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Adoptive Cell Therapy—Tumor-Infiltrating Lymphocytes, T-Cell Receptors, and Chimeric Antigen Receptors

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Advances in the field of cancer immunotherapy have enabled this therapeutic approach to enter the mainstream of modern cancer treatment. The ability of interleukin-2 (IL-2) administration to mediate complete, durable cancer regressions led to its approval by the US Food and Drug Administration for the treatment of patients with metastatic renal cancer in 1992 and metastatic melanoma in 1998. The ability of IL-2 to grow human T cells *ex vivo* led to the ability to generate tumor specific cells for the treatment of patients. Passive administration of anti-tumor T cells is referred to as adoptive cellular therapy (ACT). ACT has several advantages compared to other approaches to cancer immunotherapy. Large numbers of anti-tumor T cells can be grown *in vitro* and selected for high avidity against the desired antigen. In addition, the host can be manipulated prior to the administration of cells to provide a suitable microenvironment in the tumor. The first report of the use of tumor-infiltrating lymphocytes (TILs) for the treatment of patients was reported from the Surgery Branch, National Cancer Institute (NCI) in 1998. Extensive studies of TILs showed that cells with anti-tumor activity could be isolated from tumors derived from patients with melanoma, although using similar techniques TILs grown from most other cancer histologies did not appear to recognize tumor antigens. Further application of ACT led to the development of techniques to introduce anti-tumor T-cell receptors (TCRs) into autologous lymphocytes for use in therapy. Both conventional $\alpha\beta$ TCRs and chimeric antigen receptors (CARs) with anti-tumor specificity can be introduced into normal lymphocytes, providing them with anti-tumor activity. Clinical trials have been conducted targeting a variety of tumor histologies using this approach. In this review we will discuss the clinical experience with ACT including the use of TILs, as well as autologous lymphocytes genetically modified to recognize tumor antigens.

ADOPTIVE CELL THERAPY USING TUMOR-INFILTRATING LYMPHOCYTES

Successful use of interleukin-2 (IL-2), a T-cell growth factor, in the treatment of patients with metastatic melanoma and renal cell cancer (RCC) suggested that a manipulation of the host immune system could provoke an endogenous reaction capable of mediating cancer regression.¹ Early efforts to identify the cells that could mediate tumor regression led to murine studies using natural killer cells and lymphokine-activated killer (LAK) cells,

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followed by a human clinical trial using LAK cells in combination with IL-2. In a human clinical trial, objective regressions were observed in 11 of 25 patients treated, including patients with melanoma and RCC,² although a subsequent randomized trial in patients with melanoma and RCC did not show a significant difference in response rate compared to administration of IL-2 alone.³

Tumor-infiltrating lymphocytes (TILs) cultured from fragments of resected tumors were significantly more potent than LAK cells in mediating regression of murine metastases. Importantly, human TILs could be generated on a large scale from most tumor types.⁴ Clinical efforts in the field are summarized below and in Table 1. The first experience with adoptive cell therapy (ACT) using TILs was performed at the Surgery Branch, National Cancer Institute (NCI). Twenty patients with metastatic melanoma were treated with a single dose of cyclophosphamide, followed by adoptive transfer of autologous TILs and IL-2. Objective responses were seen in 11/20 (55%) patients, including one complete response (CR), though in general responses were of short duration. Among the five patients with disease refractory to IL-2 administration alone, two (40%) exhibited objective responses. Toxicities were similar to the administration of IL-2 alone and included hypotension, nausea, and anemia. All toxicities were transient, and there were no treatment-related mortalities.⁵

A nonmyeloablative (NMA) chemotherapy regimen consisting of cyclophosphamide and fludarabine (CyFlu) had minimal activity in melanoma but could provide a lymphodepleted host environment in which to transfer TILs and administer IL-2.⁶ In the initial report of this study, 51% (18/35) of patients demonstrated an objective response with three complete responders. In addition to the same transient toxicities seen in the first TIL study, new late toxicities were identified including rare herpes zoster infections, a patient with an Epstein-Barr virus (EBV)-associated lymphoma, and rare fludarabine-associated neurotoxicity.⁷ Long-term follow-up was reported for the 43 patients treated on a standard TIL protocol using this NMA preparative regimen prior to cell administration, and of the 43 patients treated, 49% (21/43) experienced an objective clinical responses including 12% with CRs based on Response Evaluation in Solid Tumors (RESIST) criteria.⁸

With nearly half of all patients treated with TILs experiencing objective tumor regressions, TIL ACT is currently one of the most effective treatments being studied for patients with metastatic melanoma. In a retrospective analysis evaluating all tumors resected from patients with melanoma at the Surgery Branch, NCI, viable TILs could be grown from 94% of patients following resection, and active, specific TILs screened by interferon (IFN)- γ production following co-culture with an autologous tumor cell line could be identified for 67% of patients.⁹ Using similar fragment-based techniques for TIL generation, investigators at H. Lee Moffitt Cancer Center selected rapidly growing cultures for specificity, ultimately treating 13 patients with an objective response rate of 38%.¹⁰

