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Non-Engineered Adoptive T Cell Therapy

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KEY POINTS

- Non-engineered adoptive cell transfer (ACT) is a cancer immunotherapy modality rooted in the premise that there are endogenous/existing tumor-reactive T cells that can be isolated and expanded in sufficient quantity and quality for infusion into patients, with the expectation that they will kill tumor cells and provide long-term immunoprotection.
- When combined with a lymphodepleting preconditioning regimen and a rapid expansion protocol, tumor-infiltrating lymphocyte (TIL) therapy has achieved durable responses in a subset of patients with advanced cancers, in particular metastatic melanoma.
- Endogenous T cell (ETC) therapy sources tumor antigen-specific T cells from the peripheral blood and can achieve durable responses without the use of high-dose lymphodepleting preconditioning regimens or post-infusion high-dose Interleukin-2 (IL-2).
- Challenges to application of ACT to broader patient populations include the identification of immunogenic target antigens while minimizing off-tumor toxicity, poor trafficking to and infiltration of tumor sites, and an immune-evasive, hostile tumor microenvironment.
- Combination of ACT with therapies such as checkpoint blockade, oncolytic viruses, or low-dose radiotherapy, as well as intrinsic strategies that influence T cell phenotype and function, may be used to increase the efficacy of ACT by promoting antigen spreading, improved trafficking, function, and *in vivo* persistence.
- γδ T cells have potential for antitumor activity against a broad range of tumor types, and their major histocompatibility complex (MHC)-independence reduces requirements for human leukocyte antigen (HLA)-matching while enabling them to treat tumors that escape the conventional T cell response via MHC downregulation.

INTRODUCTION

Adoptive cell transfer (ACT) is a form of cellular immunotherapy whereby tumor-reactive T cells are enriched and expanded ex vivo for the treatment of patients with cancer. This approach has become an increasingly attractive treatment modality due to its potential for high specificity, combination with other therapies, and promise of long-term immunoprotection against disease recurrence. The effector cells used for ACT may be derived from peripheral blood T cells (endogenous T cell therapy) or tumor-infiltrating lymphocytes (TIL therapy), or genetically engineered to express receptors that recognize tumor antigens (TCR-engineered T cell therapy and chimeric antigen receptor T cell [CAR T] therapy). This chapter will focus on non-engineered ACT modalities whereby the structures and signaling involved in antigen recognition and T cell activation are unadulterated; in contrast to engineered T cell approaches, non-engineered T cell therapies can offer greater flexibility in tumor targeting and opportunity for combination strategies that can lead to functional enhancement, antigen spreading, and increased T cell persistence. A variety of non-engineered ACT approaches are under clinical investigation and these are summarized in Figure 25.1.

THE BEGINNINGS OF ADOPTIVE T CELL THERAPY

Adoptive cellular immunotherapy for cancer is arguably rooted in early studies on stem cell transplantation, perhaps the crudest form of ACT, to treat leukemia in a murine model.[1] The original intent behind this therapy was to ablate the abnormal, cancerous bone marrow (leukemia) and reconstitute it with normal donor marrow. In human (allogeneic) studies, it was observed that transplant recipients who developed graft-versus-host disease trended to lower rates of relapse, and that this phenomenon was dependent on the presence of donor T cells, suggesting their role in this antileukemia effect.[2–5] In the 1970s, studies at the University of Washington showed that splenocytes from mice whose tumors had regressed could be used in conjunction with cyclophosphamide to treat tumors in recipient mice.[6,7] Simultaneously, studies on a T cell growth factor present in conditioned media of stimulated lymphocytes

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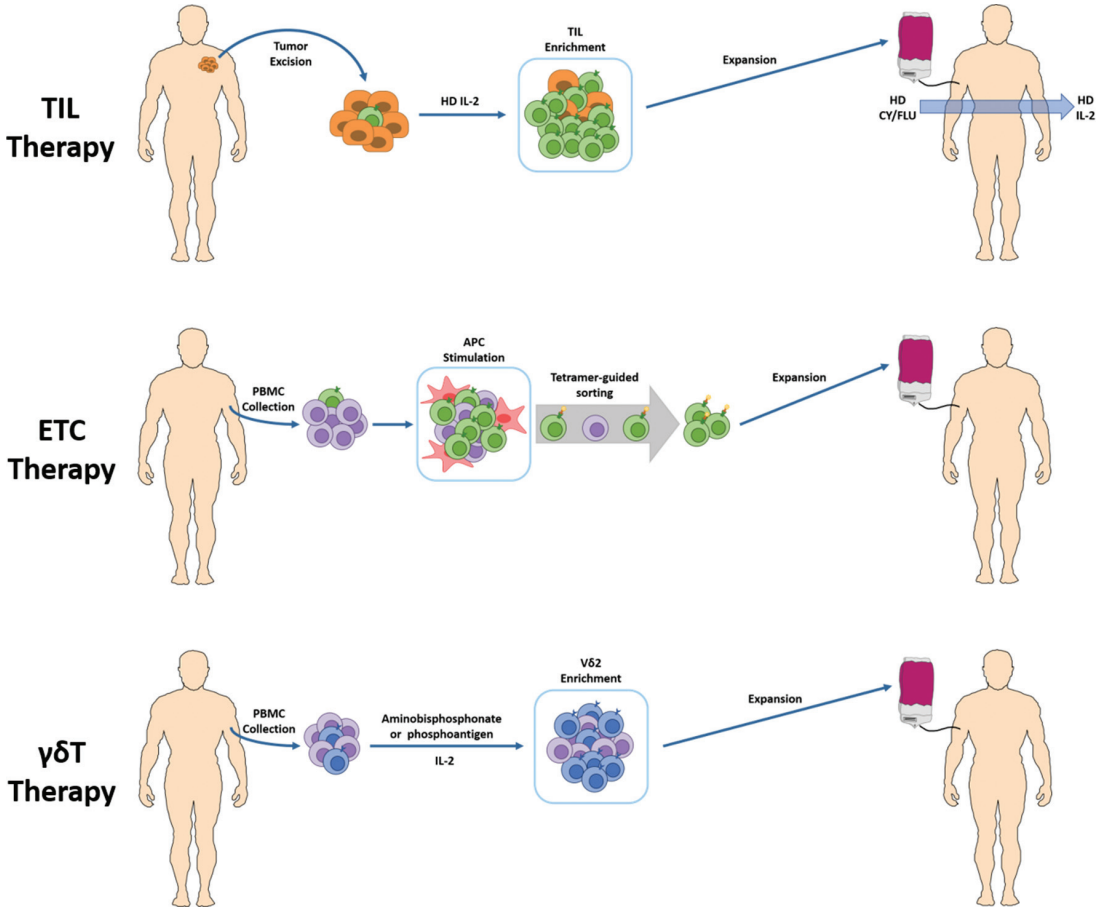

Figure 25.1 Non-engineered adoptive T cell therapy approaches. Tumor-reactive T cells used for non-engineered adoptive T cell therapy are derived from peripheral blood mononuclear cells (endogenous T cell and γδT therapies) or tumor-infiltrating lymphocytes (tumor-infiltrating lymphocyte therapy), enriched and expanded ex vivo, and infused to treat patients with cancer.

APC, antigen-presenting cell; ETC, endogenous T cell; IL-2, interleukin-2; PBMC, peripheral blood mononuclear cell; TIL, tumor-infiltrating lymphocyte.

(later characterized as interleukin-2; IL-2) enabled in vitro expansion of antitumor lymphocytes for adoptive transfer, laying the foundation for current adoptive T cell therapy paradigms, and defining three requirements operative in the elimination of cancer: lymphodepletion, a source of effector cells, and helper function.[8–14] In the 1980s, Rosenberg and colleagues at the National Cancer Institute (NCI) demonstrated that systemic administration of recombinant IL-2 could cause durable responses in some patients with metastatic cancer, providing empiric evidence for the existence of endogenous tumor-reactive T cells that can be expanded to mediate tumor regression.[15,16] Since these seminal studies, methods have been developed to isolate and expand antitumor T cells for clinical use and ACT has been actively pursued as a cancer treatment modality.

