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Commentary

Biological and Molecular Factors Predicting Response to Adoptive Cell Therapies in Cancer

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COMMENTARY

Abstract

Adoptive cell therapy (ACT) constitutes a major breakthrough in cancer management that has expanded in the past years due to impressive results showing durable and even curative responses for some patients with hematological malignancies. ACT leverages antigen specificity and cytotoxic mechanisms of the immune system, particularly relying on the patient's T lymphocytes to target and eliminate malignant cells. This personalized therapeutic approach exemplifies the success of the joint effort of basic, translational, and clinical researchers that has turned the patient's immune system into a great ally in the search for a cancer cure. ACTs are constantly improving to reach a maximum beneficial clinical response. Despite being very promising therapeutic options for certain types of cancers, mainly melanoma and hematological malignancies, these individualized treatments still present several shortcomings, including elevated costs, technical challenges, management of adverse side effects, and a limited population of responder patients. Thus, it is crucial to discover and develop reliable and robust biomarkers to specifically and sensitively pinpoint the patients that will benefit the most from ACT as well as those at higher risk of developing potentially serious toxicities. Although unique readouts of infused cell therapy success have not yet been identified, certain characteristics from the adoptive cells, the tumor, and/or the tumor microenvironment have been recognized to predict patients' outcome on ACT. Here, we comment on the importance of biomarkers to predict ACT chances of success to maximize efficacy of treatments and increase patients' survival.

Immunotherapy has revolutionized cancer management in the past years, improving patients' survival rates and reducing the risk of recurrence for many cancer types. Immunotherapy approaches, including the use of immunomodulatory agents to enhance anticancer responses, started with the use of high-dose synthetic IL-2 (aldesleukin) for the treatment of melanoma and renal carcinoma in the early 1990s (1). To date, immunotherapy has expanded to different kinds of treatments, including the use of monoclonal antibodies, immune checkpoint inhibitors, vaccines against tumoral antigens, and cell-based therapies, all of which aim to leverage and boost the patient's immune response to fight malignant cells and prevent cancer progression. However, despite impressive clinical results, many patients still cannot fully benefit from immunotherapies due to a lack of response to treatment, relapse, and/or development of resistance, or life-threatening therapy-associated toxicities (2,3). In this respect, reliable predictors of response or resistance as well as treatment monitoring markers remain a crucial need to stratify patients and maximize clinical success. Predictive markers include biological markers as well as molecular, (epi)-genomic, and transcriptomic signatures that allow rational therapeutic decision making and enhance the progress of precision medicine. Multiomics technologies are becoming a major player in the search of novel biomarkers of response to immunotherapy (2). A good example of molecular signatures that predict the chances of success to immunotherapy is the presence of the DNA methylation signature known as EPIMMUNE in metastatic non-small cell lung cancer patients. EPIMMUNE associates with good response and improved progression-free survival on treatment with immune checkpoint inhibitors (4). These DNA methylation profiles that are able to predict clinical response to immunotherapy have also been extended to

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melanoma patients (5). In addition, recent development of sophisticated computational methods allows the inference of T-cell infiltration into tumor samples from whole exome sequencing data, which can predict the response to immune checkpoint inhibitors independently of tumor mutational burden (6). Finally, the advancement of single-cell technologies also holds great potential to refine the search for predictors of treatment response, personalize patients' management, and increase the responders to nonresponders' ratio (7-9).

T Cells in Cancer Therapy

T cells are central players of vertebrates' adaptive immune system and mediate immune defense against viral infections and cancer (10). T cells are equipped with a specific receptor that recognizes foreign peptides in the context of HLA molecules on the surface of antigen presenting cells and activates effector programs to defend against infection and abnormal cells. The T-cell receptor (TCR) is a heterodimeric complex composed of 2 protein chains (usually α and β chains) that have an extracellular domain that binds to the peptide-HLA complex. The intracellular signaling module of the TCR is composed of the γ, δ, ε, and ζ subunits from 2 CD3 chains that transduce activating signals on engagement of the TCR, orchestrating specific transcriptional programs (11,12). The antitumor T-cell immunity results from the combined action of CD4+ helper T cells and the CD8+ cytotoxic cells, where specific transcriptional programs shape different stages of cell differentiation into distinct functional subsets that can be categorized as naïve T cell, stem cell memory T cell, central memory T cell, memory effector T cell, and effector T cell (13).

