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Synthetic T cell receptor-based lymphocytes for cancer therapy

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ABSTRACT

Chimeric antigen receptor (CAR) T cells have been remarkably successful in patients with hematological malignancies expressing the CD19 surface antigen, but such level of success is far from being replicated in solid tumors. Engineered T cell receptor (TCR) T cells targeting cancer antigens were first developed over two decades ago and represent an alternative adoptive T cell approach that has produced provocative clinical data in solid cancers. However, several factors may hinder this technology from realizing its full potential, including the need for HLA matching, HLA downregulation by cancer cells, the suppressive tumor microenvironment, and tissue liabilities resulting from targeting antigens shared with normal tissues. Efforts therefore continue to engineer enhanced versions of CAR T and TCR T therapies that can overcome current barriers. Furthermore, emergent novel TCR-based, HLA-unrestricted platforms may also provide unique tools that integrate the complexity of the TCR signaling cascade that can be applied to treat solid tumors. This article reviews the current state of development of TCR T cell approaches and discusses next generation improvements to overcome their current limitations.

Contents

1. Evolution of TCR T cell therapies

Adoptive transfer of engineered T cells *via* viral transfer of chimeric antigen receptors (CARs; Fig. 1) has proven transformational medicines in patients with relapsed or refractory B-cell cancers such as acute lymphoblastic leukemia (ALL), diffuse large B cell lymphoma (DLBCL) and multiple myeloma [1–3]. On August 30th, 2017, the U.S. Food and Drug Administration (FDA) made tisagenlecleucel (CTL019, Kymriah™, Novartis) the first gene therapy available in the U.S. Tisagenlecleucel, a CD19-directed CAR T cell therapy, is approved for the treatment of patients up to 25 years of age with B-cell precursor ALL that is refractory or in second or later relapse. Subsequently axicabtagene ciloleucel (KTE—C19, axi-cel, Yescarta™, Gilead) was approved for the treatment of adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy (October 17th, 2017) and tisagenlecleucel for the treatment of patients with relapsed/refractory diffuse large B-cell lymphoma (DLBCL) ineligible for or relapsing after autologous stem cell transplantation (SCT) (May 2nd, 2018). Beyond this initial success in hematologic malignancies, a large number of clinical trials are being conducted to test the activity of CAR T cell therapies against solid tumors. An alternative approach to engineer T cells is that

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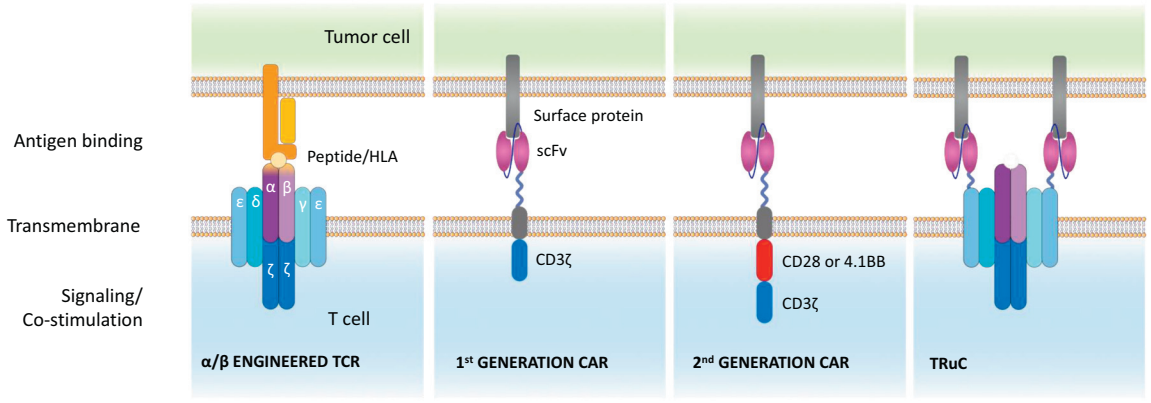

Binding domain	α/β TCR subunits	scFv, Fab, sdAb	scFv, Fab, sdAb	scFv, Fab, sdAb
HLA restriction	Yes	No	No	No
No. of ITAMs	10	6 (dimer)	6 (dimer)	10
Co-stimulatory domains	No	No	Yes	No
Regulatory domains	Yes	No	No	Yes