MODIFICATIONS TO IMPROVE DURABLE RESPONSE RATES IN MELANOMA

Efforts at the Surgery Branch and other institutes focused on experimentally altering the three components of the therapy: host lymphodepletion, characteristics of TILs associated

with response, and post-transfer support with IL-2. In murine models, increased levels of lymphodepletion enhanced the efficacy of ACT by depleting regulatory T cells (Tregs) and eliminating cellular cytokine sinks thereby increasing the serum levels of the homeo-static cytokines, interleukin (IL)-7 and IL-15, and facilitating the engraftment of the transferred T cells.^{11,12} The effect of increased lymphodepletion on clinical response was tested in two sequential TIL trials by the addition of increasing doses of total body irradiation (TBI) in combination with CyFlu. The overall response rates increased from 49% (NMA) to 52% (13/25) and 72% (18/25) when 200 and 1,200 cGy TBI, respectively, were combined with CyFlu.¹³ Of the 93 patients treated in these trials, there were 20 CRs, 19 of which are ongoing at 65–128 months. Based on the above results, a prospective randomized trial comparing NMA to CyFlu plus 1,200 cGy TBI as a preparative regimen was conducted at the Surgery Branch, NCI. This trial recently completed patient accrual and should be reported within the coming year.

Previous published data evaluating the characteristics of TILs from objective responders and non-responders determined that longer telomere length correlated with in vivo persistence and tumor regression.¹⁴ This prompted investigation into administration of minimally cultured TILs, decreasing the number of days required to culture TILs to treatment levels and consequently reducing in vitro IL-2 exposure. Using minimally cultured TIL investigators at the Ella Institute in Israel, treated 32 evaluable patients. All objective responders (four CRs, 11 partial responses [PRs], 48% overall response [OR] rate) received TILs that had been cultured <20 days prior to entering a rapid expansion phase (REP). Though the cells were not specifically enriched for CD8⁺ T cells prior to infusion, responders had both a significantly higher percentage of CD8⁺ T cells infused and a greater total number of T cells infused.¹⁵ In a trial conducted at MD Anderson Cancer Center, melanoma patients were treated with TILs following NMA preconditioning; there were 15/31 (48.4%) objective clinical responses with two complete responders. A significant correlation was again demonstrated between higher percentages of CD8⁺ T cells and response. Surprisingly, an analysis of traditional inhibitory co-receptors (PD-1, BTLA, and TIM-3) showed that responding patients had higher levels of CD8⁺/BTLA⁺ cells.¹⁶ Interestingly, these patients were treated with TILs that were not selected for tumor-specific IFN- γ secretion, and in a post hoc analysis there was no correlation between IFN- γ release and response. These results suggested that the technically challenging task of developing autologous tumor lines used for testing the specific reactivity of TILs may be clinically unnecessary, thereby reducing the time from resection to treatment for patients with advanced disease.

To further explore the correlation between CD8⁺ T cells and response, a randomized study was performed at the NCI to evaluate CD8⁺-enriched TILs in comparison to bulk TIL. There were 12 objective responders (2 CRs, 10 PR, 35% OR) in the 34 patients receiving bulk TIL, and seven responders (3 CRs, 4 PRs, 20% OR) in the 35 patients receiving CD8⁺-enriched TIL. The only significant difference between the arms was a decrease in the number of CD4⁺ TILs infused after enrichment (median 8.0×10^9 v 0.3×10^9 CD4⁺ TILs, respectively), which was the anticipated effect of the CD8⁺-selection process. The conclusion was that enrichment of CD8⁺ T cells prior to infusion did not enhance treatment.

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A potential limitation for TIL therapy as it currently exists is the transient toxicity associated with the administration of high-dose IL-2 (720,000 IU/kg every 8 hours). Interestingly, in a pilot trial at Copenhagen University, six patients were treated with TILs followed by low-dose IL-2 (2 MIU/d for 14 days). Of the six patients treated, two experienced complete objective responses.¹⁸ The use of lower dose subcutaneous administration of IL-2 may not only ameliorate some of the side effects attributable to IL-2, but potentially limit the number of inpatient hospital days the current protocols require.

The Ella Institute expanded its protocol into a formal intent-to-treat analysis, to ascertain the frequency with which a patient may undergo an operation for TILs but not receive treatment. Of the 80 patients enrolled, 22 were not treated: eight for technical reasons, three for refusal, and 11 for clinical deterioration. One patient died of cardiac arrest during lymphodepletion, leaving 57 evaluable patients. There were 23 objective responders, and the percentage of CD8⁺ T cells again correlated with response in a retrospective analysis. Of note, seven patients were given CD34⁺ peripheral blood stem cell support after NMA.¹⁹ Patients in the TBI trials at the Surgery Branch also required CD34⁺ stem cell support as a consequence of the ablating effect of irradiation.