Adoptive cell therapy (ACT) accounts for different immunotherapy modalities based on the redirection of cytotoxic T cells from the patient towards an efficient antitumor response. Therapies can be divided into those with no genetic manipulation of the T lymphocytes present in the tumor as tumor infiltrating leucocytes (TILs) (14), and those that include the genetic manipulation and reprogramming of patients' T cells to potentiate and direct killing activity towards the tumor. In the case of the latter, these are T cells expressing chimeric antigen receptor (CAR-T cells) and engineered TCRs that specifically recognize tumor antigens (15). In all ACT protocols, T cells are harvested from the patient (either from the tumor or from circulation), expanded ex vivo, and reinjected in the patient (Figure 1). Such T-lymphocyte–based treatments hold curative potential and have provided promising clinical efficacy results for certain cancers; as such, this highly evolving field with increasing therapies is being evaluated and approved at a high pace, either as standalone therapies or in combination with other drugs (2). In this regard, Supplementary Table 1 (available online) summarizes the different neoplasms that have been treated with ACT, whereas Table 1 indicates the US Food and Drug Administration (FDA)-approved therapies. In the following subsections, we will discuss the most common ACT approaches: TILs, TCRs, and CAR-T cells.

Tumor Infiltrating Lymphocytes

A hallmark of cancer is the presence of a complex immunosuppressive tumor microenvironment (TME) characterized by cells and factors that promote immune evasion by malignant cells. Tumors recruit T regulatory cells, tumor-associated macrophages, and myeloid derived suppressor cells that dampen inflammation and prevent effective antitumor responses (16). Nonetheless, certain T cells that infiltrate tumors (TILs) and recognize cancer-specific antigens can effectively kill the malignant cells and reduce cancer growth. Exploiting the cytotoxic potential of these tumor infiltrating memory-like CD8+T cells by isolating them, expanding them ex vivo in the presence of growing factors like IL-2, and adoptively transferring them back into the patient to prevent cancer progression constitutes a promising therapeutic option for solid tumors such as melanoma and some epithelial cancers (14). TIL-based therapies were pioneered by the Rosenberg team at the Surgery Branch in the National Cancer Institute, who first demonstrated that tumor-bearing mouse TILs cultured ex vivo showed in vivo cytolytic activity against tumor cells after reinjection (14) and later confirmed those findings in cancer patients (17). A major determinant of TIL therapy success is the presence of cells that recognize neoantigens expressed by tumor cells (18,19). In this regard, a meta-analysis of several cancer cohorts' datasets supports that tumor-specific CD8+ TILs are represented not only by the exhausted CD8+ T cells and their precursors (expressing thymocyte selection-associated HMG BOX (TOX), and TOX and transcription factor 7 (TCF7), respectively) but also by effector memory-like T cells with high cytotoxic capacity that could be isolated and used in TIL-based therapeutic protocols (20).

T-Cell Engineered Receptor

Despite CD8+ T cells having great cytotoxic capacity, the suppressive TME often dampens such response, constituting a mechanism for tumor immune escape (16,21). An important breakthrough in cancer immunotherapy was the application of genetic modification of normal peripheral lymphocytes from cancer patients to express TCR that recognizes specific antigen-expressing tumor cells with high affinity. This method overcomes the need to identify and isolate antitumor effector T cells specifically recognizing tumor-associated antigens (TAAs) from each patient. In the case of solid tumors, TCRs specific for the melanoma antigens melanoma antigen recognized by T cells 1 or MART-1 and glycoprotein 100 (gp100) were retrovirally transduced into melanoma patients' peripheral blood lymphocytes, successfully promoting cancer regression (22).

Chimeric Antigen Receptor T Cells

CAR-T cell therapy has shown great efficacy in the treatment of hematological cancers, with improved clinical responses since the development of first-generation CAR-T cells to the therapies approved so far, which are the second-generation CAR-Ts (23,24). In addition, many combinations are being investigated for the successful treatment of solid tumors, which has been elusive to date (25). To produce CARs, a synthetic fusion protein consisting of 2 different functional modules connected by a transmembrane domain is generated and transduced via a viral vector (most frequently retrovirus or lentivirus) into autologous or allogeneic T cells that, on expansion, are transfused into the patient to engraft in vivo and target malignant cells (26). First-generation CARs consist of a single-chain fragment variable region of antibodies extracellular domain that recognizes tumoral antigens and is connected by a linker to a transmembrane domain with a cytoplasmic signaling module from the CD3ζ chain. In the case of second-generation CAR-Ts, the CAR contains an additional costimulatory domain (from CD28 or 4-1BB) that transduces the activating signal to the T cells. To date,

