosuppression—naïve T cells have been shown to control tumor growth more than memory-derived T cells in a murine tumor model [125]. Even the presence of memory T cells during *in vitro* activation of naïve cells reduced T cell control of melanoma growth [126]. As most melanoma patients have pre-existing T cell populations in their tumors, the memory component of these pre-existing populations may influence the efficacy of adoptively transferred T cell therapies as might memory cells pre-existing in TIL or in PBMC genetically modified to generate tumor-specific T cells. Collectively, there are many mechanisms by which tumors suppress T cell-mediated destruction, and developing T cell therapies that bypass this suppression is a focus of research.

In addition to researching the relative anti-tumor efficacy of subsets (naïve, activated, memory) of $\alpha\beta$ T cell therapies, there is ongoing research investigating the efficacy of therapy with non- $\alpha\beta$ subsets of T cells. There is preclinical and clinical evidence suggesting $\gamma\delta$ T cells may play a role in tumor immunity [127, 128]. As they have little or no alloreactivity, can present antigen to other T cells, and may have some endogenous anti-tumor reactivity, $\gamma\delta$ T cells are considered a promising target population for TCR or CAR transfer [129, 130]. As previously mentioned, iNKT cells have been utilized in one clinical trial, in which the cells were well tolerated and there was modest evidence of *in vivo* reactivity [69]. As evidence is accumulated on the anti-tumor efficacy of different subsets, different subsets of T cells may be isolated for use in clinical trials of ACT for metastatic melanoma.

Innate cells and ACT

As combination therapies of T cells and other therapies are developed and tested, more information is emerging on the role of innate cells in these therapies. It has been shown that responders to CTLA-4-blockade and PD-1-blockade have fewer circulating MDSCs than nonresponders [131–133] and that the percent of MDSCs present in the blood decreases in responders to ipilimumab but increases in response to nivolumab [97, 134]. Vemurafenib can also reduce the frequency of circulating MDSCs in melanoma patients [135]. Treatment with agents that regulate MDSC frequency, including aforementioned therapies or IDO inhibitors [116], might enhance the efficacy of ACT of melanoma. Endogenous NK cells have been found to be important in mediating immunotherapy-stimulated immune responses in murine models [136–138] and haploidentical NK cell transfer has been shown to be curative in some patients with acute myeloid leukemia [139]. Despite these results, autologous NK cell transfer as a single therapy had little clinical benefit in melanoma [140] and NK cell transfer has not yet been tested in conjunction with other immunotherapies in patients.

Summary

In conclusion, there are substantial benefits to combining T cells with other immunotherapies, and there are a number of clinical trials recruiting currently to test how effective these combinations are in treating refractory metastatic melanoma patients. Combining T cells with other therapies can help transform a tumor microenvironment from a “cold” immunosuppressive microenvironment to a “hot” immunostimulatory

microenvironment that may recruit and activate more T cells that lyse more tumor cells and induce more activation, ultimately enhancing cell-mediated tumor regression (Fig. 1, summary). However, while in some clinical trials, combining immunotherapies substantially improved the efficacy of immunotherapy-mediated tumor regression [141, 142], in other trials, combining multiple therapies did not improve results over a single therapy [143]. As results of current clinical trials are published, more information about the efficacy, the mechanisms, and predictors of successful treatment will become clear, permitting improved treatment of patients with metastatic melanoma, a disease that otherwise has a very poor prognosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Combination therapies for melanoma

Combination of adoptive cell transfer therapies with other therapies can augment anti-tumor responses by promoting the transformation of a cold (immunosuppressive) tumor microenvironment to a hot (inflammatory) tumor microenvironment.

Table 1

Cytokine Therapies

molecule	mechanism	best response	toxicities
IL-2	Stimulates T cell proliferation, activation, and CD8 ⁺ T cell memory differentiation	15% objective response 7% complete regression [37]	Capillary leak syndrome, hypotension, sinus tachycardia [39] [144]
TNF- α	Leads to hemorrhagic necrosis of tumor	42% complete response, 43% partial response [145]	transient hypotension, leucopenia [145]
IFN- α	Increases HLA class I expression on tumor, enhance dendritic cell maturation, limits T _{reg} activation	22% overall response 14% complete regression [3]	
IFN- γ	Activates T cells, NK cells, and macrophages, increase expression of HLA class I and class II on tumor cells	13% complete regression [44]	No grade 3–4 toxicities, mild flu-like symptoms, lymphopenia [44]
IL-15	Stimulates T cell and NK cell activation and proliferation, enhances survival of memory T cells	27% tumor shrinkage [41]	grade 3 hypotension, thrombocytopenia, and elevations of ALT and AST [41]
IL-12	Enhances CD8 ⁺ T cell activation, proliferation, and survival		
GM-CSF	Increases dendritic cell and macrophage activation,	5.7% objective response	