AUTOLOGOUS TILs IN CANCERS OTHER THAN MELANOMA

TIL growth has been described for a variety of solid cancers other than melanoma including renal, ovarian, breast, colon, cervical, and others.^{20–24} In the IL-2 clinical trial reported by Rosenberg et al²⁵ (1998), 19% of 227 patients with renal cancer achieved an objective response. Based on these findings, which were similar to those reported for melanoma patients in the same trial, it would appear that RCC is an immunogenic tumor. However, it has been difficult to grow RCC TILs with in vitro tumor reactivity. A variety of clinical protocols evaluating TIL ACT for gastrointestinal cancers, cervical and non-cervical human papilloma virus (HPV)-associated cancers, breast cancers, and non-small cell lung cancers are currently accruing.

Early melanoma TIL trials led to the identification of a group of shared melanocyte differentiation antigens (MDA) such as MART-1, gp100, tyrosinase, and tyrosinase-related protein 1 (TRP1).^{26–31} Of 372 tumor fragments and 166 tumor digests evaluated in the Surgery Branch, NCI, 38% and 23%, respectively, showed shared MDA antigen reactivity.⁹ Patients, even those with bulky disease, treated on TIL protocols rarely exhibit autoimmune toxicities associated with recognition of MART-1 or gp100 on normal tissues. These findings could suggest that TIL recognition of MDAs is likely not responsible for mediating the tumor regressions observed following TIL ACT. In the Surgery Branch, NCI, we reported the successful treatment, using TIL isolated from a patient with heavily pretreated refractory metastatic cholangiocarcinoma.³² Of note, greater than 90% of the TIL product administered were CD4⁺ T cells reactive against a single mutated antigen, *ERBB2IP*, unique to that patient's tumor. The patient is currently experiencing an ongoing PR at 12 months. Efforts are ongoing to develop improved method for targeting the unique mutations expressed in individual tumors.

ADOPTIVE CELL THERAPY USING GENE-MODIFIED T LYMPHOCYTES

It is difficult to isolate and expand tumor-reactive TILs from many solid cancers.²³ These limitations have led to new methods by which peripheral blood mononuclear cells (PBMCs) can be gene-engineered to express an exogenous T-cell receptor (TCR) or chimeric antigen receptor (CAR) that renders them tumor-specific. Redirection of T-cell specificity using a conventional $\alpha\beta$ TCR is constrained by HLA restriction, which limits treatment only to patients expressing a particular MHC haplotype. In contrast, CARs are comprised of a monoclonal antibody single chain variable fragment (scFv) fused in frame to T-cell intracellular signaling domains capable of T-cell activation following antigen-specific binding.³³ CARs, unlike conventional TCRs, are not MHC-restricted but are limited by the requirement for the cell surface expression of the tumor antigen. Unlike TCRs, CARs can also recognize carbohydrate and lipid moieties further expanding their potential application.

TCR GENE THERAPY

The first successful application of ACT using gene engineered cells involved the use of T cells genetically modified with a conventional $\alpha\beta$ TCR targeting MART-1 for the treatment of patients with melanoma (Table 2).³⁴ MART-1 is a shared tumor-differentiation antigen overexpressed on many melanomas, but it is also expressed on normal melanocytes. Tumor-specific TCRs can be cloned directly from TILs, generated from human PBMCs following a variety of in vitro sensitization techniques or by immunization of transgenic mice expressing human leukocyte antigen (HLA) molecules. Following an evaluation of the TILs generated from the first melanoma TIL trial, a T-cell clone with a low avidity TCR recognizing MART-1 was isolated and the α and β chains cloned into a gammaretroviral vector. The vector was then used to deliver the cloned TCRs in the patients autologous PBMCs following retroviral transduction. In a clinical trial using PBMC gene-modified with this low avidity MART-1 TCR, the objective response rate was 13% (2/15) with no reported toxicities.^{34,35} In a follow-up trial with a higher avidity TCR, cloned from the same bulk melanoma TILs, the objective response rate increased to 30% (6/20).³⁶ In addition, a highly avid mouse TCR targeting gp100₁₅₄₋₁₆₂ was generated following immunization of an HLA-A*0201 transgenic mouse. Using this TCR in autologous PBMCs, a response rate of 19% (3/16) was achieved.³⁶ Patients in both trials targeting either MART-1 or gp100 melanocyte antigens experienced significant on-target, off-tumor toxicity with the destruction of normal melanocytes in the skin, eye, and ear.³⁷ In an attempt to improve response rates and decrease toxicity, investigators at the University of Copenhagen administered gene-modified PBMCs encoding a MART-1 TCRs via intratumoral injection, as opposed to systemic administration. Of 15 patients treated, one experienced a PR (1/15, 6.7%).³⁸ No skin, eye, and ear toxicities were seen in this trial.