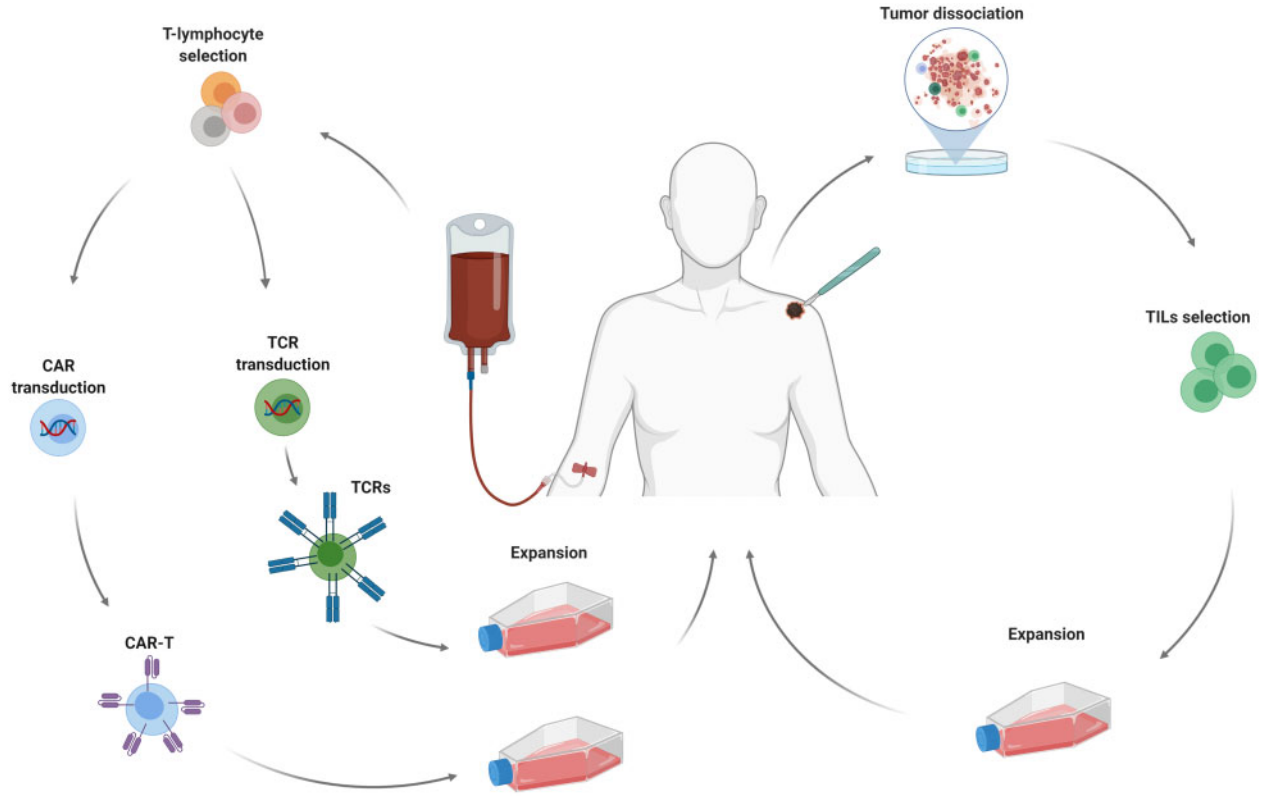

Figure 1. Adoptive cell therapy generation strategies. Tumor infiltrating leucocytes (TILs) are extracted from the tumor tissue, selected, and expanded in vitro. On expansion, TILs are reintroduced in the patient to redirect the antitumor response. T cells from the patient's circulation are selected and transduced with either an engineered T-cell receptor (TCR) or a chimeric antigen receptor (CAR). Cells are then expanded in vitro and reinfused into the patient.

autologous CAR-T cell therapy targeting CD19, and B-cell maturation antigen have been approved by the FDA for 6 types of relapsed or refractory hematological cancers —diffuse large B-cell lymphoma, follicular lymphoma, mantle cell lymphoma, multiple myeloma, and B-cell acute lymphoblastic leukemia (ALL)—in pediatric and young adult patients up to age 25 years (23). As recently as October 2021, another anti-CD19 (CAR) T-cell therapy (brexucabtagene autoleucel) named KTE-X19 was approved by the FDA for the treatment of relapsed or refractory B-cell precursor ALL in adults, indicating a long-term beneficial outcome for these patients (27).

Therapy Limitations of ACT

Despite the clinical benefit obtained from the different ACT approaches described above, some of them show short-lived remissions and develop resistance to treatment due to several factors, highlighting the importance of patients' stratification according to their risk of resistance for the rational use of these expensive and time-consuming therapies. Patients relapsing on CAR-T cell infusion has been reported to be due to either early poor CAR-T cell persistence leading to antigen-positive malignant cell expansion (28-30) or late resistance due to antigen reduction or loss (31-33).

Another major obstacle precluding the generalized use of immunotherapy, and particularly of ACT, in cancer patients is the appearance of treatment-related severe adverse effects that are potentially life-threatening (34,35).

In line with this, efforts are being made to generate less costly, allogenic universal off-the-shelf CAR-T cells that can be produced and infused in many patients. As an example, to treat B-cell malignancies, healthy donors' T cells have been engineered, using the Sleeping Beauty system to express an anti-CD19 CAR, at the time that endogenous TCR has been genetically silenced to abolish MHC-dependent TCR activation, thus preventing graft-vs-host-disease (36).