ADOPTIVE TRANSFER OF TUMOR-INFILTRATING LYMPHOCYTES

Early attempts to generate large numbers of tumor-reactive immune cells for adoptive transfer led to the development of lymphokine-activated killer cells (LAK): peripheral blood lymphocytes endowed with the ability to lyse tumor cells after exposure to IL-2.[17] However, treatment with LAK required very large numbers of cells and administration of toxicity-inducing doses of systemic IL-2 to effectively mediate regression; thus, in order to reduce these requirements, TILs were investigated as a source of more concentrated, potent tumor-reactive lymphocytes.[18,19] The initial murine studies demonstrated that TILs were 50 to 100 times more effective than LAK cells in eliminating established tumors, providing rationale for further development of TILs for the treatment of patients with cancer.[19] The standard approach to TIL expansion for adoptive therapy, pioneered by Rosenberg and colleagues at the Surgery Branch of the NCI, involves resection of tumor tissue dissected into small tumor fragments or enzymatically digested into single-cell suspensions and culture in media containing high-dose IL-2 to enable outgrowth of TILs.[20–22] The TILs can then be harvested and further expanded to numbers sufficient for infusion using a Rapid Expansion

Protocol (REP) of soluble anti-CD3, irradiated feeder cells, and IL-2—a method first established by Riddell and Greenberg and modified by the NCI Surgery Branch for TIL expansion.[22,23]

Tumor-Infiltrating Lymphocyte Therapy: Clinical Experience and Optimization

In a 1988 pilot study treating 12 patients with various advanced cancers with 0, 25, or 50 mg/kg cyclophosphamide, escalating doses of IL-2, and infusion of autologous TIL, two partial responses were observed among the patients who had received cyclophosphamide, mirroring the prior murine studies indicating that enhanced efficacy required pretreatment with cyclophosphamide and co-administration of IL-2.[19,24] Subsequent trials in metastatic melanoma patients produced encouraging results with partial responses in some patients; however, toxic side effects due to the high-dose IL-2 were common, although manageable.[20,25,26] In an effort to increase efficacy and promote engraftment and persistence of the infused TILs, a nonmyeloablative lymphodepleting preconditioning regimen of cyclophosphamide and fludarabine (adapted from stem cell transplant practices) was incorporated into clinical protocol, yielding overall response rates of 38% to 51% and durable responses of 10% to 15%.[27,28] Responses were often accompanied by vitiligo and anterior uveitis—autoimmune destruction of melanocytes in the skin and uvea, which was largely reversible. As desired, these studies provided evidence of in vivo expansion and persistence of the transferred cells; however, in some cases, the endogenous T cell repertoire was replaced almost completely with the transferred TIL population. Addition of total body irradiation (TBI) to this preconditioning regimen in a small cohort of patients reported in 2008 achieved overall response rates of 72% and dramatic and durable complete responses of up to 40%, but the additional benefit of TBI was not confirmed in a follow-up randomized trial in 2016.[29,30] Among major institutions conducting TIL therapy using a "standard" nonmyeloablative regimen of fludarabine and cyclophosphamide (Sheba Medical Center, Moffitt Cancer Center, and MD Anderson Cancer Center), the overall response rate achieved is 40% to 49% with a complete response rate of 6% to 15%.[31–36] Although temporary lymphodepletion prior to infusion seems to benefit TIL therapy, further studies are necessary to determine the optimal regimen to increase efficacy while minimizing toxicity.

The initially established procedure for TIL product generation involved culture in IL-2, selection of tumor-reactive cultures based on reactivity to autologous tumor or HLA-matched tumor cell lines, followed by further expansion, resulting in a lengthy process that could not always successfully generate an infusion product. Thus, many patients ultimately did not receive the therapy because their TIL failed testing for tumor reactivity, there was no tumor material to conduct such tests, or their disease progressed during the TIL production process.[37] To address these limitations, selection based on tumor reactivity was discontinued and a "minimal culture" method was adopted.[38,39] This simplified process reduced production time, increased the success rate for product generation (up to 94% in some series), and produced "young" TIL associated with improved efficacy.[33,37,39,40] Alternatively, the infusion of unselected TIL expanded from tumor fragments in 74 metastatic melanoma patients generated similar response rates (42%), further supporting the absence of benefit from selection based on tumor reactivity for TIL derived from melanoma.[41] Even with these improvements, requirements of tumor fragment excision, specialized production, and optimal management of adverse reactions currently limits TIL therapy to a handful of large medical centers.

Tumor-Infiltrating Lymphocyte Therapy: The Next Stage

TIL products are heterogeneous, containing $CD4^+$ and $CD8^+$ T cells with varying antigen specificity and tumor reactivity, and the composition is unique to each patient, the tumor site, and even intratumoral localization of biopsy sampling.[42] Specificity and efficacy may be improved by enriching for TIL that express markers associated with tumor reactivity. At the NCI, PD-1, which is upregulated following TCR triggering, was used as a selection marker for melanoma-reactive TILs: following PD-1 expression-based sorting and expansion, $CD8^+$ $PD\text{-}1^+$ TILs from fresh tumors displayed increased IFN-γ production and cytotoxicity after co-culture with autologous tumor cell lines.[43] Similarly, other groups have shown that expression of CD137 (4-1BB), another T cell-associated activation marker, designates tumor-specific TIL that are more reactive in vitro and more effective at controlling tumors in an NSG model.[44,45] In a related approach, the addition of agonistic anti-4-1BB antibody during the initial tumor fragment culture phase of TIL production seems to favor outgrowth of tumor-reactive $CD8^+$ TIL.[46–49] Whether the results of these preclinical studies translate to increased clinical efficacy will be determined in clinical trials.

Advances in optimization of growth conditions, such as the use of 4-1BB-agonistic antibodies, have overcome the limitation of low infiltrate and enabled successful expansion of TIL from multiple nonmelanoma solid tumors including ovarian cancer, cervical cancer, breast cancer, bladder cancer, pancreatic cancer, and even very poorly infiltrated GI cancers.[49–54] Various case reports and pilot studies have shown objective responses and clinical benefit from TIL therapy in patients with metastatic colorectal cancer, metastatic breast cancer, cervical cancer,

and metastatic ovarian cancer, but to date there have been few larger-scale studies employing current TIL therapy protocols in solid tumors, aside from advanced cutaneous melanoma.[55–59] A single-arm, Phase II trial of TIL therapy to treat metastatic uveal melanoma reported objective responses in 35% of evaluable patients, including one complete response.[60] Strategies to expand the application of TIL therapy to other solid tumors are discussed in a later section.

