Published in final edited form as:

Semin Oncol. 2012 April ; 39(2): 215–226. doi:10.1053/j.seminoncol.2012.01.006.

Advances in the Treatment of Metastatic Melanoma: Adoptive T Cell Therapy

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Abstract

Metastatic melanoma is notoriously resistant to chemotherapy and radiotherapy regimens. The prospect for newly diagnosed metastatic melanoma patients is grim with a median survival of less than a year. Currently, the only therapies resulting in long term disease free intervals, high dose Interleukin-2 (IL-2) and more recently anti-CTLA-4¹, work through activation of the immune system. However, with both therapies the response rate is low. Advances in our knowledge of how the immune system interacts with cancer have led to a number of strategies to manipulate anti-tumor immune responses through immunotherapy. This review will focus on one avenue of immunotherapy using the transfer of T cells referred to as “Adoptive Cell Therapy” (ACT), which involves the ex vivo expansion of autologous tumor-specific T cells to large numbers that are ultimately transferred back to the patient to boost anti-tumor immunity. This approach has been shown to be effective in the treatment of virally induced cancers, as well as metastatic melanoma. Recent successes with ACT hold promise and further emphasize the tremendous potential benefit of harnessing the immune system in the fight against cancer.

Treatment options for patients suffering from metastatic melanoma do not have a significant impact on overall survival except for a small proportion of patients. Systemic infusion of high dose Interleukin-2 (IL-2) results in a remarkable 5–7% of durable complete clinical responses. For patients not belonging to this privileged group, the only other FDA approved drug currently in use is the chemotherapeutic drug dacarbazine (DTIC)^{2,3}. DTIC treatment alone yields a 16% clinical response rate but responses tend to be of short duration³. Successes of IL-2 therapy warrant the investigation of strategies aiming at activating the immune system or immunotherapies. Many approaches to boost the anti-tumor immune response are at various stages of development. Adoptive cell therapy (ACT), or the transfer of large numbers of autologous tumor-specific T cells to the patient to boost the anti-tumor immunity, is gaining momentum after the publication of positive Phase II results by several groups. Tumor-reactive T cells can be derived from the blood or the tumor itself, and mediate regression of bulky tumors after re-infusion in patients. The different ACT approaches currently employed for melanoma are summarized in Figure 1. This review will

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The authors do not have any financial relationship to disclose.

briefly talk about how the field of adoptive cell therapy evolved and will focus on the recent advances and future challenges.

Early successes of Adoptive Cell Therapy in cancer treatment

Infections can lead to cancer development. Epstein - Barr virus (EBV) often causes a lymphoproliferative disease in immunocompromised hosts after hematopoietic stem cell transplant or solid organ transplant. Tumoral cells carrying EBV virus can be targeted using EBV-specific cytotoxic T cells (CTLs). Adoptive cell therapy of such cells expanded in vitro has been very successful in preventing or treating EBV-induced lymphoproliferative disease^{4,5}. A retrospective study following 114 patients for 3–15 years (median 10 years) has recently reported a stunning 100% effectiveness of EBV-specific CTLs for EBV-lymphoproliferative disease prophylaxis as well as very impressive 80% effectiveness of EBV-specific CTLs when used as therapy for EBV-lymphoproliferative disease in immunocompromised patients after stem cell or solid organ transplant⁶. EBV positive solid tumors also exist and transfer of EBV-specific CTLs has also proven beneficial in this setting⁷. Virus-specific T cells are also useful to treat viral infections in immunocompromised patients such as patients recovering from stem cell transplantation. In this regard adoptive cell therapy aimed at eliminating viral pathogens has proven to be very effective. Indeed the transfer of donor-derived CD8+ cytomegalovirus-specific T cell in immunocompromised recipients of allogeneic bone marrow effectively prevents cytomegalovirus reactivation^{8,9}. From this body of work it can be concluded that the transfer of virus-specific T cells can successfully target and kill virus infected cells. In the recipient whether these cells are cancerous or not. This strategy is now extended to cancer-specific T cells. The rationale being if we can isolate cancer-specific T cells, expand them to large number ex vivo and re-infuse them to the patient, they may eradicate cancer in the recipient.

ACT for melanoma

A prerequisite to ACT is the very existence of tumor-specific T cells in the patient. The immunogenicity of melanomas, or their capacity to trigger an immune response, became apparent as a result of two clinical observations. First, the documentation of cases of spontaneous regression and secondly the fact that the frequency of melanomas was found to be greater among immunocompromised patients, either from HIV infection or after organ transplant^{10–13}. The importance of the immune response in controlling melanoma cancer growth was further proven by the efficacy of high dose IL-2 treatment. Systemic administration of IL-2, a T cell growth factor, resulted in clinical response rates of 22% and 24% in advanced renal cell carcinoma and melanoma, respectively, while patients with colorectal or breast cancer did not respond¹⁴. Melanoma has proven to be one of the most immunogenic malignancies. A small subset (around 5–7%) of late stage melanoma patients treated with high dose IL-2 experience a durable complete response lasting for many years, which is remarkable especially that complete responses seen with chemotherapeutic drug regimens only occur in 1–2% of the patients and are generally short-lived^{14,15}.