In addition to T cells, other immune effector cells (IECs) are being engineered as alternative strategies to be used in adoptive cell therapy. Natural killer (NK) cells are well-suited with a cytotoxic machinery to fight against tumoral cells that downregulate HLA and are amenable of in vitro expansion and allogenic use. NK cells also show less potential of serious side effects, which makes them an excellent candidate for treatment design. A phase 1 and 2 clinical trial using manufactured allogenic anti-CD19 CAR-NK cells in chronic lymphocytic leukemia (CLL) and non-Hodgkin's lymphoma (NHL) patients revealed no association to cytokine release syndrome (CRS) or severe toxicities on infusion and clinical responses in 70% of patients, which could potentially make this strategy optimal for those patients at risk of serious adverse reactions to CAR-T cell therapy (37).

Other IECs being leveraged for adoptive cell immunotherapy are cells from the myeloid compartment in particular macrophages, which are promising candidates for the treatment of solid tumors, given their tumor-mediated recruitment. Adenovirus transduction of an anti-HER2 CAR into human macrophages and subsequent in vitro expansion yielded proinflammatory M1-type macrophages that, on intravenous injection in mice, were recruited to the tumor in a xenograft model of ovarian cancer showing in vivo antitumor activity that reduced tumor burden (38). Of potential interest in the clinic, induced

Table 1. ACTs FDA-approved therapies[a]

Type of therapy	Name	Trademark	Indications	Year of first FDA approval
TILs	ITIL-168	N/A	Adult melanoma stages IIB-IV	Orphan drug designation 2021
TCR	Tebentafusp-tebn	Kimmtrak	Adult unresectable or uveal metastatic melanoma	2022
CAR-T	Tisagenlecleucel	Kymriah	Pediatric and young adults ALL	2017
CAR-T	Axicabtagene ciloleucel	YESCARTA	r/r LBCL DLBCL	2017
CAR-T	Brexucabtagene autoleucel	TECARTUS	Adult r MCL Adult r/r B-cell precursor ALL	2020
CAR-T	Idecabtagene vicleucel	ABECMA	Multiple myeloma	2021
CAR-T	Lisocabtagene maraleucel	BREYANZI	r/r LBCL DLBCL	2021

[a]ACT = adoptive cell therapy; ALL = acute lymphoblastic leukemia; CAR-T = T cells expressing chimeric antigen receptor; r/r = relapse or refractory; LBCL = large B-cell lymphoma; DLBCL = diffuse large B-cell lymphoma; FDA = Food and Drug Administration; MCL = mantle cell lymphoma; N/A = not applicable; TCR = T-cell receptor; TIL = tumor infiltrating leucocyte.

pluripotent stem cells (iPSCs)-derived macrophages expressing CARs have shown promising in vitro and in vivo antitumor activity in preclinical studies but still need further investigation (39).

There is currently great interest in both academia and industry in the development of new strategies and uses, such as the evaluation of CAR cells in addition to the CD19 antigen. Moreover, as learned from viral infections, the evaluation of the optimal ratio of monotypic to polytypic effector cells is optimal for therapeutic success in each type of tumor (40). In Supplementary Table 1 (available online), we show the targets presently under investigation with more than 500 clinical trials, opening this technology beyond cancer treatment and T cells (38,41-48). Those targets represent neoantigens, TAAs, and other targetable antigens such as viral particles. Neoantigens are tumor-specific antigens induced by a mutation or genetic alteration; thus, tumors with higher tumoral load also present a higher number of neoantigens. Due to HLA restrictions, only some of those might be immunogenic and can favor the expansion of TILs and therefore can be applied to vaccines, TCRs, and CARs, but their patient specificity limits broader application. Unlike neoantigens, TAAs are not restricted to tumor tissue because they are also present in other normal cells, but their expression in an "expendable" population like CD19 in B cells, or their higher expression in the tumor compared with normal cells, poise them as good targets, especially because they can be applied to a broader number of patients, providing attainable management of toxicities. As mentioned, the target choice will have a great implication in therapy design because it will determine different technology and conditionings (49-51). To overcome current limitations, several new strategies have been developed, including optimal expansion procedures, the use of bispecific molecules, modifications in the recognition fragment, logistic strategies, modifications in the signaling section, knock-in or knock-out of several molecules including endogenous TCR, and combination with checkpoint inhibitors, immunotherapy, and other therapies (45,52-57).

Biomarkers in ACT

ACTs are still very costly and require substantial cellular manipulation, so it is essential to differentiate patients to identify potential responders and maximize the chances of therapeutic success. In addition, a better performance in most solid tumors, in which an immunosuppressive TME dampens effective antitumor immunity, remains an important challenge for this type of immunotherapy. It is therefore paramount to find robust and reliable biomarkers to establish not only which patients are more likely to benefit from ACT, but also which ones are at risk of developing serious adverse effects to rationalize the use of these costly custom-made therapeutic strategies.