Defining the antigen specificity of antitumor response in TIL products can provide a rich source of potential tumor-rejection antigens but has been challenging due to the diversity of lymphocyte repertoires and the labor-intense antigen screening process. Profiling of melanoma TIL against a panel of all known HLA-A2-restricted melanoma-associated epitopes revealed reactivity to melanocyte differentiation antigens (e.g., MART-1), germline antigens (also known as cancer-testis antigens, CTAs; e.g., NY-ESO-1 and MAGE family), and overexpressed antigens (e.g., Survivin); however, these cells comprised a small proportion of the total $CD8^+$ T cell pool.[61] Reactivity was mostly observed against MART-1, diverse germline antigens, and overexpressed antigen epitopes derived from cryptic open reading frames or alternative splicing events, suggesting a relationship to T cell escape of central tolerance.[62] Attention has increasingly shifted to neoantigens arising from tumor-specific mutations, which are postulated to be immunogenic to T cells. New prediction and screening approaches have identified melanoma neoantigens that elicit antitumor T cell reactivity, and these studies also suggest that neoantigen-specific T cells are important mediators of tumor regression.[43,63,64] Encouragingly, the NCI has reported durable complete responses in four patients who received infusions of TIL products enriched for melanoma neoantigen reactivity.[65–67] The promise of enrichment for neoantigen-specific TIL as a strategy to increase therapeutic efficacy is counterbalanced by the additional time and resources necessary to identify these cells for each patient; however, this process may be streamlined by preselection based on PD-1 or CD137 expression.[43,68]

ENDOGENOUS T CELL THERAPY

Endogenous T cell (ETC) therapy uses peripheral blood as a source of T cells for ACT and is based on the premise that there exists a population of tumor-reactive T cells that can be identified, isolated, and expanded in sufficient quantity and of desired quality for infusion into patients, with the expectation that they will kill tumor cells and provide long-term immunoprotection. The low frequencies at which circulating tumor-reactive T cells are found in patients required the development of enabling technologies to facilitate the isolation and expansion of antigen-specific T cells from peripheral blood using various in vitro strategies.

Development of Engogenous T Cell Therapy

The direct demonstration that a circulating population of potentially tumor-reactive antigen-specific T cells exists in patients with cancer first became possible following the development of peptide-MHC (pMHC) multimers.[69,70] Direct labeling of T cells recognizing tumor-associated melanocyte differentiation antigens (gp100, tyrosinase, MART-1) revealed measurable populations of circulating T cells in melanoma patients, at frequencies as high as 2%; typically, the prevalence of tumor-associated antigen-specific cytotoxic T lymphocytes (CTLs) in the peripheral circulation is <0.2% and often at the lower limits of detection (0.01%).[70,71] Tetramer-guided cell sorting enabled in vitro expansion of this population to near uniform specificity (80–100%) but would not be translated into clinical practice until more than a decade later and has since transformed the feasibility of ETC therapy.[72,73]

Initial ETC therapy studies involved a lengthy protocol of in vivo stimulation, limiting dilution cloning, screening, and expansion requiring upwards of 12–14 weeks.[74] To expedite this process, a clinical-grade cell sorter and protocol was developed.[75] On this basis, subsequent studies began using tetramer-guided cell sorting for the generation of a usable T cell product resulting in a 50% reduction in time from leukapheresis to infusion and enabling routine generation of tumor antigen-specific T cells present at very low frequencies. Although the initial sorter was a stream-in-air device, current strategies incorporate a Micro-Electro-Mechanical Systems-based microfluidics device that allows for turnkey operation and closed-path manipulation of intake and output of antigen-specific T cells.

Strategies to isolate or enrich for tumor antigen-specific T cells involve an in vitro recapitulation of in vivo priming events, namely, TCR triggering by the peptide-MHC complex presenting tumor antigenic epitopes, engagement of co-stimulatory molecules, and cytokine-induced expansion. Multiple rounds of stimulation may be necessary to expand tumor antigen-specific populations to levels that can be sorted and further expanded via Rapid Expansion Protocol (REP).[23] Autologous dendritic cells (DCs) present antigen in a relatively "physiologic" manner in vitro and can be used in various ways. Immature DCs can be loaded with tumor lysate, tumor antigen protein, or opsonized tumor particles, and then matured (using cytokine cocktails and Toll-like receptor agonists) to facilitate antigen presentation and upregulation of co-stimulatory ligands.[76] DCs may also be engineered to express the antigen of interest after introduction of RNA encoding the antigen, or, in some cases, total tumor RNA.[77–79] These approaches may provide a more comprehensive antitumor T cell response as they would present multiple epitopes on MHC class I and II to stimulate $CD8^+$ and $CD4^+$ T cells, respectively. The

most widely used approach, due to its relative facility, is to pulse mature DCs with known antigenic class I– or class II–restricted peptides. This method underlies the majority of ETC therapy clinical trials to date. Efforts are also being made in pursuit of more "universal" artificial antigen-presenting cells (APCs) based on genetically engineered insect cell lines and K562 cells, as well as artificial beads coated with anti-CD3/CD28+/– HLA-immunoglobulin domains.[80–82] Finally, addition of IL-21 during in vitro priming can significantly augment the frequency of high-affinity tumor antigen-specific CTL, as exposure to IL-21 resulted in a 10- to 100-fold increase in the frequency and absolute numbers of MART-1-specific CTLs generated in cultures.[83,84]

Endogenous T Cell Therapy in the Clinic

Initial trials of ETC therapy in the clinic generally involved infusion of monoclonal T cell products, were conducted without lymphodepleting preconditioning, and showed that in vitro generation of tumor antigen-specific T cells for adoptive therapy is feasible, infused T cells trafficked to sites of disease, and the treatment is relatively well-tolerated and safe.[74,85–87] Although not overwhelming, clinical benefit was observed in some patients as partial and mixed responses, with a complete regression that could be attributed to $CD8^+$ T cells alone. Infused T cells persisted for up to 14 days in vivo and persistence correlated with response. ETC therapy utilizing $CD4^+$ T cells has also been used to treat refractory, bulky metastatic melanoma and, impressively, a single infusion of NY-ESO-1-specific $CD4^+$ T cells resulted in a durable complete response, accompanied by T cell product persistence of greater than 80 days post-infusion with evidence of antigen-spreading.[88]

ETC therapy has also been used with some success to treat Epstein-Barr virus (EBV)-associated lymphomas and nasopharyngeal carcinomas, which express the less immunogenic EBV antigens LMP1, LMP2, and EBNA1.[89] Generation of EBV-specific T cells for infusion typically employs repeated stimulation with EBV-LCLs or APCs in the presence of IL-2, without a rapid expansion phase.[90–94] A study testing the use of EBV-specific ETC therapy in the adjuvant setting for high-risk or multiple-relapse lymphoma patients and as treatment for patients with active disease observed that 28 of 29 patients receiving adjuvant therapy remained in remission 3.1 years (median) after infusion, and there were 13 clinical responses (two partial, 11 complete) out of 21 patients with active disease.[93] There was also evidence of epitope spreading in a subset of responders. Trials of EBV-specific ETC therapy to treat nasopharyngeal carcinoma have achieved modest success with some complete responses and partial responses in those with local disease, but limited control of disease progression in patients with advanced refractory and metastatic disease.[90,92,94] Results may be improved by combination with chemotherapy, as a Phase II trial treating nasopharyngeal carcinoma with cycles of gemcitabine and carboplatin followed by infusion of EBV-specific T cells had an overall response rate of 71%.[95]

Efforts to increase the efficacy of ETC therapy focused on combination with lymphodepleting preconditioning regimens and enhancing persistence of infused T cells. As discussed earlier, nonmyeloablative but lymphodepleting chemotherapy conditioning regimens (high-dose cyclophosphamide and fludarabine) prior to TIL therapy had been shown to facilitate the in vivo engraftment and expansion of adoptively transferred cells.[35] Wallen et al. evaluated the use of fludarabine preconditioning in an intrapatient study and found a modest increase in T cell persistence without an increase in response rate.[96] Similarly, a small study of EBV-specific CTL infusion to treat nasopharyngeal carcinoma found that addition of a cyclophosphamide and fludarabine preconditioning regimen did not enhance clinical benefit.[97] Chapuis et al. evaluated the use of high-dose cyclophosphamide followed by low-dose IL-2 post-ETC infusion and found one out of 10 patients had a complete response and five patients experienced clinical benefit of stable disease up to at least week 8.[98] Plasma IL-15 levels were increased post-infusion and the infused T cells appeared to enter the cell cycle ($Ki67^+$) within 1 week of transfer, but this did not translate to in vivo persistence. Importantly, the complete response was associated with long-term persistence of the infused T cell clone and in vivo conversion to a central memory phenotype.