Successes of IL-2 therapy for melanoma point to an important role of effector T cells in the control of the disease. Efforts to capitalize on this finding and to further improve the therapy have brought about the field of adoptive cell therapy for melanoma.

Adoptive cell therapy for metastatic melanoma: Early animal models and first clinical trials

Early animal studies demonstrated that isolation of T lymphocytes from the blood and culture for extended period of time ex vivo in medium supplemented with IL-2 produces

cytotoxic T cells with high killing capacity that could be re-infused to a tumor-bearing host, mediating tumor regression when used in combination with cyclophosphamide to eliminate host lymphocytes¹⁶. Furthermore, injection of IL-2 into the host at the time of cell transfer greatly enhanced the efficacy of the transferred cells¹⁷. Parallel studies demonstrated that T lymphocytes expanded with high dose IL-2 from melanoma patient's blood were cytotoxic against autologous tumor cells in vitro¹⁸. T cells isolated directly from surgically resected tumors (Tumor Infiltrating Lymphocytes or TIL) were later showed to be enriched in tumor-specific T cells as compared to T cells from the peripheral blood of tumor-bearing mice¹⁹. The transfer of ex vivo expanded TILs to cyclophosphamide treated tumor-bearing mice along with IL-2 could mediate the regression of lung and liver metastasis¹⁹. Studies by Overwijk et al. demonstrated that the addition of a vaccination step post-transfer with a virus encoding an epitope recognized by the transferred CD8+ T cells causes regression of large established tumors and long term cure of the animals²⁰.

Methods have been developed to isolate TILs from human tumors and expand them for ACT²¹. In 1994, the results of the first clinical trial on the use of TILs in adoptive cell therapy demonstrated a 34% objective response among a cohort of 86 metastatic melanoma patients²². The therapy was able to induce regression of bulky metastatic disease at diverse anatomical sites in patients that had previously failed IL-2 single therapy and were refractory to chemotherapy. The treatment regimens evaluated were adoptive transfer of TILs plus high dose bolus IL-2 with or without pre-conditioning cyclophosphamide treatment. Surprisingly there was no significant difference between the two groups. Infused TILs were found to preferentially localize to tumor sites as compared to infused peripheral blood lymphocytes, and increased tumor localization of TILs was noted with cyclophosphamide pre-treatment^{23,24}.

Addition of a lymphodepleting regimen given to the patient immediately prior to cell transfer has substantially improved persistence of the transferred cells. The removal of endogenous lymphocytes is thought to eliminate immune regulatory cells, alleviate competition for growth factors and also cause a surge in serum concentrations of cytokines that help T cell growth such as Interleukin-7 and -15²⁵. Cyclophosphamide was first used in animals as a lymphodepleting agent in conjunction with cell transfer, and was shown to be required for transferred cell effectiveness and persistence. But when used in humans, the persistence of the transferred cells was not optimal, with cells becoming undetectable in the blood in a matter of few weeks. Fludarabine used alone as a mild lymphodepleting agent, leaving lymphocyte counts of about 150/mm³ of blood at time of T cell transfer, was able to modestly increase the persistence of transferred T cells²⁶. Cyclophosphamide and fludarabine combination used as a non-myeloablative lymphodepleting regimen induced a transient but profound lymphodepletion (lymphocyte counts in the blood fall to 0 on day of T cell infusion) that translated into much ameliorated persistence of the transferred cells. Specific T cell clones in the TIL infusion product were tracked using T cell receptor (TCR) variable beta region specific antibodies or tetramers (for melanoma antigen specific T cells), and later sequencing of TCR CDR3 region in blood samples from infused patients at different time points post-infusion. The analysis revealed that specific TCR clones in the original TIL infusion product could be detected months and even up to two years after infusion. Not only were these persisting T cells detectable in the blood, but they could actually represent a major fraction of the total CD8+ T cell population for months to years following cell transfer²⁷.

Improved TIL persistence in the blood correlated with better clinical response rate (climbing to 51%) and longer duration of the response (11.5 +/- 2.2 months) in a group of 35 metastatic melanoma patients^{28,29}. Improved clinical response rate in turn coincided with the appearance of autoimmunity manifestations in patients. Indeed 75% of the responding

patients whose TIL culture was shown to contain T cells reactive to melanocyte differentiation antigens experienced autoimmune melanocyte destruction in the form of vitiligo or uveitis, or both. It has to be kept in mind that tumors are a hyperproliferative defect of an otherwise normal cell, therefore T cells recognizing tumors may also recognize normal cells to some extent and cause collateral damage.