Carcinoembryonic antigen (CEA) is another shared tumor-differentiation antigen. CEA is highly overexpressed on many epithelial cancers, most notably colorectal adenocarcinoma.³⁹ No toxicities (and no clinical responses) were observed following several clinical trials using anti-CEA antibody or vaccines.⁴⁰⁻⁴³ In a clinical trial reported by Parkhurst et al (2011), a murine TCR directed against CEA₆₉₁₋₆₉₉ with a single amino acid substitution in the CDR3 α to enhance recognition was tested in three patients.³⁹ It has been shown that

modifications to the complementary determining regions (CDR) of a TCR can enhance antigen-specific T-cell functions by altering the avidity of the TCR binding to the peptide/MHC complex.^{44,45} While one patient experienced an objective partial response, all three patients experienced transient but dose-limiting colitis and colonic mucosal destruction that could be reversed by corticosteroid administration. These trials highlight the importance of selecting appropriate tumor antigens to target in order to minimize normal tissue toxicities. Antigens expressed on normal tissues, but overexpressed on tumors, may not be suitable targets for ACT. Alternatively, shared antigen expression on non-essential normal tissues such as breast, prostate, thyroid, or ovary could potentially render such toxicities more acceptable in the face of tumor regression, as loss of these tissues is not life-threatening.

Unlike shared antigens which might be expressed on normal tissues, cancer germ-line antigens (CGA) are expressed only on germ cells during fetal development and then re-expressed on cancers. Normal tissue expression is confined to the testes and because the testes do not express class I MHC molecules, it is an immunologically protected site.⁴⁶ NY-ESO-1 is a CGA overexpressed on melanoma, as well as, a variety of solid epithelial cancers including synovial cell sarcoma.⁴⁷⁻⁴⁹ A high-avidity human TCR containing two amino acid substitutions in CDR3 α to enhance recognition of NY-ESO-1₁₅₇₋₁₆₅ was developed.⁴⁴ Patients with metastatic melanoma or synovial cell sarcoma were treated following ACT using autologous lymphocytes transduced with a gammaretrovirus encoding this receptor.⁵⁰ In updated results from this trial, 11 of 20 (55%) patients with melanoma showed objective tumor responses, four of which were CRs, three ongoing at 31–58 months. Ten of 18 (56%) patients with synovial cell sarcoma showed objective tumor response, only one of which is complete and ongoing at 16 months. Of note, no toxicities were observed in this trial.

MAGE-A3 is another CGA that has been targeted with TCR gene therapy. A high-affinity murine TCR was raised against MAGE-A3₁₁₂₋₁₂₀ in which the CDR3 α was modified by a single amino acid substitution to improve antigen recognition.⁵¹ In a clinical trial of nine patients treated with autologous T cells transduced with this receptor, 56% (5/9) of patients experienced an objective tumor response, one of which was a CR. However, three of nine (44%) patients also experienced severe neurologic toxicities, including two deaths. The cause of death has been linked, in part, to possible cross-reactivity with a similar epitope present in MAGE-A12 within the brain.⁵² In a separate clinical trial targeting MAGE-A3, an HLA-A1-restricted TCR was affinity-enhanced by making four amino acid substitutions in CDR2 α .^{53,54} The first two patients treated on this trial developed cardiogenic shock and died within a few days of T-cell infusion. Follow-up studies revealed TCR recognition of the striated muscle-specific protein, titin, as the cause of the acute cardiac toxicity. These findings demonstrated the potency of TCR-transduced cells to mediate tumor killing as well as significant on-target, off-tumor toxicities. Thus, while targeting NY-ESO-1, MAGEA3, and other CGA is an attractive strategy for the application of ACT for the treatment of solid cancers, caution must be taken to ensure lack of cross-reactivity with vital normal tissues. In addition, modifications of the CDR regions of TCR must be performed with caution, as modified receptors, similar to receptors generated following immunization of HLA-transgenic mice, have not undergone negative selection in the thymus and could have potential reactivity against unrelated normal host proteins. A need exists to develop better

screening methods to avoid such toxicities in the future. As more antigen-specific TCRs are identified targeting other cancer histologies, more data will become available to allow a better understanding of how to use TCR gene-engineered cells to better treat patients with cancer.

CAR GENE THERAPY FOR SOLID CANCERS

To date, there has been limited success using CAR-based ACT for the treatment of solid cancers (Table 3). One of the first antigens targeted by CAR ACT was carbonic anhydrase IX (CAIX), which is highly expressed on renal cell carcinomas (RCC).^{55–57} In a small trial, patients with metastatic RCC were treated with CAIX CAR–T cells.⁵⁸ The first three patients received up to seven increasing doses of cells over 19 days in combination with IL-2. All three patients showed signs of liver toxicity. A liver biopsy from one patient showed cholangitis with T-cell infiltration around bile ducts suggesting that the toxicity was mediated by an influx of CAIX CAR–T cells.⁵⁸ In a follow-up study an additional nine patients were treated and similar toxicities were observed. These toxicities were on-target but off-tumor as they could be minimized by pre-infusing the anti-CAIX monoclonal antibody, G250.⁵⁹ There were no objective responders in these trials.