While most of the focus on generation of persistent memory T cells centered on IL-15 on the basis of murine studies, in 2005 a unique role was discovered for IL-21 for human $CD8^+$ T cells: exposure to IL-21 during in vitro priming led to the development of a central memory pool of tumor antigen-specific $CD28^+$, $CD127^+$, CD8 T cells with high replicative potential.[83,84] These tumor-reactive helper-independent CTLs produced antigen-driven autocrine IL-2. The clinical significance of these findings was confirmed when WT1-specific T cells generated in the presence or absence of IL-21 were administered to high-risk of relapse acute myeloid leukemia (AML) patients following stem cell transplant: only IL-21–primed T cells demonstrated long-term in vivo persistence and maintained or acquired phenotypic and functional characteristics associated with long-lived memory $CD8^+$ T cells.[99] The transcriptional and epigenetic mechanisms underlying the effects of IL-21 in human $CD8^+$ T cell priming were recently described.[100] Subsequent studies utilizing IL-21-primed ETC therapy approaches have confirmed persistence of the cells after transfer and in some cases have achieved durable responses.[101–103] Although clinical trials of ETC therapy have thus far been small in

scale and achieved varying degrees of success, current ETC therapy offers several advantages as a therapeutic modality: it requires only access to peripheral blood, sourcing a wider, unbiased TCR repertoire, and does not require high-dose IL-2 or high-dose lymphodepletion, avoiding toxicities associated with those treatments and allowing for potential outpatient therapy. In addition, ETC therapy has shown efficacy with minimal, self-limited toxicities, and the neurotoxicity and cytokine release syndrome sometimes observed with CAR T therapy do not occur with ETC therapy, though there may be a mild, transient, lymphopenia. As with other ACT modalities, greater ETC therapy efficacy will likely be achieved through further enhancing the persistence of adoptively transferred cells and incorporating combination therapies to mitigate the impact of a hostile tumor microenvironment and augment T cell function (discussed in later sections). Table 25.1 summarizes the key differences between TIL and ETC adoptive therapy.

ADOPTIVE T CELL THERAPY FOR SOLID TUMORS BEYOND MELANOMA

To date, most successes observed for TIL and ETC therapies have been observed in patients with metastatic melanoma; this section will briefly discuss some of the major challenges to their application in other solid tumor types.

Target Antigen Identification and Selection

Identification and selection of target tumor antigens is of central importance to effective ACT. Being that cancerous cells are originally derived from normal cells of "self" tissue origin, the preponderance of antigens that may be targeted are weakly immunogenic (i.e., poorly recognized by T cells). The consequence of this is illustrated by the observations that cancers with higher mutational burden like melanoma, non-small cell lung cancers, and mismatch repair deficient colorectal cancers are more likely to be successfully treated with immune checkpoint therapy than less "immunogenic" tumors.[104–108] For antigen-specific ACT, the number of TCR targets are few, limited to a handful of tumor types such as melanoma and sarcoma, for which immunogenic epitopes to differentiation antigens (e.g., MART-1, gp100) or CTAs (e.g., NY-ESO-1, MAGE-A4) have been identified. Chapters 4 and 37 on human tumor antigens and tumor antigen profiling provide a more comprehensive discussion; for the purposes of TIL and ETC therapy, we focus on mutated and non-mutated tumor-associated antigens.

Neoantigens as TCR Targets for Adoptive T Cell Therapy

Mutations resulting in neoantigens that are processed and presented are postulated to be more amenable to T cell recognition. TIL targeting neoantigens may improve efficacy in solid tumors, and as more neoantigen epitopes are identified they may also be used in other T cell-based modalities.[109] Neoantigens have been identified in a variety of solid tumors such as gastrointestinal and ovarian cancers, and the screening of TIL for neoantigen reactivity has shown that mutations are very frequently recognized by $CD8^+$ and/or $CD4^+$ TIL.[63,110,111] Preliminary studies have shown adoptive transfer of TIL enriched for reactivity to neoantigens can result in objective responses in patients with solid tumor malignancies including colorectal cancer and breast cancer.[53,55,57,112,113] It is important to note that mutations may be found in all tumor cells (clonal), or a fraction of the tumor mass (subclonal). A complete response to TIL therapy was achieved in a metastatic breast cancer patient using TIL enriched for recognition of four distinct private mutations, including two clonal mutations.[57] This result suggests that even for mutated antigens, attention should be given to select antigens that are uniformly expressed across the whole tumor mass to maximize the odds of response. In addition to intratumoral heterogeneity of expression, neoantigens resulting from somatic mutations are rarely shared across patients, restricting this approach to highly personalized therapy requiring ad hoc identification and screening for neoantigen expression on patient tumor before generating a T cell product—a process that can have significant constraints on sample acquisition, manufacturing, and patient selection.[111] Ideally, antigens derived from more ubiquitous gene mutations, such as those that support oncogenesis, would be identified and targeted, and for this purpose TIL serve as a valuable resource, exemplified by studies targeting neoantigens of mutant KRAS and TP53, some of the most common mutations in epithelial cancers.[55,110,114]

Nonmutated Cancer-Testis Antigens as TCR Targets for Adoptive T Cell Therapy

Germline antigens (more commonly known as cancer-testis antigens, CTA), which have expression restricted to germ cells and placental cells but can be reexpressed by tumor cells of various tissues, are another promising class of antigens. The most recognizable CTA are probably NY-ESO-1 and the MAGE family antigens. Recent studies have identified additional families of CT and CT-like antigens whose derived immunogenic epitopes can be presented by a broader array of HLA alleles.[115] Due to the restriction of their expression among adult somatic tissues to immune-privileged sites such as testis, fetal ovary, and placenta, CTA are expected to be immunogenic, and this is supported by the presence of CTA-reactive T cells among endogenous TIL from melanoma patients.[61] CTA have the advantage of being more widely expressed across patients and tumor types, but their expression may be low or heterogeneous within tumors; however, demethylating

Table 25.1 Comparison of Tumor-Infiltrating Lymphocyte Therapy Versus Endogenous T Cell Therapy

	TIL THERAPY	ETC THERAPY
Source	Tumor biopsy (T cell infiltrated)	Peripheral blood mononuclear cells
Target antigen	Undefined, mixed, or neoantigen	Defined antigen (known immunogenic epitopes)
In vitro processing	Tumor disaggregation	Stimulation with antigen-presenting cells (autologous or artificial)
	Culture with high dose IL-2	Low dose IL-2
		Low dose IL-21 (in some protocols)
Production time (*current standard)	Standard TIL: 7–8 weeks	Monoclonal product: 8–12 weeks
	Young TIL*: 4–5 weeks	Polyclonal cultures*: 4–6 weeks
Cell therapy product	Heterogeneous, $CD4^+$ and $CD8^+$ T cells, varying antigen specificity and tumor reactivity	Generally uniform, tumor-reactive $CD8^+$ T cells; central memory phenotype (IL-21 protocols)
Pre-infusion conditioning	High dose cyclophosphamide	Low dose cyclophosphamide (optional)
	Fludarabine	
Post-infusion cytokine administration	High dose IL-2	Low dose IL-2 (optional)
Tumor types treated (to date)	Melanoma	Melanoma
	HPV-associated carcinomas	EBV-associated lymphomas and carcinomas
	Uveal melanoma	Uveal melanoma
	Ovarian cancer	Ovarian cancer
	Gastrointestinal cancers	Pancreatic and gastrointestinal cancers
	Breast cancer	Merkel cell carcinoma
		NSCLC
Toxicities		
Cytokine-induced	Pulmonary failure	Fever
	Renal failure	
	Hypotension	
	Fever	
On-target	Vitiligo (melanoma)	Vitiligo and rash (melanoma)
	Uveitis (melanoma)	

EBV, Epstein-Barr virus; ETC, endogenous T cell; HPV, human papillomavirus; NSCLC, non-small cell lung cancer; TIL, tumor-infiltrating lymphocyte.