Interestingly, Laurent and colleagues noted a much superior proliferative potential of CD8+ T cells over CD4+ T cells when PBMCs containing melanoma-specific T cells were infused to metastatic melanoma patients following cyclophosphamide and fludarabine lymphodepletion³⁰. Taken together, these data suggest that deeper lymphodepletion afforded by cyclophosphamide and fludarabine combination increases the persistence of polyclonal TIL populations in vivo and may facilitate the homeostatic expansion of cytotoxic CD8+ TILs in the circulation post-infusion.

To further go down the path of lymphodepletion and to build on pre-clinical data showing that total body irradiation (TBI) was comparable to cyclophosphamide at increasing persistence of TILs after transfer to the animal¹⁹, TBI (2 or 12Gy) was tested in humans in a clinical trial in combination with cyclophosphamide and fludarabine before TIL transfer and using high dose bolus IL-2 to support TIL growth after infusion²⁵. Clinical response rate was 72% in the group receiving the highest irradiation dose (12Gy), 18 responses out of 25 patients, compared to 49% (21 responses out of 43 patients) in a separate group of non-randomized patients receiving only cyclophosphamide and fludarabine. This is to date the best clinical response reported in TIL ACT for metastatic melanoma treatment. Patients exposed to 12Gy of TBI experienced more treatment related toxicities and since this radiation dose is myelosuppressive, it required the transfer of autologous stem cells. Therefore the improved clinical response came at a cost of increased toxicities. Although initial response rate with TBI combination is encouraging, longer follow-up, greater number of patients and randomized clinical trials are needed to definitively conclude that TBI addition to the lymphodepleting regimen has a beneficial effect in ACT.

Recent attempts to improve TIL ACT: “Young TIL” and CD8+ selection

From the first report of TIL adoptive cell therapy (ACT) in 1994, it was observed that the duration of ex vivo culture of the TILs correlated with clinical response, where TILs cultured for shorter time generated better clinical responses in patients²². It was later found that TILs that persist in vivo after transfer tend to have longer telomeres at the moment of infusion, which means such TILs have been through less cell division cycles or constitute “younger” TILs³¹. To expand on this finding and to streamline the procedure for generating TIL cell products, the so-called “Young TILs” protocol was optimized. This revised version of TIL culture simplifies derivation of T lymphocytes from tumors by the use of enzymatic tumor digestion and setup of a unique T cell culture. The protocol generally used so far expands T cells from small tumor fragments that are individually plated in wells of 24 well plates in media containing IL-2. Using the traditional approach, TILs are generally successfully grown from 50–60% of the patients. The young TILs protocol can derive successful TIL cultures from 80% of patients in a shorter period of time (2–3 weeks instead of 5 weeks) but does require a larger tumor specimen. Another major change in the “Young TILs” protocol is the elimination of the in vitro testing of TILs for reactivity to autologous tumor, on the basis that no difference in clinical response rates have been found between TIL “selected” for tumor reactivity by using interferon gamma secretion assays in vitro and “unselected” TILs. Practically speaking, the use of the Young TIL protocol will accelerate the production of the TIL product, which will diminish the drop-out rate of patients that experience progression during the manufacturing time of their TIL product and have to turn to other treatment options. It may also allow enrollment of patients previously ineligible,

because it can grow TILs from a substantially greater fraction of samples. In 2010, Besser and colleagues announced the results of the first clinical trial using the Young TILs protocol in conjunction with cyclophosphamide and fludarabine pre-conditioning regimen in ACT for metastatic melanoma, and reported a 50% clinical response rate in a cohort of 20 patients (2 complete responses and 8 partial responses)³². These results are very similar to previously reported clinical response for TILs grown using traditional methods²⁵ and indicate that TILs derived using the “Young TILs” protocol appear equally effective. Thus, while the shorter ex vivo culture period did not translate into better clinical response rate so far, it considerably eases the process of generating the TIL product and thus potentially renders the treatment available to a larger patient population.