There are no clear factors dictating whether a particular infused cell therapy is going to be successful. Most of the data reported correspond to TILs and, in the last few years, to CAR-Ts, but little has been reported on engineered TCRs, although common features may be shared by all T-cell therapies. Due to the limited studies and heterogeneity of cellular products, it is difficult to identify a universal predictive set of biomarkers. The main areas explored for the identification of biomarkers correspond to adoptive transferred cells' intrinsic factors, the tumor-specific characteristics, and the microenvironment (Figure 2). A summary of biomarkers of ACT response, persistence, and toxicity is shown in Table 2, and its value for TILs and CAR-T cells is discussed in the following subheadings.

Biomarkers of TILs Therapies

Unlike other types of cancer therapies, ACT is generated from a pool of heterogeneous T-cell subtypes at diverse ratios and stages of differentiation. Although the phenotype(s) and ratios responsible for clinical success are not completely clear, reports suggest that the presence of a stem-like fraction, a bias towards CD8+ T cells with the company of CD4+ T lymphocytes, and the presence of functional markers are indicators of more effective antitumor response in vivo.

From the early 1990s, it was noted that shortened T-cell culture periods were associated with a better response to TILs (58). Shorter culture times were found to correlate with telomere length provided that these regions got shorter in every cycle of cell division and have downstream influences on cellular function and antitumoral activity (59,60). TIL products that are rich in naïve T cells, memory T cells, and stem cell memory T cells exhibit stronger antitumor responses and long-term persistence in vivo, with higher capacity for self-renewal and expansion (9). A higher proportion of CD8+ T cells within the TILs population has been shown to correlate with a