Table 2

Antibody-mediated immunotherapies

Molecule	Mechanism	Best response	Toxicities
PD-1	Inhibitory receptors on T cells	40% objective response [146, 147]	Autoimmune-like/inflammatory side-effects seen at skin, gastrointestinal, hepatic, pulmonary, mucocutaneous, and endocrine systems [147–149]
PD-L1	Ligand for PD-1 (PD-L1 on tumor cells)	39% overall response 5.7% complete response	Autoimmune-like/inflammatory side-effects seen at skin, gastrointestinal, hepatic, pulmonary, mucocutaneous, and endocrine systems [148, 149]
CTLA-4	Inhibitory receptors on T cells [150, 151]	28.5% overall response, 14% complete response [152, 153]	Skin lesions (rash, pruritus, and vitiligo), colitis, and less frequently hepatitis, hypophysitis, and thyroiditis [91, 149, 152, 154–156]
LAG-3	Decreases cytokine and proliferation of antigen specific CD4 ⁺ T cells	0% [7]	Hematologic toxicity, leucopenia, and CD4 ⁺ T cell lymphopenia [7]
TIM-3	Inhibitory receptor on T cells	Recruiting, NCT02817633	
OX40	Prevents apoptosis of T cells, increases cytokine production	0% PR [157]	Lymphopenia, fatigue, rash, flu-like symptoms [157]

Table 3

Recruiting Clinical Trials of Combination Therapies (As of 9/7/2017)

Clinical Trial Number & Title	Status	Phase	T cell source (specificity)	Other treatments	Results and publication
NCT00338377: Lymphodepletion Plus Adoptive Cell Transfer With or Without Dendritic Cell Immunization in Patients With Metastatic Melanoma	Recruiting	II	TIL	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine High-dose IL-2 Vaccination with MART-1-peptide-pulsed dendritic cells 	42% ORR (11/31 PR, 2/31 CR) [158]
NCT00604136: Treatment of Metastatic Melanoma Patients With Tumor Infiltrating Lymphocytes and IL-2 Following a Regimen of Non-myceloablative Lymphocyte Depleting Chemotherapy	Recruiting	II	TIL	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine High-dose IL-2 	N/A
NCT00910650: Adoptive Transfer of MART-1 F5 TCR Engineered Peripheral Blood Mononuclear Cells (PBMC) After a Nonmyeloablative Conditioning Regimen, With Administration of MART-126*35-Pulsed Dendritic Cells and Interleukin-2, in Patients With Advanced Melanoma	Recruiting	II	MART-1 F5 TCR-transduced PBL T cells	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine IL-2 Vaccination with MART-1 (126–135)-peptide pulsed dendritic cells 	0% ORR (0/13) [159]
NCT01586403: Transfer of Genetically Engineered Lymphocytes in Melanoma Patients: A Phase 1 Dose Escalation Study	Recruiting	I	PBL transduced with (1383) tyrosinase-reactive TCR	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine IL-2 	17% ORR (1/6 PR) (unpublished data)
NCT01740557: A Pilot Study of Lymphodepletion Plus Adoptive Cell Transfer With T-Cells Transduced With CXCR2 and NGFR Followed by High Dose Interleukin-2 in Patients With Metastatic Melanoma	Recruiting	I-II	TIL transduced with Nerve Growth Factor Receptor and CXCR2	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine High-dose IL-2 	N/A
NCT01807182: Cellular Adoptive Immunotherapy Using Autologous Tumor-Infiltrating Lymphocytes Following Lymphodepletion With Cyclophosphamide and Fludarabine for Patients With Metastatic Melanoma	Recruiting	II	TIL	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine High-dose IL-2 	N/A