Overall, the “Young TILs” protocol has aimed to limit the number of cell divisions of TILs in high dose IL-2 before the cells undergo the “rapid expansion protocol” (REP) to generate the final TIL infusion product. This approach may result in less differentiated cells undergoing expansion and produce a fundamentally different final TIL product in terms of differentiation status and telomere length of the cells infused. However, this needs to be confirmed by careful phenotypic and functional studies. Although TIL in the “Young TIL” protocol may be “younger” when placed into a REP situation, they may expand to a greater extent (more cell divisions) during the REP, resulting ultimately in a spectrum of cells with similar biological properties for adoptive transfer as with current approaches. In the recently reported clinical trial by Besser et al³² using the “Young TIL” approach, it was found that clinical response was correlated with infusion of higher numbers of post-REP TIL (thus, more extensively divided TIL). Since no improvement in clinical response has been seen so far with the “Young TIL” protocol it is unclear whether starting the REP earlier in the process makes a difference biologically^{31,32}. Nevertheless, from a practical standpoint, the decreased TIL product production time is an advantage. More studies will be needed to define markers of efficacy of the TIL product in order to tailor an expansion protocol preserving the desired characteristic.

The composition of the TIL product has also been scrutinized. T cells can be roughly divided in two types according to surface molecule expression: CD8+ or CD4+. Historically, CD8+ T cells have been considered cytotoxic and directly able to kill their target, whereas CD4+ TILs are either of the helper (Th0, 1, 2, 9, 17 or 22) type and mainly secrete cytokines to promote activation and effector function of the CD8+ T cells, or of the regulatory type, in which case they would inhibit CD8+ T cell proliferation and function. A recent retrospective analysis of TIL ACT clinical trials conducted at the NIH revealed a trend towards cultures containing more CD8+ TILs having less CD4+ TILs and better clinical response. The same study validated that for in vitro experiments, enrichment of CD8+ TILs before rapid expansion leads to improved autologous tumor-reactivity³³. Therefore, to help tip the balance toward a favorable CD8+/CD4+ TILs ratio, the “Young TILs” protocol has been further optimized to enrich CD8+ TILs via magnetic cell separation before rapid expansion for clinical use. Clinical trials are currently in progress to assess whether CD8 enrichment enhances antitumor immune responses.

Fine-tuning cancer specificity: Use of T cell clones in ACT for melanoma

The identification of melanoma-associated antigens displaying restricted expression to melanomas and normal melanocytes, more specifically MART-1(Melan-A) and gp100 (glycoprotein 100),^{34–36} and the realization that CD8+ T cells recognizing these antigens have a high prevalence both in TILs and peripheral blood lymphocytes of melanoma patients,³⁷ spurred interest in the selective infusion of T cells bearing such specificity. The success of TIL therapy for some patients has been highly correlated with the presence of TILs reactive against MART-1 or gp100^{38,39}. In a Phase II/III randomized adjuvant clinical

trial of stage III melanoma patients, the median survival of HLA-A2+ patients infused with a TIL product containing MelanA-specific TILs was 53.5 months as compared to 3.5 months for patients whose TIL product lacked MelanA-specific TILs³⁹.

The derivation of CD8+ T cell clones from PBMCs or TILs involves the limiting dilution of the product to the point of actually setting up individual T cell cultures from one cell. The growth is encouraged by the use of allogeneic irradiated PBMCs (referred to as feeder cells) that will secrete growth factors necessary for T cell outgrowth but will eventually die and leave a pure T cell culture. This process is very time consuming and requires the individual testing of all clones showing reactivity to the desired antigen and eventually picking only one or a few highly reactive clones for large scale expansion. At the end of the process the expanded clones have been in culture for 3–5 months⁴⁰. The first clinical trials conducted to test the efficacy of melanoma-antigen specific CD8+ cytotoxic T cell clones derived from patient's blood or TILs reported poor success in terms of clinical response. Those trials were designed to transfer cells without any lymphodepleting regimen. CD8+ T cell clones usually had a good initial expansion after transfer, could migrate to tumor sites and could perform effector function, but had very limited persistence, being typically undetectable in the blood after few weeks^{41–43}. Clinical responses achieved were mainly minor responses, mixed responses or stable disease, with the exception of one complete response⁴². Differences among clinical protocols in methods to derive clones and the different IL-2 doses used to support transferred cells growth makes it difficult to directly compare results from the different studies. A study where cyclophosphamide and fludarabine lymphodepleting regimen was used prior to ACT using a gp100 specific CD8+ clone derived from TILs has reported no objective clinical response⁴⁴.

One notable exception is a case report of an NY-ESO-1 specific CD4+ T cell clone that led to a durable clinical remission in one metastatic melanoma patient without any pre-infusion conditioning or post-infusion IL-2 administration⁴⁵. This is the first report of the clinical use of CD4+ T cell clones in ACT for melanoma. A number of pre-clinical studies have recently shown that CD4+ T cell clones can cure mice with B16 melanomas⁴⁶. These mouse models have shown that ACT with CD4+ T cells in a lymphopenic host require a smaller number of cells than with CD8+ T cells and that the transferred CD4+ T cells acquire cytotoxic capability.