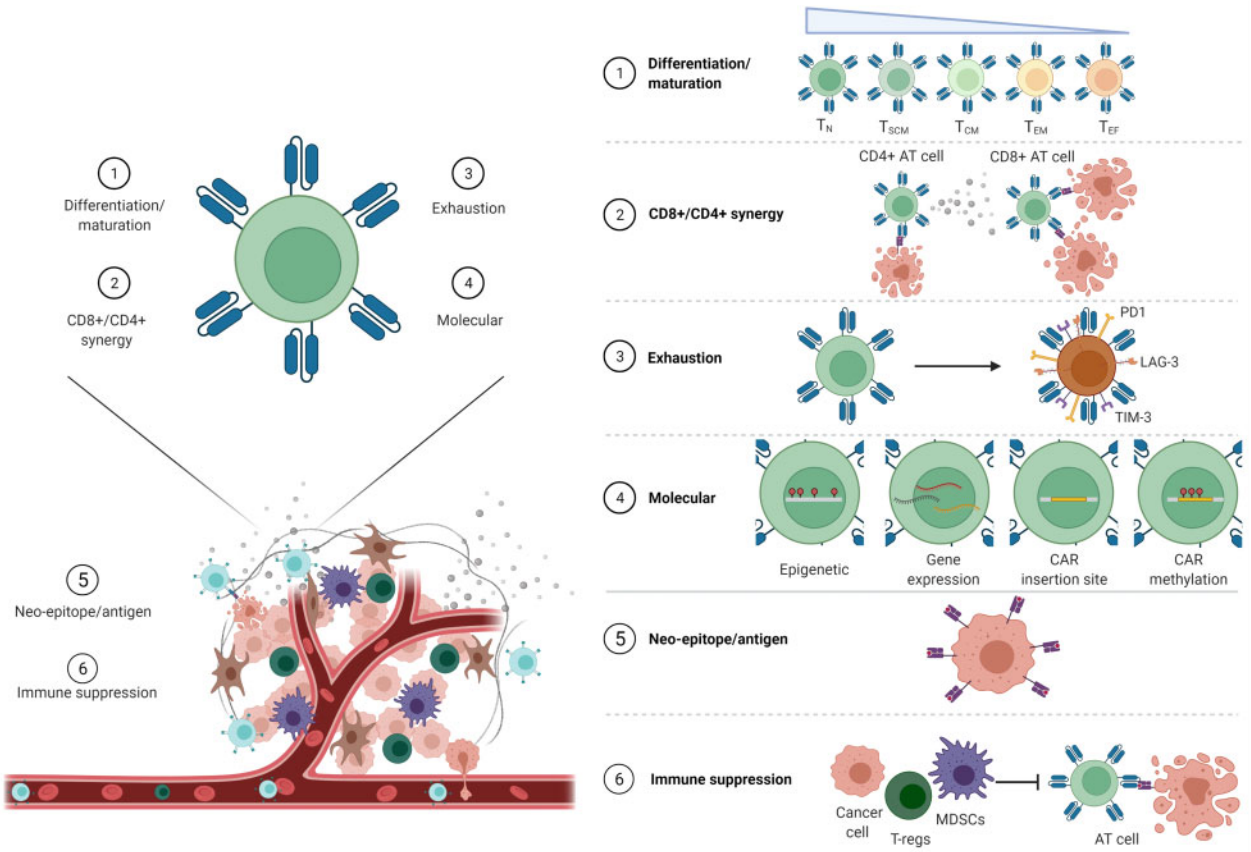

Figure 2. Adoptive cell therapy outcome biomarkers. Several characteristics of adoptive transfer (AT) cells, the tumor, and tumor microenvironment depicted in the figure have been linked with patient outcomes. These include adoptive transferred cell maturation, the presence of both CD8+ and CD4+, and the level of exhaustion and genomic, epigenetic, and transcriptomic markers. In the tumor, the specificity, amount, and dependence of the antigen are very important to induce a strong durable response, as are the tumor and the microenvironment suppressive strength. T_N = naïve T cells; T_{SCM} = stem memory T cells; T_{CM} = central memory T cells; T_{EM} = effector memory T cells; T_{EF} = terminal effector T cells; T-regs = regulatory T cells; MDSCs = myeloid derived suppressor cells.

better response to treatment (58), probably due to higher in vitro killing activity shown by those cells. CD4+ T cells, known as helper T cells, promote higher CD8+ T-cell activity and are now recognized as crucial for improving the response of CD8+ cytotoxic TILs (61,62). Moreover, single-cell gene expression of long-lasting TILs allowed identification of a profile enriched in surface markers and transcription factors (IL7R, ITGB1, KLF2, and ZNF683) (7).

In addition to TIL composition, there are biomarkers related to the tumor and its microenvironment that can also determine the chances of therapeutic success. Currently, TIL therapy is only applied to immunogenic tumors, where the higher the capacity of achieving antigenic-mediated activation, the higher chance of tumor killing by the T cells. In TIL and TCR cell therapies, one of the main predictors of response remains the specificity of the TCR against tumor antigens, especially against neo-epitopes. Sequence variation such as mutations, frameshifting insertions or deletions, and tumor-specific alternative splicing have been found to correlate with a successful immunotherapy response (63,64). TILs are effective for patients with melanoma, who usually present numerous neo-epitopes, whereas in tumors with low average mutational load, an optimization of TILs cultures to enrich T cells that recognize neo-epitopes is required to potentiate tumor killing (63).

Biomarkers of CAR-T Therapies

Given their common origin, a number of biomarkers are shared by CAR-T cells and TILs. For instance, the therapeutic efficacy and in vivo persistence of CAR-T cells statistically significantly correlate with their differentiation stage. CAR-T cell products that are rich in stem-like cells mediate more efficacious antitumor responses and improve patients' outcomes (65,66). However, infusion of CD8+ anti-CD19 CAR-T cells alone is sufficient for long-term B-cell eradication in lymphoma patients (67). CD4+ T cells are also shown to be crucial in CAR-T cells (68-70). CD4+ CAR-T cell dynamics showed initial slower tumor killing and a reduced granzyme B secretion but presented better persistence following antigen exposure compared with CD8+ counterparts (68-70). Most current approaches use CAR-T formulations that include a heterogeneous random composition of CD4+ and CD8+ cells. A defined composition of CD4 to CD8 CAR-T cells in a 1:1 ratio augments antitumor activity in vivo and supports the synergism between both subsets (65,71). T-cell biology complexity is worth considering; CD4+ T cells can be further subdivided into Th subsets, and the expansion of Th1 cells expressing T-bet and Th9 cells during CAR-T cells manufacturing has a positive impact on the antitumor response (72,73).

Table 2. TILs and CAR-T cells biomarkers[a]

ACT	Origin	Biomarker	Output	References
TILs	TILs	T cells stemness and memory	Response, persistence	(9, 58-60)
		CD8+ and CD4+ T cells	Response, persistence	(58, 61, 62)
		Surface markers and transcriptomic phenotype	Response, persistence	(7)
	Tumor and TME	Abundance of neoantigens	Response, persistence	(63, 64)
		Suppressive TME	Response, persistence	(63)
CAR-Ts	CAR-Ts	T cells stemness and memory phenotype	Response, persistence	(65, 66)
		CD8+ and CD4+ T cells	Response, persistence	(65, 67-73)
		CAR design	Response, persistence, toxicity	(23, 43, 88, 94, 95, 116)
		Cytokine production	Response, persistence, toxicity	(88, 93-102, 106, 108-110, 117)
		Tumor infiltration	Response, persistence	(88, 99, 101)
		Epigenetics (signatures)	Response	(79)
		Surface markers and transcriptomic phenotype	Response, persistence	(52, 77, 78)
		CAR integration site	Response, persistence	(30, 80)
		CAR methylation	Response, persistence	(81)
	Tumor and TME	Tumor load	Response, persistence	(82, 83)
		Antigen escape	Response, persistence	(32, 84)
		Suppressive TME	Response, persistence	(52, 87-91)
		Immunological clearance	Response, persistence	(102)
		Inflammatory cytokines	Response, persistence, toxicity	(93, 111-113, 117)

[a]CAR-T = T cells expressing chimeric antigen receptor; TIL = tumor infiltrating leucocyte; TME = tumor microenvironment.