Source: Adapted with permission from Yee C. The use of endogenous T cells for adoptive transfer. *Immunol Rev.* 2014;257(1):250–263. doi:10.1111/imr.12134

agents and other epigenetic modifiers may be used to enhance their expression in tumor cells.[116,117] Furthermore, emerging evidence indicates that CTA have functional roles in tumor cells and may contribute to oncogenic processes.[116] Several clinical trials are evaluating CTA-specific ETC therapies, alone or in combination with the demethylating agent decitabine. Immune escape by tumor antigen loss should be less of an issue for polyclonal TIL and ETC therapies, compared to more homogenous TCR-T and CAR T approaches; however, they could be further improved by discovery of target antigens which confer some oncogenic advantage to tumor cells and survival dependency, as well as combination therapies that encourage antigen spreading. It must be noted that tumors may still escape recognition by T cells via diminished antigen presentation, such as by downregulation of MHC.[118,119]

Nonmutated Tumor-Associated Differentiation Antigens as Targets for Adoptive T Cell Therapy

Toxicities to normal tissues are a serious concern in target antigen selection, as many shared tumor-associated differentiation antigens are expressed by both malignant and normal cells. An example of this is the autoimmune vitiligo that often accompanies response to immunotherapies in melanoma patients, which is due to T cell-mediated destruction of normal melanocytes.[120] Such "on-target/off-tumor" toxicity is further illustrated by studies using TCR-engineered T cells (TCR-T). Administration of autologous T cells engineered to express a TCR recognizing CEA, an antigen which is also present in normal colonic epithelia, resulted in transient but severe colitis.[121] Treatment of melanoma using autologous PBMCs transfected with a nonmutated MART-1 TCR led to uveitis and decreased hearing, presumably due to MART-1 expression among pigmented cells in the uvea and stria vascularis of the inner ear.[122,123] No such toxicity was observed in responding patients receiving non-engineered MART-1-specific endogenous T cells, suggesting that overexpression of even a nonmutated TCR can lead to unwanted on-target/off-tumor toxicities. That ETC therapy can effect tumor regression without inducing toxicity to tissues expressing nominal levels of antigen demonstrates that a "goldilocks" window of therapeutic efficacy can be achieved without undue autoimmune toxicity using peripheral blood-derived, naturally occurring MART-1-specific T cells. When targeting differentiation antigens, shared expression with normal tissues may not be prohibitive if such tissues do not represent critical organ sites such as the CNS, heart, lungs, and so on. For example, tumor-associated antigens shared by breast cancer or prostate cancer and their normal tissue counterpart may be targetable especially if patients have already undergone a mastectomy or prostatectomy.[124,125] Finally, a shared tissue antigen selectively overexpressed in uveal melanoma, but not mature melanocytes, may offer a wider therapeutic window than differentiation antigens, which are expressed at high levels in both melanoma and melanocytes (such as gp100 and MART-1).[126]

Trafficking and Infiltration

After infusion, adoptively transferred T cells must find and enter tumors to exert their antitumor response; however, poor trafficking to and infiltration of tumors is currently a significant barrier in solid tumors. T cells navigate to tissues in response to chemokine gradients and extravasate via interactions with adhesion molecules on endothelial cells; however, tumors can subvert this process. By decreasing T cell-homing chemokines and increasing chemokines for which T cells have low cognate receptor expression and/or that attract immunosuppressive cells, tumors can render an unfavorable tumor microenvironment.[127–129] Preclinical studies indicate that engineering ACT products to express receptors for tumor-produced chemokines could improve trafficking, but this approach would be tailored to the varying chemokine milieus of different tumor types.[130–133] Tumors may also inhibit T cell infiltration via aberrant vasculature and downregulation of endothelial adhesion molecules.[134] Anti-angiogenic therapies may normalize tumor vasculature, and use of VEGF-neutralizing antibody reportedly increased lymphocyte infiltration in a murine melanoma model.[135] The enhanced efficacy of adoptively transferred T cells in this study may also have been due in part to mitigation of various immunosuppressive effects of VEGF.

The tumor control provided by TIL therapy in metastatic melanoma patients strongly suggests that TIL have the ability to home back to the tumor tissue. Additionally, the fact that TIL grown from one tumor lesion can eliminate not only the lesion that they were grown from but also other lesions throughout the body, including brain metastasis, demonstrates their ability to traffic to various tumor sites and suggests that the breadth of antigens targeted by TIL is sufficient to circumvent tumor heterogeneity, at least in the subset of patients deriving long-term benefit. Recent work has demonstrated a correlation between the presence of tissue-resident memory (T_{RM}) phenotype T cells in tumor tissue and survival in several solid tumor types, suggesting that there may be a distinct, tissue homing, population of TIL that is most desirable to select for expansion.[136,137] The potential of tissue-resident lymphocytes to expand ex vivo has not yet been thoroughly investigated owing to a variety of technical challenges: the propensity for T_{RM} cell death due to tissue processing, a lack of T_{RM} markers that are maintained during conventional expansion protocols, and/or a paucity of in vitro culture methods suitable for T_{RM}; as a result, it is unclear if T_{RM} comprise a substantial portion of the expanded TIL infused to patients and whether

they retain their tissue imprinting.[138–140] The elucidation of the molecular underpinning of tissue-resident programming also offers the possibility of engineering T cells with qualities that facilitate tissue homing.

The Immunosuppressive Tumor Microenvironment

Once T cells enter the tumor they must contend with an immunologically hostile microenvironment. Soluble mediators such as IL-10, TGF-β, and VEGF can inhibit the antitumor effector response and upregulate inhibitory checkpoint molecules on T cells. These factors may be countered through the use of neutralizing antibodies, receptor blocking antibodies, or genetic approaches involving expression of dominant-negative receptors that can act as cytokine sinks without signaling, or chimeric cytokine receptors that combine the exodomain of the suppressive factor's receptor with stimulatory or pro-survival endodomains, thereby redirecting an inhibitory ligand to trigger positive signals.[141–143] Metabolites produced by tumor cells and other cells in the tumor microenvironment can also inhibit T cell function. The ectonucleotidases CD39 and CD73 contribute to immunosuppression by reducing pro-inflammatory extracellular ATP and producing adenosine, which can directly inhibit T cells through the adenosine A2A receptor.[144] These effects may be mitigated using CD39/CD73 blocking antibodies, small-molecule inhibitors targeting A2AR, or gene editing of A2AR in T cells.[145,146] Indoleamine 2,3-dioxygenase (IDO) depletes the T cell-essential amino acid tryptophan and produces regulatory T cell (T_{reg} cell)-promoting kynurenine.[147] To address this, IDO inhibitors are currently in clinical development, but results have not yet fulfilled expectations.[148] Suppressive immune cells such as myeloid-derived suppressor cells (MDSCs) and T_{reg} cells are responsible for production of the inhibitory factors detailed previously, are sources of ligands for inhibitory checkpoint receptors, and interventions to modulate their function or eliminate such cells would be expected to improve antitumor T cell response.[149] Finally, T cell competition with tumor cells for glucose, which constrains effector functions in the glucose-deficient tumor microenvironment, might be overcome by augmenting metabolic fitness via checkpoint blockade, genetic strategies such as expression of phosphoenolpyruvate carboxykinase 1 (PCK1), and other innovative approaches.[150,151]

ADOPTIVE T CELL THERAPY IN COMBINATION STRATEGIES

To disable many of these immunoevasive mechanisms, combination strategies that target suppression must be implemented so that transferred T cells can achieve their full effector potential. Given the ever-increasing number of targetable pathways, the ability to track and rigorously analyze a highly uniform T cell population with defined specificity and phenotype, while exposed to immunologic countermeasures in vivo, would be invaluable in understanding the reasons for success or failure of a given combination strategy. In this way, the role of adoptively transferred T cells as a "transferrable cellular biomarker" becomes experimentally and empirically important in immune-based therapies not only as a therapeutic modality, but an analytical one as well.