Dreno and colleagues reported a 50% objective clinical response rate including 2 durable complete responses (28 months and over 5 years, respectively) in a small cohort of 14 stage IIIB/C or IV melanoma patients after the transfer of autologous MART-1 or gp-100 specific CD8+ clones derived from PBMCs⁴⁰. Patients on this protocol did not receive lymphodepleting conditioning prior to cell transfer. All patients received DTIC treatment for 3 or 4 cycles before T cell infusion, while the clones were prepared in vitro. In this study, T cells were supported by a combination of IL-2 and IFN-alpha, an active combination in melanoma. Therefore, it is not clear whether the responses were due to the T cell infusion.

T cell clones bear a unique specificity, which is both appealing and problematic. Infusing clones allows for highly selected targeting of tumor cells. Although the antigen recognized has been carefully chosen for selective and potent expression in the tumor, the intensity of expression will vary from one tumor cell to another, with a fraction of tumor cells potentially being negative for the given antigen. Cases of antigen loss on recurrent tumors after transfer of CD8+ T cell clones have been documented, and are a major argument for the use of more polyclonal T cell populations^{42,43,47}. The lack of persistence of T cell clones seen in different protocols may be related to the extended culture period ex vivo to isolate and expand clones through multiple rounds of activation that could lead to T cell exhaustion. In addition, lack of CD4+ T cell "help" may be an issue. In a mouse model of ACT, CD4+ T

helper cells have been shown to be important for the persistence of CD8+ T cells in vivo. Therefore pure CD8+ cytotoxic T cell clones could fail to persist due to lack of CD4+ T cell help⁴⁸.

So far, the transfer of T cell clones has not surpassed the clinical response rates and durability achieved with the transfer of polyclonal TIL populations. The derivation of T cell clones and their expansion to large numbers is very labor intensive. Moreover, evidence of possible tumor antigen loss leading to resistance also argues that the use of T cells bearing a single specificity might be too risky. However the recent successes of ACT using T cell clones derived from peripheral T cells could serve patients from whom TILs cannot be grown.

Targeting TILs to the tumor: Gene modification of infused T cells

TIL ACT for the treatment of metastatic melanoma has yielded good clinical response rates, but not every patient undergoes tumor regression. Efforts to maximize efficiency of the therapy are focusing on improving persistence of the TILs after transfer, augmenting the proportion of TILs recognizing antigens specifically expressed by melanomas, and increasing the trafficking of the infused TILs into tumor sites. Various gene modification strategies are being developed to address these issues.

Since persistence of the TILs in the circulation after transfer has been shown to be dependent on the presence and levels of IL-2 in the body, genetic modification of the TIL product through the viral transduction of a vector coding for IL-2 was attempted. However, patients receiving TIL products modified to secrete IL-2 did more poorly (1PR out of 7 treated patients, 4 months duration), and when exogenous IL-2 was co-administered for an additional 5 patients one PR was seen (4 months duration) along with two mixed responses⁴⁹. The authors attribute this poorer outcome to the extended in vitro culture period required to reach sufficient cell numbers of gene-modified TILs. It is also possible that the effects of localized high doses of IL-2 could have led to activation-induced cell death of the T cells. Overall, IL-2 production by the TILs did not improve TILs persistence or clinical response. The expression of other cytokines in TILs, such as IL-12 and IL-15, or expression of anti-apoptotic molecules such as bcl-2 or bcl-xL to ameliorate persistence or function of the TILs is currently evaluated in pre-clinical models^{50–52}. TIL cells have also been modified to counterbalance the effects of immunoregulatory factors from the tumor microenvironment such as transforming growth factor (TGF)-beta. TGF-beta resistant CTLs have been engineered through the expression of a dominant negative TGF-beta receptor that acts as a decoy and have been found to have enhanced antitumor activity in vivo⁵³.

Each melanoma tumor is different and TILs grown out of one patient's tumor are often reactive to autologous tumor but not always to melanoma tumors from HLA-matched allogeneic patients. Clinical trials have suggested that increased frequencies of TILs specific for known and usually shared melanoma antigens in the final product leads to more favorable clinical outcome^{38,39,54}. T cell specificity is governed by the TCR molecule expressed on the surface of the T cell. The unique range of specificities of each TCR molecule is the product of random rearrangement of TCR alpha and beta chains. Study of T cell populations infiltrating into tumors have permitted us to isolate genes coding for specific TCR molecules involved in tumor regression. The possibility of conferring defined melanoma-antigen specificity to any T cell through the TCR gene has gained popularity, and appears to be a good alternative for patients from whom TILs cannot be grown.