Clinical Trial Number & Title	Status	Phase	T cell source (specificity)	Other treatments	Results and publication
NCT01883323: Phase II Study Evaluating The Infusion Of Autologous Tumor-Infiltrating Lymphocytes (TILs) And Low-Dose Interleukin-2 (IL-2) Therapy Following A Preparative Regimen Of Non-Myeloablative Lymphodepletion Using Cyclophosphamide And Fludarabine In Patients With Metastatic Melanoma	Recruiting	II	TIL	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine IL-2 	N/A
NCT01946373: A Phase I Study to Evaluate Safety, Feasibility and Immunologic Response of Adoptive T Cell Transfer With or Without Dendritic Cell Vaccination in Patients With Metastatic Melanoma	Recruiting	I	TIL	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine NY-ESO-1-peptide- and tumor-lysate-pulsed DC vaccination IL-2 	17% ORR (1/6 CR) [160]
NCT01955460: A Pilot Study of Lymphodepletion Plus Adoptive Cell Transfer With TGF-beta Resistant (DNRII) and NGFR Transduced T-Cells Followed by High Dose Interleukin-2 in Participants With Metastatic Melanoma	Recruiting	I	TIL transduced with dominant negative TGF-beta receptor (DNRII) and Nerve Growth Factor Receptor (NGFR)	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine High-dose IL-2 	N/A
NCT01967823: Phase II Study of Metastatic Cancer That Expresses NY-ESO-1 Using Lymphodepleting Conditioning Followed by Infusion of Anti-NY ESO-1 Murine TCR- Gene Engineered Lymphocytes	Recruiting	II	PBL transduced with NY-ESO-1-specific TCR	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine High-dose IL-2 	N/A
NCT01993719: A Phase II Study for Metastatic Melanoma Using High Dose Chemotherapy Preparative Regimen Followed by Cell Transfer Therapy Using Tumor Infiltrating Lymphocytes Plus IL-2 With the Administration of Pembrolizumab in the Retreatment Arm	Recruiting	II	Young TIL	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine High-dose IL-2 PD-1 blockade (pembrolizumab) 	N/A
NCT02070406: NY-ESO-1 TCR Engineered Adoptive Cell Transfer Therapy With CTLA4 Blockade	Recruiting	I	NY-ESO-1-transduced T cells	<ul style="list-style-type: none"> IL-2 NY-ESO-1 (157-165)-peptide-pulsed DC vaccination CTLA-4 blockade (ipilimumab) 	N/A

Clinical Trial Number & Title	Status	Phase	T cell source (specificity)	Other treatments	Results and publication
NCT02096614; Multi-center, Investigator Initiated Phase 1 Study of MAGE-A4 Specific TCR Gene Transferred T Lymphocytes With Solid Tumors	Recruiting	I	MAGE-A4-specific TCR-transduced PBL T cells [TBI-1201]	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine 	N/A
NCT02111850; Phase I/II Study of the Treatment of Metastatic Cancer That Expresses MAGE-A3 Using Lymphodepleting Conditioning Followed by Infusion of HLA-DP0401/0402 Restricted Anti-MAGE-A3 TCR-Gene Engineered Lymphocytes and Aldesleukin	Recruiting	I/II	MAGE-A3-specific-TCR-transduced PBL T cells	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine High-dose IL-2 	N/A
NCT02153905; Phase I-II Study of the Treatment of Metastatic Cancer That Expresses MAGE-A3 Using Lymphodepleting Conditioning Followed by Infusion of Anti-MAGE-A3 HLA-A*01 Restricted TCR-Gene Engineered Lymphocytes and Aldesleukin	Recruiting	I/II	MAGE-A3-specific TCR-transduced PBL T cells	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine High-dose IL-2 	N/A
NCT02278887; Randomized Phase III Study Comparing a Non-myceloablative Lymphocyte Depleting Regimen of Chemotherapy Followed by Infusion of Tumor Infiltrating Lymphocytes and Interleukin-2 to Standard Ipilimumab Treatment in Metastatic Melanoma	Recruiting	III	TIL	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine IL-2 CTLA-4 blockade (ipilimumab, given to different cohort than TIL) 	N/A
NCT02354690; T-cell Therapy in Combination With Vemurafenib for Patients With BRAF Mutated Metastatic Melanoma	Recruiting	I/II	TIL	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine IL-2 	N/A
NCT02360579; A Phase 2, Multicenter Study to Assess the Efficacy and Safety of Autologous Tumor Infiltrating Lymphocytes (LN-144) for Treatment of Patients With Metastatic Melanoma	Recruiting	II	TIL	<ul style="list-style-type: none"> Lymphodepletion with fludarabine and cyclophosphamide IL-2 	33% ORR (1/9 CR, 2/9 PR) [161] (Poster)
NCT02366546; Multi-center, Investigator Initiated Phase 1 Study of NY-ESO-1 Specific TCR Gene Transferred T Lymphocytes With Solid Tumors	Recruiting	I	NY-ESO-1-specific TCR-transduced PBL [TBI-1301]	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine 	N/A