Lymphodepleting Preconditioning Regimens

ACT combination strategies were first evaluated more than 20 years ago, beginning with studies by North et al. demonstrating the requirement for lymphodepleting preconditioning regimens to augment ACT efficacy in tumor-bearing murine models.[13] These regimens are thought to improve ACT by multiple mechanisms, including lymphopenia-induced elevation of homeostatic cytokines such as IL-15, removal of cytokine sinks (endogenous effectors), depletion of suppressive cells such as T_{reg} cells, bacterial translocation across GI barriers leading to Toll-like receptor activation, and increased tumor antigen availability and presentation as a result of tumor cell death.[152–156] These conditioning regimens continue to be practiced today (see the previous text), but rationale for their use should be contextualized for any given cell therapy approach. For example, while host immune suppression for gene-modified T cells may contribute to extended in vivo persistence by reducing the probability of endogenous immune rejection of the transgene, a finding that was noted even before CAR T cell-based therapies, it is less clear if such an immunosuppressive regimen is necessary for non-engineered T cell therapy.[157]

Clearing not only suppressive T cells, but also endogenous effector cells, with high dose cyclophosphamide and fludarabine also eliminates any possibility of antigen spreading. Antigen spreading, whereby targeting a specific antigen (in this case, with a tumor antigen-specific T cell), can elicit T cell responses to nontargeted antigens by tumor destruction, cross-presentation of immunogenic proteins and induction of a broad effector response in the presence of a favorable milieu is associated with a durable clinical response in the absence of antigen immune escape, even when the antigen targeted is dispensable for tumor survival or metastasis.[88,102,103] The absence of antigen spreading may be one reason for the high rates of relapse seen in patients receiving T cell therapy preceded by high-dose immunosuppressive conditioning.

Checkpoint Blockade Therapy

Immune checkpoint blockade and ACT have achieved some early successes in melanoma when checkpoint inhibitor therapy fails, and their combination holds

promise in extending effective immunotherapy to more cancer types. Underpinning this strategy is the concept that in cancers where endogenous tumor-reactive T cells are sparse, infusing a bolus of tumor antigen-specific T cells would provide effectors that can respond more vigorously in vivo following the reduced inhibition and increased metabolic fitness afforded by checkpoint blockade. In a small, recent trial testing the combination of anti-CTLA-4 and standard TIL therapy in patients with metastatic melanoma, of the 12 patients who received both therapies five had objective responses, four of which were durable, and one of which later became a complete response.[158] Importantly, this trial began anti-CTLA-4 administration weeks before TIL infusion and had a reduced rate of attrition due to progression during the ACT production process (7% compared to 32%).

In a first-in-human, prospectively designed study evaluating the combination of memory MART-1-specific ETC therapy and CTLA-4 blockade in 10 patients with refractory metastatic melanoma, three of whom had failed prior anti-CTLA-4 monotherapy, seven out of 10 patients received clinical benefit.[103] Two patients achieved a durable complete response, one of them despite failing prior anti-CTLA-4 monotherapy. T cell persistence was observed for all patients for as long as samples could be collected (>500 days), accompanied by upregulation of central memory markers (CD28, CD127, CD62L, and CCR7) among all seven responders. In addition, all responders showed increased reactivity to nontargeted melanoma-associated antigens, indicating antigen spreading. In this study, upregulation of central memory markers and the emergence of antigen spreading was positively associated with clinical response.

PD-1 blockade represents an attractive option for combination ACT, as PD-1 expression can mark tumor-reactive T cells, and its antagonism may be used to relieve T cell exhaustion in the tumor microenvironment.[43,159,160] Indeed, at the NCI, treatment of a single metastatic breast cancer patient with neoantigen-specific TIL therapy and anti-PD-1 resulted in a complete durable regression.[57] Multiple larger studies are planned or underway to evaluate the combination of PD-1 pathway blockade and ACT.

Effects of Prior Checkpoint Blockade Therapy on Adoptive T Cell Therapy

As immune checkpoint blockade therapy becomes more commonly used, it is important to consider its effects on later adoptive cellular therapy treatment. Although earlier studies seemed to suggest that prior treatment with anti-CTLA-4 therapy had no effect on later TIL therapy, a larger, more recent study of 43 checkpoint-naïve metastatic melanoma patients and 31 patients who had received prior checkpoint blockade therapy reported reduced survival, overall response, and duration of response, and less expansion of TIL during product generation in patients who had received prior checkpoint blockade.[33,41,161] It is unclear whether these results were a function of an inherent patient population refractory to anti-CTLA-4, effects of anti-CTLA-4 treatment on endogenous lymphocytes and/or tumor stroma, or both. One study of patients with regionally advanced melanoma receiving neoadjuvant anti-CTLA-4 therapy reported increases in circulating tumor antigen-specific T cells and increased tumor infiltration by activated T cells.[162] Interestingly, another study of TIL grown from patients with metastatic melanoma reported higher CTLA-4 expression but also broader, more frequent tumor antigen responses in T cells from patients who had received anti-CTLA-4 therapy.[163] Perhaps one advantage to pre-TIL harvest PD-1 therapy may be the infiltration and replacement of TILs with T cell clones that are more likely to be functionally tumor-reactive.[164] Further studies will be important in helping to determine treatment sequences and patient populations amenable to the combination of checkpoint inhibitor therapy and ACT.

Genetic Inhibition of Checkpoint Molecules

To avoid possible unwanted systemic effects of immune checkpoint blockade, intrinsic modification of transferred T cells ex vivo may be achieved by gene editing. The advent of CRISPR-Cas9 gene editing technology has stirred great interest in genetic approaches to checkpoint inhibition, in particular deletion of (PD-1), with a number of recent clinical trials testing this approach in ACT.[165] In preclinical studies, genetic disruption of PD-1 improved antitumor function in both CAR T cells and ETC; however, this may be at the expense of generating memory cells from the infused T cells.[166–169] A study in a chronic viral infection model showed PD-1 knockout in virus-specific T cells resulted in accumulation of terminally differentiated cells and greater contraction due to decreased survival, indicating an inability to establish memory, which may be due to a deficiency in upregulation of fatty acid oxidation.[170,171] These results were mirrored in a recent Phase I clinical trial testing the safety of PD-1-edited TCR-T cells (also edited for endogenous TCR alpha and beta chains).[172] Infusion of a mixed product containing PD-1 knockout and PD-1 sufficient cells could allow for enhanced antitumor function without sacrificing memory formation. Use of self-delivering small interfering RNA to knock down PD-1, rather than knock out, is an interesting alternative that has been successfully applied to TIL in a preclinical in vitro study.[173]