TCR recognizing melanoma-associated antigens have been cloned from TIL cultures of patients responding to the therapy and could be successfully transferred to T cells from patient's PBMC^{55–58}. Although initially rather disappointing results (13% clinical response

rate in the treatment of 15 patients with autologous PBMCs engineered to express a TCR recognizing MART-1⁵⁹), transfer of a TCR with higher avidity for MART-1 or gp100 in a follow-up clinical trial induced a higher clinical response rate. In that trial, 30% of patients receiving MART-1 TCR responded, while 19% of the patients who were given T cells expressing gp100 specific TCR had a clinical response. These clinical responses were also associated with autoimmune attack of melanocytic cells of the eye, the ear and the skin⁵⁸. The engineered T cells were shown to persist in responding patients and most responses were durable. Although these results are still not as impressive as that seen with the transfer of polyclonal TILs and the occurrence of autoimmunity is elevated, it remains an option for patients from whom TILs cannot be grown. T cells from the blood may not be endowed with tumor homing capability as much as TILs are; thus, future TCR engineering of T cells isolated from tumors may help address this issue.

Another approach in targeting TILs to the tumor site is to make them express a chimeric antigen receptor (CAR). These artificially engineered receptors are composed of an extracellular antigen recognition domain, usually made of a single chain variable fragment of an antibody specific to a cancer antigen, coupled to the transmembrane and intracellular signaling domain of CD3 zeta chain of the TCR complex alone or in combination with the intracellular signaling domain of co-stimulatory molecules such as CD28, OX40 or 4-1BB. Upon binding of the CAR expressed on a T cell to its target antigen, the T cell will become activated and kill the target cell. This approach combines an antibody's high affinity antigen recognition with the killing machinery of a T cell. The antigen recognition is therefore non HLA-restricted, and since antibodies bind to their antigen with very high affinity it requires very little antigen expression to trigger T cell activation. This is a major difference from normal antigen recognition mediated through the TCR, which has to happen in association with defined HLA molecules. Full activation of the T cell requires a second co-stimulatory signal, through molecules such as CD28, OX40 and 4-1BB not readily available in the tumor microenvironment. Recognition through the TCR can also be turned off by loss of HLA class I expression by the tumor cells. The absence of HLA restriction in the CAR strategy would allow the use of the same CAR for all patients regardless of their HLA type. This a major advantage over TCR transduction strategies, where each TCR has an HLA restriction element and therefore can only be used in patients carrying this particular HLA subtype. As far as application of the CAR approach for the treatment of melanoma, pre-clinical models have shown promising results with the targeting of ganglioside GD2 or of a high molecular weight melanoma-associated antigen^{60,61}.

The CAR strategy has now been used in the clinic. Initial studies used CAR that were lacking intracellular co-signaling domains of co-stimulatory molecules and saw a lack of persistence of the CAR modified T cells^{62,63}. Recent studies using CAR incorporating such domains have started to show clinical benefit⁶⁴. However, excitement in the field has been dampened with reports of serious adverse events and even fatalities after ACT using CAR modified T cell⁶⁵⁻⁶⁷. A colon cancer patient was infused with CAR modified T cells recognizing ERBB2 antigen and died 5 days after treatment of what appears to be a cytokine storm and respiratory failure triggered by the recognition of low levels of antigen on lung epithelial cells⁶⁷. This unexpected targeting of the normal lung by the transferred cells emphasizes the increased sensitivity of CAR modified T cells, and demonstrates that low levels of antigen expression on normal cells can be sufficient to trigger T cells with this approach. Another cancer patient treated in a Phase I trial of CAR modified T cells, this time recognizing CD19, developed hypotension, dyspnea and renal failure shortly after infusion and died 44 hours after T cell transfer. Cause of death was unclear but circumstantial evidence points to T cell involvement⁶⁵.

The affinity of CAR modified T cells for their specific antigen is unprecedented. The challenge ahead will be to select antigens with expression exclusively restricted to the tumor. Careful dose escalation studies need to be performed to scale down the amount of CAR modified T cells infused and find levels that maintain clinical effectiveness but spare normal tissues. These adverse events, while advising caution in the design of future clinical trials, also point out the power of T cell therapy.