Fig. 1. Overview of engineered T-cell platforms. The first adoptive cell therapies (ACTs) utilized tumor-infiltrating T cells, which were isolated from tumor and expanded and re-invigorated *ex vivo* in the presence of IL-2 prior to re-administration to patients. However, the use of *ex vivo* expanded TILs is limited by the paucity of isolated TILs and restrictions to certain types of tumors. To increase the engraftment of TILs, in more recent clinical trials, patients have been treated with IL-2. (A) Subsequently, more sophisticated approaches have emerged. Affinity-matured TCRs recognizing cancer-testis antigens, such as NY-ESO-1, or differentiation antigens are introduced into autologous T cells isolated from PBMCs using viral or non-viral gene insertion and then expanded *ex vivo*. This process allows for the expression of tumor-specific TCRs and the production of greater T cell numbers for adoptive transfer. While this approach has demonstrated activity in hematological and solid tumor, it is hampered by a limited number of tumor-specific TCRs and the requirement HLA matching. (B) CAR-T cells cancer-associated antigen in an HLA-independent manner. CARs comprise an antibody based binding domain, mostly a scFv, which tethered to a plasma membrane by a spacer/hinge sequence that provides flexibility followed by a transmembrane domain and intracellular signaling domains. First-generation CARs harbor only the CD3ζ domain to trigger T cell activation. This design failed to show robust anti-tumor activity in clinical studies. Therefore, additional co-stimulation signal domains derived from CD28 or 4.1BB, respectively, were added to promote a more robust and durable CAR-T cell response. The second-generation CAR-T have shown great efficacy in hematological malignancies and resulted in the approval of two new T cell products by the FDA. Unfortunately, these gains have not been observed in solid tumor treatment. Current efforts to increase the efficacy of CAR-T cells in solid tumors focus on additional genetic modifications and enhancement of activity by co-expression of cytokines or interference with the immunosuppressive PD-1 signal. (C) T cell receptor fusion constructs (TRuCs) present a novel engineered T cell platform that is not HLA-restricted and different from CAR-T cells utilize the entire T cell receptor for T cell activation. By fusing an antibody -based binding protein to one or more subunits of the CD3 complex, the specificity of the TCR is reprogrammed to recognize cell surface antigens expressed on tumor cells. Thereby, the use of all natural TCR subunits enables a more comprehensive and controlled activation of T cells. As an example, the fusion of a scFv tethered to the CD3ε subunit is shown.

of affinity maturing synthetic T cell receptors (TCRs) that are then transduced into T cells to more selectively recognize and destroy cancer cells (Fig. 1). This approach actually predates CAR T cell approaches and was first pioneered by investigators at the National Cancer Institute and has proven very potent against solid cancers where they have already shown that they can persist over time and produce long-lasting responses. The therapeutic use of T cells was first developed during the 1990s by two groups, one led by Steve Rosenberg at the National Cancer Institute (NCI) and the other by Phil Greenberg at the University of Washington in Seattle [4]. Both groups isolated antigen-specific T-cell clones from peripheral blood for adoptive immunotherapy in both cancer and viral infections. Several clinical trials showed the potential of tumor infiltrating lymphocytes (TILs) expanded *ex vivo* for the treatment of human cancers such as malignant melanoma [5,6] or ovarian cancer [7]. Proof-of-concept regarding the therapeutic potential of targeting tumor-associated antigens by TILs was provided by studies at the National Cancer Institute (NCI), where this approach rendered objective responses in 72% of patients with metastatic melanoma [8]. However, it soon became apparent that successful TIL manufacture was only possible in half the patients with melanoma, with very limited success in other malignancies. This realization propelled the development of techniques to genetically engineer T cells expressing TCRs specific against antigens expressed in multiple human cancers. In the next few years, the above-mentioned research groups isolated the specific TCRs imparting tumor reactivity and developed recombinant TCRs against tumor-associated antigens. The first trial involved the use of TCRs cloned from the T cells of a patient who had an almost complete response (CR) to TIL therapy. Patients were administered autologous T cells previously expanded *ex vivo* and transduced with TCRs specific for the MART-1 tumor antigen. The next step was to develop the technology to clone TCRs and delivery into T cells *via* viral vectors. In the meantime, other studies with recombinant TCRs were launched against other antigens such as MAGE (melanoma-associated antigen) [9]. A critical milestone in the development of TCR T cell therapies was reached in 2000, when it was shown that the affinity of TCRs could be increased by mutating the TCR complementarity-determining region (CDR) [10]. In the next few years a number of companies were launched to develop TCR T cell technologies, including Adaptimmune (2008) and Kite Pharma (2009, part of Gilead since 2017), which partnered with the NCI to develop their pipelines. More recently Juno Therapeutics (part of Celgene since 2018) was formed in Seattle in 2013 to develop both CAR T and TCR T cell programs in collaboration with Memorial Sloan Kettering Cancer Center (New York, NY) and the Fred Hutchinson Cancer Research Center (Seattle, WA). These and other companies and academic institutions are developing clinical programs with TCR T cell products both in solid tumors and hematologic malignancies (Table 1).