Oncolytic Viruses

Oncolytic viruses are designed to selectively replicate in cancer cells and induce immunogenic cell death that can act as an in situ vaccine and, more importantly for

combination ACT strategy, as a means to enhance trafficking and in situ T cell activation.[174] Being viruses, they provide ligands for pattern recognition receptors and can promote a pro-inflammatory environment via induction of the antiviral response, resulting in the production of cytokines and chemokines that improve T cell infiltration and tumor antigen presentation, among other effects. They may also be used as vectors for gene delivery to tumor cells, modifying them to express desired cytokines, chemokines, co-stimulatory ligands, antigens, or other molecules.[174,175] Currently, there is one U.S. Food and Drug Administration (FDA)-approved oncolytic virus therapy: talimogene laherparepvec (T-VEC), an attenuated herpes simplex virus type 1 (HSV-1) encoding granulocyte-macrophage colony-stimulating factor (GM-CSF), approved for the treatment of some melanomas.[176] The successes of combined checkpoint blockade therapy and T-VEC suggests that combination of ACT with oncolytic viruses may also be effective.[177,178] A preclinical study identified adenovirus as the most effective virus when combined with TIL transfer; an oncolytic adenovirus encoding IL-2 and TNF-α (TILT-123) is currently being evaluated in combination with TIL therapy for melanoma in an ongoing clinical trial.[179]

Radiation Therapy

Early evidence supporting the combination of radiation therapy and ACT was the observation of synergy between TIL therapy and localized radiotherapy in a murine model of colon adenocarcinoma.[180] In similar manner to oncolytic viruses, radiation causes DNA damage-induced immunogenic tumor cell death that releases tumor antigens and promotes pro-inflammatory cytokines and the interferon (IFN) response, and may also enhance T cell infiltration by inducing the production of chemokines and tumor vasculature remodeling.[181,182] More recent preclinical studies in models of glioblastoma and pancreatic adenocarcinoma have shown that low-dose (subtherapeutic), local radiotherapy can increase the efficacy of adoptively transferred CAR T cells by improved trafficking to the tumor and sensitization of the tumor cells to T cell-mediated cytotoxicity.[183,184] Although the combination strategy of ACT and radiation therapy has yet to be evaluated in clinical trials, a case report of durable response in a single myeloma patient who was treated with localized radiotherapy for spinal cord compression shortly after infusion of CAR T cells shows promise.[185] Increases in inflammatory cytokines and TCR diversity were also observed post-radiation therapy.

DESIRABLE T CELL PHENOTYPES FOR ADOPTIVE T CELL THERAPY

Selection of Functional Cells

In contrast to vaccine-based or immune checkpoint therapies, ACT allows for ex vivo selection and enrichment of a desired effector phenotype for adoptive transfer. As T cells can develop a progressive state of dysfunction known as "exhaustion" following chronic in vivo or ex vivo stimulation, a means to evaluate and mitigate this phenotype would be desirable. Exhausted T cells are characterized by expression of several immune checkpoint molecules and a gradual loss of effector functions: proliferative capacity and production of IL-2, TNF-α, and IFN-γ.[186] Exhaustion was originally studied in models of chronic viral infection, but it has also been found that T cells isolated from tumor models and cancer patients exhibit many of the characteristics of T cells exhausted from chronic infection. In the context of tumors, in addition to persistent antigen and inflammation, causes of T cell dysfunction may be attributable to poor antigen presentation and/or priming, deficient co-stimulation, and immunosuppressive soluble mediators, which can lead to upregulation of inhibitory receptors including checkpoint molecules.[159,186] Multiple studies have shown that tumor antigen-specific T cells isolated from the peripheral blood, tumor, and lymph-node metastases of melanoma patients express checkpoint molecules, in particular PD-1 and TIM-3, and are deficient in cytokine production.[187–189] Interestingly, it has been reported that expression of PD-1, TIM-3, and LAG-3 designate tumor-reactive TIL, as measured by IFN-γ production and cytotoxicity in response to autologous tumor cell lines.[43,190] In this latter case, the cells were tested after a two-week period of in vitro expansion, suggesting that expression of checkpoint molecules indicated activation of these cells in vivo by tumor antigens and possible restoration of some effector function during in vitro culture.

Studies in chronic viral infection and tumor models have shown that exhausted T cells are a heterogeneous population consisting of a spectrum of different cell states, and significant efforts have been made to characterize these various states based on marker expression. A study comparing single-cell RNA sequencing profiles of T cells from chronic LCMV infection and melanoma models found both conditions produced T cells that were enriched for the canonical exhaustion signature defined by Wherry et al.[191,192] Several groups have identified that within TIL there are "progenitor" and "terminal" exhausted T cells which can be distinguished by their expression of PD-1, TIM-3, and TCF1, and determined that progenitor exhausted T cells (PD-1$^+$ TIM-3- TCF1$^+$) mediate tumor control and response to PD-1 checkpoint blockade.[192–194] As TCF1 is a transcription factor and therefore an intracellular marker, SLAMF6 has been identified as a surrogate cell-surface marker, based on high co-expression of SLAMF6 and TCF1, enabling the selection of live progenitor exhausted T cells.[192] Strategies to enhance ACT by sorting progenitor exhausted TIL based on these markers are being developed.[195] Even more recently, Beltra, Wherry, and colleagues have further defined four

subsets of exhausted T cells based on their expression of SLAMF6 and CD69, with SLAMF6 marking the progenitor subsets.[196] Selection of tumor antigen-specific T cells that are not terminally exhausted could prove to be a fundamental requisite for ACT efficacy, especially in combination with PD-1 pathway blockade therapies.

Enhancing Persistence of Transferred Cells

Commensurate with these research studies are clinical studies demonstrating that in vivo persistence of adoptively transferred T cells correlates with patient response to ACT.[88,98,102,103,197–199] As discussed earlier, one of the original goals of lymphodepleting preconditioning regimens was to enhance persistence of transferred cells; however, this may also be achieved by selection of less differentiated T cells or manipulation of T cells in vitro to enrich for less differentiated or memory phenotypes. Preclinical studies have shown that less differentiated T cells, such as stem cell memory T cells (T_{scm}) or effectors derived from naïve or central memory precursors, have increased proliferative capacity, persistence, and antitumor function.[200–204] These cells are typically identified by their expression of CD62L, CCR7, CD95 (Fas), CD127, CD28, and/or CD27. Stem-like T cells may also be identified based on their mitochondrial membrane potential.[205]

Most current ACT production protocols involve (sometimes repeated) stimulation and extended culture in IL-2 in order to produce a sufficient number of cells for infusion; however, this can drive differentiation of effector T cells in vitro resulting in suboptimal performance in vivo.[203] The first demonstration, in 2005, that it was possible to modulate the phenotype of human CD^{8+} T cells was achieved by IL-21 exposure during priming and led to expansion of helper-independent central memory type T cells, which were later demonstrated to be critical for in vivo persistence of these cells at high frequencies in patients for more than nine months after adoptive transfer.[83,99] Since then, several studies using a combination of gamma-chain receptor cytokines (IL-7, IL-15, and IL-2) with IL-21 have reportedly resulted in T cells with a less differentiated phenotype, combinations which were later revealed to be counterproductive once an understanding of the signaling mechanisms leading to central memory development were elucidated.[100,206–208] Limitation of differentiation and preservation of "stemness" may also be achieved by inhibition of Akt signaling, limiting ROS metabolism with antioxidants such as N-acetylcysteine, or exposure to extracellular potassium-inducing autophagy.[209–211] Lastly, alteration of T cell mitochondrial dynamics by culturing with fusion promoting and fission inhibiting chemicals or enforced expression of the mitochondrial inner membrane fusion protein Opa1 can promote a memory phenotype.[212]

The in vitro priming used in ETC therapy offers an opportunity to influence T cell phenotype. A pilot study using artificial APCs expressing co-stimulatory ligands and IL-2/IL-15 to generate infusion products resulted in a mixture of effector memory and central memory phenotype T cells that persisted post-infusion, reminiscent of the phenotypic and functional attributes of memory T cells.[81,213] As discussed in an earlier section, exposure to IL-21 during priming promotes $CD28^{+}$ central memory phenotype with high replicative potential and enhanced persistence without negatively affecting cytolytic activity.[83,84,99] Additionally, the effects of IL-21 are exerted predominantly through enhancement of tumor antigen-specific CTL derived from naïve $CD8^{+}$ T cells, resulting in a younger, less differentiated ACT product.[83] It has also recently been shown that IL-21 can be combined with epigenetic remodeling via histone deacetylase inhibition to reprogram effector CTL to central memory phenotype cells.[100] Enhancing persistence of ACT cells should not only increase antitumor efficacy, but also provide a long-lived population of tumor-reactive T cells to protect against metastasis and recurrence.