In 2007, CAR ACT was used to target the L1-cell adhesion molecule (CD171) overexpressed on meta-static neuroblastoma. Of six patients treated, one experienced a partial objective response.⁶⁰ In 2008, another successful CAR trial targeting the diasialoganglioside, GD2, for the treatment of neuroblastoma was reported.⁶¹ In this trial, EBV-specific cytotoxic T lymphocytes and activated T lymphocytes were transduced with a GD2 CAR and both populations of cells were administered to patients. In the initial report, 4/8 (50%) patients with evaluable tumor experienced tumor regression or necrosis with one complete responder. A second report detailed the long-term follow-up of 19 patients, of which eight patients were in remission and 11 had active disease at the time of infusion. The OR rate was 36% [CR, 3/11 (27%), PR, 1/11 (9%)] for patients with active disease. EBV-specific and T-lymphocyte populations from non-immunized patients persisted for up to 192 and 96 weeks, respectively.⁶²

In an attempt to treat cancer patients with *ERBB2*-overexpressing tumors, a CAR targeting the receptor tyrosine-protein kinase erbB-2 (*ERBB2*) was generated using a single-chain Fv fragment based on the widely used humanized monoclonal antibody, trastuzumab (Herceptin, Genentech, San Francisco, CA).⁶³ *ERBB2* is overexpressed on a variety of cancers, including breast, colon, ovarian, kidney, and melanoma, making it an attractive immunotherapy target. However, in the first patient treated on this trial, following cell infusion the patient experienced respiratory distress and a dramatic pulmonary infiltrate likely due to CAR–T-cell recognition of low-level antigen expressed in the lungs. The patient showed marked increases in serum cytokine levels including IFN- γ , granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor- α (TNF- α), IL-6, and IL-10.⁶⁴ Despite intensive medical intervention, the patient died 5 days post-treatment. Moving forward, it will be important to conduct more restricted dose-escalation studies to minimize the effects of the potential on-target, off-tumor toxicities.

The development of CAR-based ACT for the treatment of solid cancers has been slow due to the difficulty of identifying suitable target antigens. Mesothelin, normally expressed on mesothelial cells lining the pleura, pericardium, and peritoneum, is overexpressed on mesotheliomas, as well as pancreatic and ovarian cancer.^{65,66} In an effort to reduce normal tissue toxicity, Beatty et al (2014) at the University of Pennsylvania utilized mRNA-engineered CAR-T cells to treat patients with mesothelin-positive solid tumors.⁶⁷ The mRNA-engineered CAR-T cell expressed the anti-mesothelin CAR transiently for about 1 week. In order to maintain persistence of the CAR-T cells, the protocol involved multiple intravenous infusions followed in one patient with two intratumoral injections of the CAR-T cells. A patient with malignant pleural mesothelioma (MPM) experienced near lethal anaphylactic shock following repeated intravenous administration of mRNA-engineered CAR-T cells due to antibodies against the murine portion of the CAR. Clinical responses were minimal.⁶⁷ At the NCI Surgery Branch, a clinical trial targeting mesothelin using a second-generation anti-mesothelin CAR is currently accruing. In addition, there is an active clinical program using a CAR to target the mutated epidermal growth factor receptor, EGFRvIII, a unique mutated antigen expressed on approximately 40% of glioblastomas, as well as head and neck cancers,⁶⁸ but not expressed on normal tissues. Similarly, there is a clinical effort to target tumor vasculature using a CAR directed against the vascular endothelial growth factor-2 receptor, VEGFR-2.⁶⁹

CAR GENE THERAPY FOR HEMATOLOGIC MALIGNANCIES

CAR ACT for the treatment of hematologic malignancies is an active area of research given the effectiveness of monoclonal antibody therapies targeting CD20⁺ B-cell malignancies.^{70–74} In a trial using a first-generation CAR targeting CD20 that was electroporated into T cells, seven patients were treated, of which five were evaluable (Table 4). Of these five, one experienced an objective PR lasting 3 months.⁷⁵ In a second trial using a third-generation CAR targeting CD20 along with the administration of low-dose IL-2, three patients were treated. All three had low levels of CD20 CAR-T cells persisting up to 12 months post-cell transfer. Of the three patients treated on this trial only one had evaluable disease and achieved a PR lasting 12 months.⁷⁶

To date, the most widely studied B-cell antigen targeted by CAR ACT is CD19. CD19 is expressed on virtually all B-cell malignancies, whereas normal tissue expression is limited to B-cell precursors, mature B cells, plasma cells, and follicular dendritic cells, but not pluripotent hematopoietic stem cells.^{77–79} Kochenderfer et al (2010) were the first to report the successful treatment of a patient with B-cell lymphoma (follicular, FL) using autologous T cells transduced with a gamma-retrovirus encoding a second-generation CD19 CAR containing both CD28 and CD3 ζ signaling domains.⁸⁰ This patient is an ongoing progression-free responder at nearly 5 years duration following two treatments. An additional seven patients were treated on this trial, with five of the patients experiencing an objective PR.⁸¹ This trial was associated with transient but significant toxicities including hypotension and neurological toxicity, which correlated with increased levels of serum inflammatory cytokines.⁸¹ More recently, in a trial treating patients with chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies (lymphoma and chronic lymphocytic leukemia), 15 patients were treated with an OR rate of 12/15 (80%).