2. Differences between TCR T cell and CAR T cell platforms

Whilst the clinical development of TCR T cell approaches preceded that of CAR T cell therapies, currently the latter significantly outnumber the former in terms of sheer clinical trial volume in the 3 main geographic cell therapy hubs: the U.S. Europe, and China. It is important to note that the latter are two fundamentally different adoptive T cell therapy platforms. Initial results from clinical trials suggest that TCR T cell approaches are more efficacious against

Table 1
NY-ESO-1 TCR T cell clinical trial in synovial sarcoma (NCT01343043). N: number; Flu: fludarabine; Cy: cyclophosphamide; CR: complete response; PR: partial response; DOR: duration of response; PFS: progression-free survival; AEs: adverse events; CRS: cytokine release syndrome.

	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Total
	High	Low	High	High	$N = 28$
	NY-ESO-1 Expression	NY-ESO-1 Expression	NY-ESO-1 Expression	NY-ESO-1 Expression	
	High Flu/Cy	High Flu/Cy	Only Cy	Intermediate Flu/Cy	
	$N = 12$ (%)	$N = 5$ (%)	N = 5 (%)	$N = 6$ (%)	
N/Accrual status	15/Complete	9/Ongoing	5/Complete	10/Ongoing	39
N Screened	120	116			236
Transduced cells × 10^9, Median (range)	3.6 (0.5–14.4)	3.5 (1.8–3.8)	3 (1.5–5)	2.2 (1.3–3.8)	3.2 (0.5–14.4)
CRS	2 (17)	1 (20)	1 (20)	0 (0)	4 (14)
ORR: Confirmed, CR + PR: N (%)	6 (50)	2 (40)	1 (20)	3 (50)	12 (43)
Median PFS: weeks (range)	15 (8, 38)	12 (3–14)	12 (8, 38)	NA	NA
Median DOR weeks (range)	30.9 (13, 72)	7.5 (6–9)	21--	NA	NA
Serious AEs (SAEs)	6 (50)	3 (60)	4 (80)	2 (33)	15 (54)
Treatment related SAEs	6 (50)	2 (40)	2 (40)	1 (17)	11 (39)

solid tumors compared to CAR T cell therapies. Furthermore, TCR T cell therapies result in lower rates of cytokine release syndrome (CRS) and neurotoxicity, the latter being the two more frequent severe adverse events reported with engineered T cell therapies. These differences in clinical outcomes may be due to differences in mechanism of activation and/or antigen engagement (Fig. 1). CARs are activated and operate independently from the TCR and largely lack autoregulatory mechanisms, whereas synthetic TCRs follow the same activation and autoregulatory paradigm that applies to native TCRs. Notably, TCRs can potentially recognize all peptides processed and presented in the context of HLA molecules, thus allowing TCRs to target some intracellular antigens. Conversely, the scope of targetable antigens is significantly more limited for CARs as the latter can only engage cell surface proteins (approximately 10% of all potential targets), albeit in an HLA unrestricted manner. This is important because TCR T cell therapies can only be successful in patients expressing the specific HLA proteins that the synthetic TCRs have been designed against. Most available TCR T cell therapies are directed against antigens presented in the context of HLA-A*02, which is present at most in 40–45% of patients of Caucasian descent, thus preventing the use of these therapies in the majority of cancer patients. Notwithstanding their limitations in terms of the number of targetable antigens, CAR T cells can be administered to all patients expressing the cancer antigen against which they are designed. Moreover, immune evasion *via* loss or downregulation of HLA molecules or of other elements of the antigen presenting machinery is a common mechanism of escape in human cancer that may potentially limit the success of TCR T cell approaches, but not of CAR T cells.

3. TCR generation for adoptive T cell therapy

The first TCR T cell therapies (*i.e.* those against the MART-1 antigen) utilized naturally occurring TCRs obtained from patients with metastatic melanoma experiencing objective responses to TIL therapy [8,9]. It must be noted that high affinity TCRs (in the nM to pM range) against antigens expressed on normal tissue (*i.e.* self-antigens) undergo thymic deletion and therefore are almost impossible to obtain [12,13]. Such TCRs would allow the targeting of developmental antigens such as carcinoembryonic antigen (CEA), differentiation antigens such as glycoprotein-100 (gp100), or tumor-associated antigens such as overexpressed growth factor receptors like ERB/HER. Given the difficulty of isolating naturally occurring high-affinity TCRs, more frequently these TCRs are used scaffolds that are further matured *ex vivo* to increase their affinity following a stepwise amino-acid replacement within the CDRs followed by empirical testing against target antigen [14,15]. This process rendered the c259 TCR that has been successfully tested in patients whose cancers express the cancer germline antigen NY-ESO-1. This TCR recognizes a nonapeptide (NY-ESO-1157-165) presented by HLA-A*02 [16]. An alternative way of isolating high affinity TCRs is that of immunizing HLA-A*02 transgenic, or HLA-A*02/human TCR chromosomal double transgenic mice [17] with antigens that do not match the murine homolog. The aim is to generate peptide-MHC complexes whose cognate TCRs avoid thymic deletion, and therefore T cells bearing those TCRs can be collected from lymphoid organs and further expanded *ex vivo* [18,19].