ADOPTIVE TRANSFER OF GD T CELLS

Most adoptive T cell therapy approaches focus on "conventional" αβ T cells; however, "unconventional' γδ T cells are also being actively pursued for antitumor therapy. As their name indicates, γδ T cells are defined by their T cell receptors (TCRs), which are composed of a gamma (γ) chain and delta (δ) chain associated with CD3 complexes. γδ T cells generally do not express CD4 or CD8 coreceptors and recognize antigens independent of MHCs. While the antigen and antigen-presenting molecule for most γδ T cells are unknown, a subset of γδ T cells directly bind to an antigen's superstructure, whereas other minor subsets recognize lipid presented by CD1d and possibly metabolites presented by MR1.[214] γδ T cells comprise 1% to 5% of T cells circulating in human peripheral blood and are also found in peripheral tissues.[214] These unconventional T cells are typically distinguished by the variable (V) domain of their TCRδ chains, with Vδ2 (preferentially paired with Vγ9) cells predominating in the blood and Vδ1 cells commonly found in tissues.[214] γδ T cell antigens have not yet been comprehensively characterized but it is known that Vγ9Vδ2 T cells recognize nonpeptide phosphoantigens, such as isopentenyl pyrophosphate (IPP), that are metabolites derived from the mevalonate pathway.[215] Tumor cells with increased HMG-CoA reductase activity have increased endogenous phosphoantigens; this can also be achieved by exposure to aminobisphosphonate drugs such as Zoledronate, which trigger accumulation of phosphoantigens via disruption of the mevalonate pathway, rendering tumor cells susceptible to γδ T cell recognition.[215]

Conformational changes in CD277 (BTN3A1) appear to be involved in γδ T cell detection of phosphoantigens. Vγ9Vδ2 T cells also reportedly recognize stress-induced cell surface proteins on tumor cells such as heat shock protein 60 and FI-ATPase.[216]

In addition, some γδ T cells can express DNAM-1, NKG2D, CD244 (2B4), KIRs, NKp30, and/or NKp44 markers more commonly associated with natural killer (NK) cells, and recognize their ligands Nectin-2 and PVR, MIC A/B, and ULBPs. Hence, γδ T cell recognition of tumor cells likely involves more than one ligand and possibly concurrent detection of conformational changes. Following activation, the γδ T cell response is similar to that of conventional T cells by initiating cytotoxic pathways that produce IFN-γ, TNF-α, perforin, and granzymes to directly kill tumor targets. It has been suggested that they may form memory cells.[217] Thus, γδ T cells may be potentially effective against a variety of cancers and are especially attractive effector populations for treating tumors that escape the conventional T cell response by MHC downregulation.

In an early study of leukemia, patients who received allogeneic partially HLA-mismatched bone marrow transplants depleted of αβ T cells (to reduce graft-versus-host reactions) there was an association between high levels of circulating γδ T cells and improved disease-free and overall survival rates, suggesting that transferred γδ T cells can mediate an antitumor effect.[218,219] Most γδ T cell ACT studies to date have focused on Vγ9Vδ2 T cells, as they are the most common and accessible subtype in peripheral blood and can be expanded in vitro using cytokines and aminobisphosphonate drugs (e.g., Zoledronate) or synthetic phosphoantigens.[214] No studies used lymphodepleting preconditioning; however, most employed in vivo Zoledronate to sensitize tumor cells to the transferred γδ T cells.[217] Unfortunately, these trials had very limited success. While the trials have shown that γδ T cell ACT is generally well-tolerated, transferred cells can traffic to tumor sites and can achieve partial responses; complete responses were rare—although the few observed have been in varied tumor types (RCC, CRC, and breast cancer).[220–222]

Improving the Efficacy of γδ T Cell Adoptive T Cell Therapy

In vitro studies suggest the possibility of combination γδ T cell ACT with approved treatments. For example, when exposed to the chemotherapeutic temozolamide, GBM cells can upregulate cell-surface NKG2D ligands, enabling γδ T cell-mediated lysis.[223] Similarly, treatment of colon cancer cells with 5-fluorouracil or doxorubicin sensitizes them to γδ T cell cytotoxicity via a mechanism dependent on NKG2D.[224] Combining tumor-targeting antibodies such as rituximab (anti-CD20) or trastuzumab (anti-HER2) with γδ T cell therapy could increase efficacy by promoting tumor cell killing via antibody-dependent cellular cytotoxicity (ADCC) and allowing γδ T cells to present tumor antigens to conventional αβ T cells.[225–227] Furthermore, γδ T cells from tumor sites can express PD-1 and reportedly increase degranulation (CD107 expression) in vitro in response to anti-PD-1, suggesting γδ T cell ACT may also benefit from combination with PD-1 blockade therapy.[228]

Efforts to improve the efficacy of γδ T cell ACT are generally hampered by the dearth of knowledge regarding γδ T cell antigens, target cell recognition, activation, and regulation, as well as the considerable diversity among γδ T cells.[217] As with conventional T cells, γδ T cells are functionally diverse; in some tumor types, they can play important immunosuppressive and/or pro-tumorigenic roles, though the data regarding this has been contentious.[229,230] The endogenous suppressive or IL-17-producing γδ TIL in these reports were primarily of the Vδ1 subtype, but in vitro studies have shown that particular cytokine milieus can direct Vγ9Vδ2 T cells toward regulatory or IL-17+ phenotypes, and it is unknown whether adoptively transferred Vγ9Vδ2 T cells might undergo phenotypic alterations in the tumor microenvironment.[231,232] Although the expansion approach used in most γδ T cell ACT trials to date results in a bulk product that contains primarily Vγ9Vδ2 T cells, the individual clones can vary widely in TCR affinity as well as their expression of activating and inhibitory NK receptors; thus, identification and enrichment of a population with high antitumor reactivity might lead to improved efficacy.[233,234] An interesting alternative approach is to introduce high-affinity γδTCRs into αβ T cells, creating T cells engineered with defined γδTCRs (TEGs).[217,233] Transgenic expression of γδTCRs leads to downregulation of endogenous αβTCR expression on the cell surface, and this phenomenon, combined with γδTCR MHC-independence, opens the possibility for use in a broad patient population with reduced requirements for HLA-matching.[234] A Phase I trial using this approach in patients with AML or multiple myeloma is currently underway (NTR6541). Finally, methods have recently been developed for clinical-grade expansion of Vδ1 T cells, which are characterized favorably for tissue localization properties and enhanced cytotoxicity, and may have some advantages for ACT such as greater persistence and resistance to activation-induced cell death.[235–237] Overall, the lack of MHC-restriction and potential for antitumor activity against a broad range of tumor types will continue to fuel interest in γδ T cells (and their TCRs) for cancer immunotherapy, and a greater understanding of their biology should inform and improve future γδ T cell ACT approaches.

CONCLUSION

Over the last 30 years, ACT has developed from a boutique-type therapy, limited to selected patients with

anecdotal clinical responses, to an established treatment modality. The reinvigoration of cancer immunotherapy by the advent of checkpoint blockade therapy has set the stage for a new era of ACT, aided by scientific advances, enabling technologies, and judicious application of combination strategies to enhance trafficking, function, and persistence of adoptively transferred antitumor T cells. Successes of ACT will be extended to broader patient populations through improvements in target antigen selection and strategies to overcome T cell dysfunction and the immunosuppressive tumor microenvironment.

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