Eight of the 15 patients treated (53.3%) achieved a CR and 4/15 (26.6%) achieved a PR.⁸² Numerous other institutions have also reported clinical trials using autologous T lymphocytes expressing an anti-CD19 CAR for the treatment of B-cell malignancies. In 2011, results from a trial conducted at the University of Pennsylvania were reported in which three chronic lymphocytic leukemia (CLL) patients treated with anti-CD19 CAR-T cells transduced using a lentiviral vector encoding the same anti-CD19 scFv (FMC63) used in the NCI/Surgery Branch trials, but the CD28 signaling domain was replaced by 41BB.^{83,84} Two of the three patients experienced complete objective responses. Similarly, in two patients with acute lymphocytic leukemia (ALL) treated with the same CAR, both patients had CRs, only one of which was durable but was associated with severe cytokine-release syndrome (CRS).⁸⁵ The other patient relapsed with CD19⁺ blasts after 2 months. Memorial Sloan Kettering Cancer Center has reported two clinical trials that used a second-generation CAR with the scFv derived from 19z1 but with CD28 and CD3 ζ signaling domains.^{86–88} In the first report, nine patients with CLL or ALL were treated CD19 CAR-T cells.⁸⁷ The first three patients received CD19 CAR-T cells and no lymphodepletion and none of these patients responded to this therapy. Of the five additional patients treated (four CLL, one ALL), one patient with CLL experienced a PR. In a follow-up study evaluating the efficacy of anti-CD19 CAR ACT in five patients with ALL, all five patients had complete objective responses, four of which went on to receive allogeneic hematopoietic stem cell transplants (HSCTd).⁸⁶ Most recently, results from two phase I clinical trials were reported for the treatment of children and young adults with relapsed acute lymphoblastic leukemia (B-ALL). In the Pediatric Oncology Branch at the NCI, in the first intent-to-treat analysis using retroviral-transduced CD19 CAR-T cells to treat B-ALL, this therapy induced complete responses in 70% of the patients treated and an minimal residual disease (MRD)-negative response in 60% of patients.⁸⁹ Similarly, in a trial sponsored by the University of Pennsylvania, lentiviral-transduced CD19 CAR-T cells induced complete remissions in 90% (27/30) of patients treated.⁹⁰ In both trials, a major toxicity was CRS, the severity of which appeared to be correlated with the burden of disease. The CRS was reversible and managed with supportive care and the anti-IL-6 receptor antibody, tocilizumab. Disease relapse and opportunistic viral infections are major concerns following HSCT.⁹¹ An interesting approach to control such adverse outcomes involves the transfer of donor-derived CD19 CAR virus-specific T cells (CD19-VSTs) following HSCT. A phase I trial evaluated eight patients (six with relapsed disease, two in remission) treated with CD19-VSTs 3 months to 13 years following HSCT. Of the six patients with relapsed disease, two experienced an objective response (one CR lasting 3 months and one PR lasting 8 weeks). In addition, the CD19-VTSs persisted in all patients for a median duration of at least 8 weeks with no evidence of graft-versus-host disease (GVHD).⁹¹ Interestingly, 3/8 patients had viral reactivation (one adenovirus [AdV] and two EBV). While the frequency of AdV-specific T cells increased in response to reactivation, the frequency of CD19-VTSs did not; however, for the two patients with reactivation of EBV, there was a positive correlation with an increased frequency of EBV-specific T cells and CD19-VTS and a corresponding drop in EBV viral load, suggesting that CD19-VTSs can control opportunistic viral infections and mediate objective tumor regressions.

There are other CARs in development targeting hematologic surface makers such as CD22, CD23, receptor tyrosine kinase-like orphan receptor-1 (ROR1), the immunoglobulin kappa light chain, and B-cell maturation antigen.^{92–97}

CONCLUSIONS

ACT using TILs is an effective therapy for the treatment of patients with metastatic melanoma. The objective response rate from patients treated with standard TILs is greater than 50% with many of these patients experiencing durable CRs beyond 5 years. The effort to extend TIL therapy for the treatment of other solid cancers is an ongoing effort. New approaches include the selection of tumor reactive cells in TILs using phenotypic markers such as PD1 and 41BB,⁹⁸ as well as the means to foster a more permissive tumor microenvironment by the local secretion of IL-12.⁹⁹ In addition, identification of TILs capable of recognizing patient-specific mutated antigens in melanoma and cholangiocarcinoma suggests that mutated antigens, rather than shared tumor antigens, are likely the primary targets of TILs. This finding suggests that TILs targeting mutated antigens can be applied to treat other solid cancers. As technologies improve, it may also be possible to clone TCRs from mutation-reactive TILs for use in ACT using gene-modified autologous lymphocytes. The major challenge confronting the continued development of ACT for cancer will be the identification of target antigens that minimize “off-tumor, on-target” toxicities.