During in vitro expansion and after therapeutic infusion, repeated antigen stimulation can induce CAR-T cells to enter an exhaustion state (74), which is characterized by poor self-renewal, expansions, and effector function; sustained expression of inhibitory receptors (eg, PD1, TIM-3 and LAG3); and epigenetic and gene expression alterations (75,76). Higher levels of the exhaustion markers PD1, TIM-3, and LAG3 at the moment of apheresis, preinfusion, and postinfusion have been reported in patients whose CAR-T cells persist for shorter periods of time and fail to respond to treatment (52,77,78).

At the molecular level, in addition to cell surface markers, some studies have identified indicators of response associated with CAR-T cell gene expression, CAR integration site, and its epigenetic regulation. Recently, our group described a DNA methylation signature in CAR-T cells that we named EPICART (79). EPICART, like a previously described gene expression signature (77), associates good outcome with a signature enriched in naïve-like or early memory cell populations, thus reinforcing the importance of the presence of these subsets at preinfusion to determine overall response. CAR integrates in the genome, thereby marking cell lineages and modifying the cellular genome by insertional mutagenesis. Interestingly, it has been shown that vector integration into the TET2 locus and subsequent clonal expansion is associated with clinical success (30). Consequently, a wider study demonstrated that therapy responders presented integration sites enriched in genes coding for cell-signaling and chromatin modifiers, suggesting that insertional mutagenesis into these pathways promoted therapeutic T-cell proliferation and preinfusion products able to support a better outcome (80). Finally, retroviral vectors encoding foreign genetic material have been shown to be prone to progressive acquisition of DNA methylation, leading to subsequent epigenetic silencing of the CAR, which abrogates CAR-T cell function (81).

Regarding predictors of CAR-T therapies related to the tumor characteristics and its microenvironment, an earlier long-term follow-up study in adult ALL patients receiving CD19 CAR therapy showed that high tumor burden correlates with relapse (82). An article describing opposite risk factors for CD19-negative vs CD19-positive relapse on anti-CD19 CAR-T treatment of pediatric relapsed or refractory BCP-ALL has identified high tumor burden as the major factor that correlates to increased risk of CD19 negative relapses in the presence of increased CAR-T cell persistence. On the other hand, low tumor burden was found to be associated with decreased persistence and risk of CD19-positive relapses (83). In addition, downregulation or loss of the targeted antigen is associated with reduced or abrogated response to treatment. This evasion mechanism occurs via 2 distinct pathways: antigen escape (84), where there is a lack of surface expression of the antigen, or lineage switch (80), where the patient relapses with a phenotypically different malignancy (32). To avoid immune elimination, cancer cells can present 1 or several immune evasion mechanisms. Tumor cells can express inhibitory ligands such as PD-L1 and release immune antiinflammatory cytokines, including arginase, transforming growth factor beta (TGF-β), indoleamine 2,3-dioxygenase (IDO), and IL-4, as well as orchestrate and sustain an inhibitory TME containing T regulatory cells and myeloid derived suppressor cells (16,85,86). All these immunomodulatory mechanisms diminish CAR-T efficacy by reducing their proliferation, cytotoxicity, and persistence (52,87-91).

Persistence of CAR-T Therapy

Persistence remains an important impediment for the development of effective CAR-T therapies in cancer and clearly predicts the effectiveness of the treatment because long-lasting CAR-T cells aim for the complete eradication and avoid tumor relapse. However, in some cases, transient rather than prolonged persistence is desirable. Such is the case for the use of in vivo–

generated CAR-T cells to eliminate fibrosis heart injuries that peak at 24 hours, post delivering modified mRNA in T cell–targeted lipid nanoparticles and rapidly dropping over the following days, where transient presence of CAR-Ts is required to avoid affecting the normal injury-healing process (43). Several factors affect the persistence of CAR-T cells in patients, including activation-induced cell death, peripheral tolerance, peripheral ignorance, and immunological clearance (92). Some of these issues are being addressed by engineering better CARs, but as mentioned above, the complex composition of the products being used is especially important, and T-cell stemness and T-cell exhaustion are key markers of persistence (65,66,69,93). On infusion, the lack of increase in inflammatory markers such as interferon gamma (IFN-γ), IL-6, and IL-10 and the absence of tumor infiltration or the elimination of the CAR-T cells by the immune system have been identified to be poor markers of persistence and response (88,94-102).