Persistence of the transferred CAR-modified T cells in vivo was increased by making sure the T cell gets optimally activated upon antigen encounter endowing it with maximum proliferative and differentiation signals (addition of intracellular signaling domains of major co-stimulatory molecules). Another factor controlling T cell persistence is the prevalence of its antigen. T cell populations will surge in the presence of the antigen they are made to recognize and kill, then quickly contract to infinitely smaller levels, potentially undetectable, when the antigen is no longer present. Generally, the specificity of the TCR from the CAR-modified T cells is not considered when preparing cells for therapy. One novel approach to try to enhance the survival of the CAR modified T cells is to use only T cells whose TCR recognize a virus that is known to persist in the host, like Epstein Barr Virus (EBV), which most people carry in a latent form. This strategy will take advantage of the constant low antigen exposure through the TCR by endogenous antigen presenting cells, maintaining a sizable population of CAR modified T cells in the circulation ready to be triggered to kill whenever CAR antigen is found. A first clinical trial using this strategy with a ganglioside GD2-recognizing CAR for the treatment of neuroblastoma has demonstrated increased survival of virus-specific CAR-modified T cells over non virus-specific CAR-modified T cells. The treatment appeared to be safe and was associated with tumor regression⁶⁸.

Another challenge is to enhance the ability of TIL to migrate to the tumor site. Whole body imaging of radioactively labeled TILs after intravenous infusion in patients showed a massive and rapid migration of infused TILs to the lungs, immediately followed by accumulation in spleen and liver before a complete disappearance from the peripheral circulation^{23,24,69}. Some TIL infiltration of the tumor is seen, but it clearly does not represent the majority of the infused cells. The natural migration of cells in the body happens through a sophisticated and tightly regulated network of chemoattractant cytokines called chemokines. The specific expression of their receptor controls how a responding cell type will migrate towards a gradient of the secreted chemokine and be brought in close proximity to the chemokine secreting cell to perform its function. Cancer cells can also secrete chemokines. Melanoma cells have been found to secrete elevated amounts of CXCL-1(Gro- α), facilitating tumor growth^{70,71}. CXCL1 binds CXCR2 receptor, which is expressed mainly by neutrophils but not by T cells, except on CD4+ T cells in large granular lymphocytic leukemia⁷². TILs have been engineered to express CXCR2 receptor, and transduced cells were shown to be able to migrate towards CXCL1 chemokine in vitro⁷¹. When transferred to melanoma bearing mice, CXCR2 transduced TILs demonstrated enhanced tumor localization and could mediate improved tumor clearance⁷³. These promising pre-clinical results have paved the way for a clinical trial testing the effects of expanded melanoma TIL transduced with the CXCR2 gene currently underway at MD Anderson Cancer Center.

Proposed engineering of the TILs may considerably augment affinity of the cytotoxic T cells for the tumor, ameliorate their ability to migrate towards their target and increase the long-term persistence of the infused cells. Carefully designed dose-escalation trials are needed to safely elucidate the impact of CAR-engineered T cells to move this field forward.

Enhancing feasibility of TIL ACT

Establishment of a TIL ACT program in an institution requires important resources and a considerable Good Manufacturing Practices (GMP) infrastructure. In its current format, a TIL ACT program can probably only be set up in major centers. Additionally the process of TIL culture can be technically challenging. This is even more challenging when transduction of genes into TILs is also contemplated. Development of such expertise can be time-consuming.

A handful of centers have established TIL ACT programs thus far and are actively treating patients. As the field moves forward this expertise could be exploited. A model of multiple centers acting as separate manufacturing sites could provide TIL ACT products for a large population of patients and be suitable for the realization of a Phase III randomized trial. This model has the advantage of making use of existing resources but would need careful harmonization of all procedures.

An alternative approach to the issue of manufacturing TIL cell products for a large number of patients in the case of a Phase III trial would be to have one or two centralized TIL manufacturing/expansion sites solely dedicated to TIL expansion. Tumor samples could be directly shipped to these centralized facilities, commercial or academic, which would culture and expand the TILs and would ship the final product back to the ordering clinician, ready for infusion. A clear advantage of this system is the standardization of TIL growth for all patients, which is not as easily achievable in disseminated centers. In the context of a Phase III trial, it would allow centers that may not have the resources to grow TILs to enroll patients and therefore speed up the accrual. This model is similar to Dendreon's manufacturing of Provenge (sipuleucel-T), the first autologous cellular immunotherapy product for the treatment of prostate cancer approved by FDA. Following FDA approval, Dendreon has now been building more manufacturing facilities to accommodate the growing demand for this treatment. For Dendreon's product generation, leukapheresis products from patients are shipped to the manufacturing facility where the peripheral blood mononuclear cells are activated with a fusion protein of a prostate cancer antigen (prostatic acid phosphatase) fused with granulocyte-macrophage colony-stimulating factor for few days before being shipped back for infusion into the patient. This process involves cell processing and a relatively short time in culture, which differs from typical stem cell transplant programs more widely available where cells are collected and frozen, and at a later point thawed and infused without requiring further cell manipulation.

TIL generation might be amenable to a central or a multi-center production scenario, and plans for a Phase III multi-center trial involving large patient numbers may require the development and optimization of this approach.

ACT in the treatment of metastatic melanoma; where do we go from here?