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Table 1.
The History of Adoptive Cell Transfer Using TILs for the Treatment of Solid Cancers

Reference	Histology	Patients (n)	Response ⁺			Significance
			OR (%)	CR (%)	PR (%)	
Rosenberg et al, 1988	Melanoma	20	11 (55%)	1 (5%)	10 (50%)	First in human trial (TILs + IL-2)
Dudley et al, 2005	Melanoma	43	21 (49%)	5 (12%)	16 (37%)	Pre-conditioning regimen to improve TIL engraftment using “modern-era” lymphodepletion (NMA) and high dose interleukin-2 (IL-2)
Dudley et al, 2008		25 (2 Gy TBI)	13 (52%)	5 (20%)	8 (32%)	In sequential trials, response rate directly proportion to depth of pre-conditioning lymphodepletion, prompting evaluation in a randomized trial.
Rosenberg et al, 2011		25 (12 Gy TBI)	18 (72%)	10 (40%)	8 (32%)	
Dudley et al, 2010	Melanoma	33 (NMA)	19 (58%)	3 (9%)	16 (49%)	Minimally cultured CD8-enriched TILs can mediate effective tumor regression.
		23 (6 Gy TBI)	11 (48%)	2 (9%)	9 (39%)	
Itzhaki et al, 2011	Melanoma	31	15 (48%)	4 (13%)	11 (35%)	Minimally cultured bulk TILs can mediate effective tumor regression.
Pilon-Thomas et al, 2012	Melanoma	13 ^a	5 (38%)	2 (15%)	3 (23%)	Bulk TIL screened for IFN- γ secretion can mediate durable tumor regression.
Radvanyi et al, 2012	Melanoma	31	13 (42%)	2 (6%)	11 (35%)	TIL, particularly differentiated effector cells (CD8+/BTLA+) can mediate durable tumor regression.
Ellebaek et al, 2012	Melanoma	6 ^b	2 (33%)	2 (33%)	0	Complete and durable responses were induced after TIL treatment using NMA in combination with low-dose IL-2
Besser et al, 2013	Melanoma	80 ^c	23 (29%)	5 (6%)	18 (23%)	Complete and durable responses with NMA TIL. First intent-to-treat analysis, demonstrating a 29% dropout rate, mainly due to disease progression
Tran et al, 2014	Cholangiocarcinoma	1	1 (100%)	-	1 (100%)	First successful treatment of a solid epithelial cancer using TILs targeting a mutated antigen.

⁺ As measured by WHO or RECIST criteria

^a An additional 6 patients resected, but not treated

^b An additional 5 patients resected, but not treated

^c 23 (29%) patients enrolled, but not treated

ACT Clinical Trials Employing T Lymphocytes Engineered to Express Specific T-Cell Receptors for the Treatment of Solid Cancers

Table 2.

Reference	Histology	Antigen (epitope)	HLA ^a	Patients (n)	Response			Significance
					OR(%)	CR(%)	PR(%)	
Morgan et al, 2006	Melanoma	MART-1 (aa 27–35)	A*02	15	2 (13%)	0	2 (13%)	First demonstration of use of TCR-engineered T cells to mediate tumor regression. No treatment-related toxicities.
Johnson et al, 2009	Melanoma	MART-1 (aa 27–35)	A*02	20	6 (30%)	0	6 (30%)	Demonstrated importance of clonal selection. Grade 3 ototoxicity in 8 patients.
	Melanoma	Gp100 (aa 154–162)	A*02	16	3 (19%)	1 (6%)	2 (13%)	Demonstrated use of TCR with murine constant regions. Grade 3 ototoxicity in 1 patient.
Parkhurst et al, 2011	Colon	CEA (aa 691–699)	A*02	3	1 (33%)	0	1 (33%)	Recognition of CEA in normal colonic mucosa resulted in 3 patients with severe colitis.
Robbins et al, 2011	Synovial sarcoma, melanoma, breast, ovarian prostate, thyroid	NY-ESO-1 (aa 157–165)	A*02	17	9 (53%)	2 (12%)	7 (41%)	First report using of TCR-engineered T cells targeting a cancer germline antigen to mediate tumor regressions. Modification of CDR2 of TCR alpha chain to increase TCR avidity without altering antigen specificity. No major toxicities observed.
Morgan et al, 2013	Melanoma	MAGE-A3 (aa 112–120)	A*02	9	5 (56%)	1 (11%)	4 (44%)	Previously undescribed MAGE-A12 expression in brain tissue resulted in 3 patients with severe neurologic toxicity, including 2 TRM ^b .
Linette et al, 2013	Melanoma, myeloma	MAGE-A3 (aa 168–176)	A*01	2	0	0	0	Off-target activity against myocardial protein titin resulted in 2 TRM secondary to cardiogenic shock.