Clinical Trial Number & Title	Status	Phase	T cell source (specificity)	Other treatments	Results and publication
NCT02379195: T Cell Therapy in Combination With Peginterferon for Patients With Metastatic Melanoma	Recruiting	I/II	TIL	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine IL-2 Peginterferon α-2b 	N/A
NCT02424916: Adoptive Transfer of CD8+ T Cells, Sorted With HLA-peptide Multimers and Specific for Melan-A and MELOE-1 Melanoma Antigens, to Metastatic Melanoma Patients. A Phase I/II, Non-randomized, Open Monocentric Study	Recruiting	I-II	Melan-A and MELOE-1 melanoma antigen-specific T cells (tetramer-sorted)	<ul style="list-style-type: none"> IL-2 	N/A
NCT02457650: Phase I Study of Malignancies That Express NY-ESO-1 With T Cell Receptor-transduced T Cells Targeting NY-ESO-1	Recruiting	I	NY-ESO-1-specific TCR-transduced PBL	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine 	N/A
NCT02482532: Phase I Study of Vaccine Enriched, Autologous, Activated T-Cells Redirected to the Tumor Marker GD2 in Patients With Relapsed/Refractory Melanoma	Recruiting	I	GD2-CAR (14g2a.Ç) transduced PBL from patients vaccinated against selected infectious antigens/pathogens	<ul style="list-style-type: none"> Repeat vaccination against selected infectious antigens/pathogens to stimulate transduced vaccination-specific T cells 	N/A
NCT02500576: Phase II Study of MK-3475 in Conjunction With Lymphodepletion, TIL, and High or Low Dose Interleukin-2 (IL-2) in Patients With Metastatic Melanoma	Recruiting	II	TIL	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine IL-2 PD-1 blockade (pembrolizumab) 	N/A
NCT02621021: A Prospective Randomized and Phase 2 Trial for Metastatic Melanoma Using Adoptive Cell Therapy With Tumor Infiltrating Lymphocytes Plus IL-2 Either Alone or Following the Administration of Pembrolizumab	Recruiting	II	Young TIL	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine High-Dose IL-2 PD-1 blockade (pembrolizumab) 	N/A
NCT02652455: A Pilot Clinical Trial Combining PD-1 Blockade, CD137 Agonism and Adoptive Cell Therapy for Metastatic Melanoma	Recruiting	I	TIL	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine High-dose IL-2 PD-1 blockade (nivolumab) OX40 costimulation (in vitro) 	N/A

Clinical Trial Number & Title	Status	Phase	T cell source (specificity)	Other treatments	Results and publication
NCT02654821: Multicenter Phase I/IIa Study Using T-cell Receptor Gene Therapy in Metastatic Melanoma	Recruiting	I/II	MART-1-specific [1D3 HM CysTCR] TCR-transduced PBL	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide 	N/A
NCT02830724: Phase I/II Study Administering Peripheral Blood Lymphocytes Transduced With a CD70-Binding Chimeric Antigen Receptor to Patients With CD70 Expressing Cancers	Recruiting	I/II	CD70-specific-CAR-transduced PBL T cells	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine High-dose IL-2 	N/A
NCT02869217: Phase Ib Study of TBI-1301 (NY-ESO-1 Specific TCR Gene Transduced Autologous T Lymphocytes) in Patients With Solid Tumors	Recruiting	I	NY-ESO-1-specific TCR-transduced PBL [TBI-1301]	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide 	N/A
NCT02870244: Adoptive T Cell Immunotherapy for Advanced Melanoma Using Engineered Lymphocytes: A Phase Ib Study	Recruiting	I	Tyrosinase-specific TCR (1383D)-transduced PBL T cells	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine IL-2 	17% ORR (1/6 PR) (unpublished data)
NCT02959905: Phase I Clinical Trial of TSA-CTL (Tumor Specific Antigen-Induced Cytotoxic T Lymphocytes) In the Treatment of Metastatic Melanoma	Recruiting	I	Tumor-specific-antigen induced cytotoxic T lymphocytes	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine 	N/A
NCT03166397: A Phase 2, Single-Center, Open Label Study of Autologous, Adoptive Cell Therapy Following a Reduced Intensity, Non-myeloablative, Lymphodepleting Induction Regimen in Metastatic Melanoma Patients	Recruiting	II	TIL	<ul style="list-style-type: none"> Lymphodepletion with fludarabine High-dose IL-2 2Gy TBI 	N/A
NCT02498756: A Study of Ipilimumab Plus Cytokine-induced Killer Immunotherapy for Stage II Melanoma Patients	Not yet recruiting	II	Cytokine-induced killer cells	<ul style="list-style-type: none"> CTLA-4 blockade (ipilimumab) 	N/A
NCT03068624: Phase Ib Study of Cellular Adoptive Immunotherapy Using Autologous CD8+ Antigen-Specific T Cells and Anti-CTLA4 for Patients With Metastatic Uveal Melanoma	Not yet recruiting	I	CD8+ T cells reactive to SLC45A2 (melanoma antigen AIM1)	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide IL-2 CTLA-4 blockade (ipilimumab) 	N/A
NCT03158935: Phase Ib Trial of Pembrolizumab Administered in Combination With or Following Adoptive Cell Therapy- A Multiple	Recruiting	I	TIL	<ul style="list-style-type: none"> Lymphodepletion with cyclophosphamide and fludarabine IL-2 	N/A

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Clinical Trial Number & Title	Status	Phase	T cell source (specificity)	Other treatments	Results and publication
Cohort Study; The ACTIVATE (Adoptive Cell Therapy InVigorated to Augment Tumor Eradication) Trial				• PD-1 blockade (pembrolizumab)	