TIL ACT in combination with lymphodepletion and high dose bolus IL-2 has so far been demonstrated to produce clinical response rates averaging 50% for metastatic melanoma patients in non-randomized phase II clinical trials in studies from the NCI, and more recently from a separate group in a different country (Sheba Medical Center, Israel)^{29,32}. Those results, although promising, remain to be validated in a randomized Phase III clinical trial and compared to IL-2 alone or the current standard of care. Genetic modification of the transferred T cells might lead to improved clinical outcome, but this still remains to be seen. Thus far, only half of the patients receiving ACT benefit from the treatment. Further increasing clinical response may require the integration of drugs targeting different aspects of the disease or working through different mechanisms.

Other therapies aimed at melanoma have shown promising results in the clinic in the last few years. Ipilimumab is an antibody binding to Cytotoxic T lymphocyte Antigen-4 (CTLA-4) on the surface of activated T cells. This molecule is normally upregulated once the T cell has been activated and acts as negative regulator of TCR activation returning the T cell to a resting state. Ipilimumab is a blocking antibody preventing the de-activation of the T cell or “taking the breaks off” T cell activation. The treatment aims at maximally activating T cells and will target all T cells, not only anti-tumor T cells. The possible combination of TIL with ipilimumab makes sense, since TIL are a population of T cells enriched for tumor specificity.

Another area to consider is the provision of costimulation to adoptively transferred TILs and the need to protect them from activation-induced cell death after antigen contact in vivo. We have found that post-REP TILs lose critical positive costimulatory molecules such as CD28 after extensive expansion, and become hyporesponsive to further activation and susceptible to activation-induced cell death⁷⁴. IL-2 driven TIL expansion may be partially responsible for this, as expansion with a combination of IL-15 and IL-21 preserved CD28 expression and improved post-REP TIL responsiveness to additional antigenic stimulation⁷⁴. We have found that alternative costimulatory pathways, such as through the TNF-R family member 4-1BB/CD137 using agonistic anti-CD137 antibodies, can protect melanoma TILs after TCR re-stimulation and further enhance their anti-tumor effector function⁷⁵. Thus, treatment with anti-CD137 following TIL infusion using currently available fully human antibodies may be an option to improve TIL persistence and anti-tumor activity in vivo.

Targeted therapies have also made significant progress against melanoma in the last few years. In melanoma, a majority of the cases are found to contain an activating mutation in one member of the MAP kinase pathway; either B-RAF (41%) or N-RAS (18%)^{76–78}. The lead agent showing the most significant clinical effectiveness for metastatic melanoma patients thus far is a small molecule (PLX4032) specifically inhibiting the mutated form of B-RAF kinase. A Phase I-II clinical trial reported an 80% response rate on a cohort of 32 metastatic melanoma patients whose tumor had the B-RAF V600E mutation⁷⁹. Studies looking at the effects of PLX4032 on the immune system have so far not found any inhibition of function^{80,81}. Combination of mutated B-RAF inhibition and immunotherapy to increase duration of the response appears to be a very reasonable option.

In summary, new treatment options for metastatic melanoma should be available relatively soon. Although these new agents induce clinical response in some patients, the response can be short-lived and response rates can be low for single agents. Combination therapy, including ACT, may increase the response rates and further increase survival.

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Figure 1. Adoptive Cell Therapy for metastatic melanoma

Derivation of tumor-specific T cells from the tumor (upper panel) or the blood (lower panel) of melanoma patients for use in adoptive cell therapy. **Upper panel** –In the “Young TIL protocol,” the whole tumor is digested enzymatically and T cells are pooled into one culture, while the original protocol involves mincing a small part of the tumor with the individual tumor fragments being placed into different wells and expanded separately. Both protocols use high concentrations of IL-2 (3000–6000 units/ml) to expand TILs. TILs derived from tumor digests reach minimum number of cells required for ACT in less time than TILs expanded from tumor fragments. After initial expansion to desired numbers by culture in IL-2-containing media, typically 50–100 million cells, TILs are put through a Rapid Expansion Protocol (REP) consisting of polyclonal stimulation of T cells through CD3 bound to allogeneic PBMCs (feeders) and high concentrations of IL-2. Patients will typically be infused with 50–100 billion of expanded TILs. **Lower panel**– Derivation of tumor-specific T cells from the peripheral blood of melanoma patients. Naturally occurring tumor reactive T cells are present in the blood at very low frequency and can be cloned by limiting dilution and later amplified using the REP protocol or similar expansion strategy. However, this approach is fastidious and requires 3–5 months for cell preparation before the patient can be treated. T cells from the blood can also be engineered to express a TCR or CAR recognizing a tumor antigen and therefore be given a tumor specificity ex vivo.