^aHLA: Human leukocyte antigen restriction element recognized by the TCR.

^bTRM: Treatment-related mortality

Table 3.
ACT Clinical Trials Employing T Lymphocytes Engineered to Chimeric Antigen Receptors for the Treatment of Solid Cancers

Reference	Histology	CAR Generation	Target	Patients (n)	Response ^a			Significance
					OR (%)	CR (%)	PR (%)	
Laners et al, 2006	Clear Cell RCC	1 st	CAIX	3	0 (0%)			First clinical experience targeting CAIX.
Laner et al, 2013				12	0 (0%)			Reported on-target off-tumor reactivity of CAR-T cells, which could be attenuated by pre-treatment with CAIX monoclonal antibody (G250).
Park et al, 2007	Neuroblastoma	1 st	L1-CAM	6 ^b	1 (17%)	1 (17%)	0	First successful treatment of a solid tumor using a CAR.
Pule et al, 2008	Neuroblastoma	1 st	GD2	8 ^c	1 (12.5%)	0	1 (12.5%)	Tumor regression and <i>in vivo</i> persistence of EBV specific CAR-T cells for the treatment of neuroblastoma.
Louis et al, 2011				11 ^d	4 (36%)	3 (27%)	1 (9%)	
Morgan et al, 2010	Colon adenocarcinoma	3 rd	ErbB2	1				Mortality because of on-target, off-tumor reaction to low expression in normal lung tissue
Beatty et al, 2014	Mesothelioma, pancreatic adenocarcinoma	2 nd	mesothelin	2 ^e	1 (50%)	0	1 (50%)	Mesothelin-specific CAR mRNA-engineered T cells induce anti-tumor activity

^aOR, overall response; CR, complete response; PR, partial response; RCC, renal cell carcinoma; CAIX, carbonic-anhydrase IX; L1-CAM, L1 cell adhesion molecule; EBV, Epstein Barr virus; CAR, chimeric antigen receptor; NE, not evaluable for response after treatment.

^b 4 additional patients enrolled (2 ineligible prior to treatment and unable to generate cell product for 2)

^c 3 additional NED patients treated

^d 8 additional NED patients treated

^e 1 additional NE patient

Table 4.

ACT Clinical Trials Employing T Lymphocytes Engineered to Chimeric Antigen Receptors for the Treatment of Hematologic Malignancies

Reference	Histology	CAR Generation	Target	Patients (n)	Response ^a			Significance
					OR (%)	CR (%)	PR (%)	
Till et al, 2008	NHL, MCL	1 st	CD20	5 ^b	1 (20%)	1 (20%)	00	First report using electroporated CAR-T cells.
Till et al, 2012	Follicular B-cell lymphoma	3 rd		3 ^c	1 (33%)	1 (33%)		Increased persistence of CAR-T cells with IL-2 administration.
Kochenderfer et al, 2010	B-cell lymphoma	2 nd	CD19	1	1 (100%)	1 (100%)	-	First report of tumor regression in lymphoma patient treated with CD19 CAR.
Kochenderfer et al, 2014	DLBCL, CLL, SMZL, PMBCL, NHL			13 ^d	12 (92%)	8 (62%)	4 (30%)	First successful treatment of patients with DLBCL using CD19 CAR.
Kalos et al, 2011	CLL	2 nd	CD19	3	3 (100%)	1 (33%)	2 (67%)	Demonstrated in vivo expansion, persistence and establishment of memory for CAR-transduced T cells.
Porter et al, 2011								
Brentjens et al, 2013	ALL	2 nd	CD19	14 ^e	12 (75%)	10 (63%)	2 (13%)	First report of clinical response in ALL.
Davila et al, 2014								
Cruz et al, 2013	CLL, ALL	2 nd	CD19	6 ^f	2	1 (17%)	1 (17%)	Successful use of donor-derived CD19-redirection virus-specific T cells for the treatment of B-cell malignancies following allogeneic stem cell transplant.

^a Abbreviations: OR, overall response; CR, complete response; PR, partial response; NHL, non-Hodgkins Lymphoma; MCL, mantle cell lymphoma; NED, no evaluable disease; NE, not evaluable for treatment response; CAR, chimeric antigen receptor; NCI, National Cancer Institute; DLBCL, diffuse large B cell lymphoma; CLL, chronic lymphocytic leukemia; SMZL, splenic marginal zone lymphoma; PMBCL, primary mediastinal B cell lymphoma; MSKCC, Memorial Sloan Kettering Cancer Center; ALL, acute lymphoblastic leukemia; EBV, Epstein-Barr virus.

^b 4 additional NE patient treated, 2 NED and 2 NE

^c 1 additional NE patients treated

^d 2 additional NE patients treated

^e 2 additional patients with molecular CR prior to cell infusion, CR defined as MRD⁺, PR as MRD⁺ (morphological residual disease)

^f 2 additional NED patients treated