CAR-T Adverse Side Effects and Toxicity

Toxicities or adverse side effects associated with CAR-T cell therapies can be severe and even life threatening if not managed properly, and are less common and milder in TIL and TCR regimes. For this reason, there has been a great interest in understanding the mechanisms underlying these adverse effects and identifying predictors and markers for their early detection in CAR-T cell treatment. The main described toxicities include CRS, immune effector cell associated neurotoxicity syndrome (ICANS) and hematologic toxicities and infections, with CRS and ICANS being the most frequent. CRS is a major complication of CAR-T cell therapy and is characterized by systemic inflammation (34). The symptoms of CRS vary depending on the severity and range from mild to serious illness, the latter presenting high fever, hypotension, shock, disseminated intravascular coagulation (usually in the absence of bleeding), and even multiple organ dysfunction (103-105). CRS generally develops in response to the engagement of the CARs by its specific antigens, which subsequently stimulate other immune and nonimmune cells (106,107). The IL-6, IL-10, and IFN-γcytokines are the strongest contributors to CRS development, and IL-6 is a core cytokine in CRS pathophysiology (106,108-110). Cytokine responses generated by CAR-T and bystander macrophage activation can lead to Macrophage Activation Syndrome that contributes to therapy-derived immunotoxicity (111). A murine xenogeneic model of ALL with human 1928z CAR-T cell infusion was used to interrogate the role of different cell types in the production of inflammatory cytokines such as IL6 and IL1β that drive acute CRS identified macrophages as the main contributors to the pathogenesis of CRS (112). In this line, INF-γ produced on CAR-T cell activation has been shown to activate macrophages triggering a CRS-like cytokine release, and IFN blockade abolished macrophage-mediated cytokine production, diminishing cytokine-derived toxicities, increasing CAR-T persistence, and improving overall antitumor response (111). Fever and high monocyte chemoattractant protein 1 (MCP-1) levels within 36 hours after the infusion of CAR-T cells are considered to be predictors of severe CRS and ICANS with the best sensitivity and specificity (113).

ICANS is another primary adverse event during CAR-T cell therapy and is characterized by encephalopathy, aphasia, delirium, seizures, and tremors (114,115). The mechanism underlying the development of ICANS is not fully understood. The massive release of inflammatory cytokines and changes in blood-brain barrier permeability have been suggested to play key roles in the unfolding of ICANS, but recent results from a single-cell study identified mural cells that surround the endothelium and are critical to the integrity of the blood-brain barrier, express CD19, and present an off-target for CAR19 strategies (116). The incidence of ICANS was associated with a high pretreatment disease burden, a rate of CAR-T cell expansion, higher levels of proinflammatory cytokines, and endothelial markers that can be used as predictors and for personalized monitoring (93,117).

Conclusions and Perspectives

The refinement of related protocols to IECs in cancer management has opened up an unprecedented opportunity to reach therapeutic success through tailoring of the patients' immune cell response to therapies that fight the tumor. Such highly personalized therapies are costly and labor intensive and are not exempt from generating potentially life-threatening adverse side effects. This risk underscores the need for reliable and robust predictive and prospective biomarkers of response that inform of individual characteristics, enabling rational decision making in therapeutic options to expand the responder population and increase survival. In addition, further research is needed to discover novel biomarkers in specific T-cell products to improve efficacy and safety through the identification of patients who are greater risk of developing severe toxicities related to treatment.

Off-the-shelf modalities have started to emerge, to eliminate the waiting period for patients to receive the treatment, normalize therapeutic agents, reduce costs, and allow these therapeutic options to reach a broader spectrum of individuals. Solid tumors also represent an unmet need because they, unlike in hematological malignancies, do not express an ideal uniform target for adoptive cell therapy, increasing the risk of on-target off-tumor toxicity (118). Finding predictive markers that inform of the risk of an unwanted immunological response in each case remains a major challenge. In addition, tumor heterogeneity, mainly due to genomic instability and the accumulation of mutations, in solid tumors makes it difficult to select the best TAA to target in each patient (119). In that respect, the target selection for the rational design of immunotherapeutic products deserves further investigation to pinpoint targets that may warrant clinical success as well as approaches to surpass the immunosuppressive TME. Clinical breakthroughs in the biomarkers arena will likely come from currently used multiomics technologies and the analytical computational tools that are constantly being advanced. Such biomarkers will also contribute to the optimization of the production process to generate affordable and scalable therapies that reduce the risk of relapse and the appearance of resistance, coming closer to the ultimate goal of achieving curative responses.

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Data Availability

The data underlying this article are available in the article.

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