4. Antigens amenable to TCR T cell approaches

The first step towards the generation of TCR T cells is the identification of a suitable target antigen. The ideal target for adoptive T cell therapies is one that is expressed exclusively on cancer cells but not on normal tissue. Few targets exist that meet these stringent criteria. Instead, most targeted antigens are overexpressed on cancer cells and expressed at low levels on healthy tissue, thus creating a therapeutic window. In the latter example, lowering the TCR affinity may aid in the selective killing of high-antigen expressing cancer cells while sparing low-antigen bearing normal cells [20,21]. The types of antigens targeted by TCR T cell therapies in clinical trials can be divided in three categories: cancer germline antigens, differentiation antigens, and viral antigens. The expression of cancer germline antigens is typically limited to fetal tissues and to germ cells which lack HLA class I expression, which makes those tissues immune to T cell attack. Targeting cancer germline antigens is the current focus of Adaptimmune Therapeutics which is currently testing their proprietary TCR T cell technology against tumors expressing NY-ESO-1, MAGE-A10, MAGE-A4, and PRAME. The main advantage of targeting these antigens is that they are not processed into peptide on adult tissue, with the exception of PRAME which can be detected at very low levels on healthy tissue [22], which minimizes off-tumor toxicity. However, deaths induced by TCR T cells targeting cancer germline antigens due to cross-reactivity with related [23] and unrelated proteins have been described and the number of malignancies sufficiently expressing such antigens is limited, which makes clinical development quite challenging [24]. Differentiation antigens are typically overexpressed on cancerous tissue and expressed at low levels on normal tissue from which the tumors arise like MART-1 in melanoma, EGFR in non-small cell lung cancer (NSCLC), or PSMA and PSCA in prostate cancer. In this case, the amplitude of the therapeutic window is critical to prevent on-target/off-tumor toxicity. Targeting differentiation antigens is the preferred strategy at Juno Therapeutics (currently Celgene). They are developing TCR T cell programs that target WT1, which is found overexpressed in acute myeloid leukemia, mesothelioma and NSCLC, and mesothelin, which is overexpressed in pancreatic cancer. Viral antigens, on the other hand,

appear to be safe, but TCR T cells can only be designed against tumors arising as a consequence of viral infection (*e.g.* HPV- and HBV-related cancers or EBV- and CMV-induced post-transplant lymphoproliferative disorders). The NCI and Kite Pharma (currently Gilead) are developing TCR T cell programs against cancers overexpressing HPV-16 E7.

In addition to the above described main antigen classes, others are being considered as targets for TCR T cell therapy. Advances in genome sequencing techniques, mass spectrometry, and data analysis have facilitated the identification of specific neoantigens unique to individual cancers. This has allowed the generation of autologous TCR T cells reactive against such neoantigens with clinical activity [25–27]. While many of these neoantigens are generated by classical mutations, others are created as a consequence of post-translational modifications (*e.g.* phosphorylation, methylation) that can also be targeted by TCR T cells [27,28,29]. Finally, T cell epitopes associated with impaired peptide processing (TEIPP) are a novel class of antigens present in cancer cells with defects in the antigen presenting machinery that are amenable to TCR T cell targeting approaches [30].

5. Developing safe synthetic TCRs

The identification of antigens amenable to TCR T cell targeting is challenging even for targets that have been extensively validated in monoclonal antibody studies given the significantly higher potency of T cells when used as therapeutics as well as the potential for untoward cross-reactivity events observed in trials employing synthetic TCRs [23,24,31]. Unfortunately, such events cannot be experimentally predicted *in vivo* given differences between murine MHC and HLA, the scarce number of target epitopes shared by both mouse and human, thus making it difficult to determine potential normal tissue toxicity [32, 33], and the limitations or absence of murine models of CRS and neurotoxicity. Off-target toxicity is even more difficult to predict, particularly upon TCR affinity maturation as this process may artificially promote untoward binding to peptide/HLA complexes derived from other proteins expressed on normal cells, which otherwise would have been edited out by the thymus. The standard implementation of *in vitro* and *in silico* assays to predict cross-reactivity profiles has drastically increased the safety of novel affinity matured TCRs, as shown in recent trials where most toxic events derive from the lymphodepleting chemotherapy administered prior to TCR T cell infusion.

6. Clinical development of engineered TCR T cell therapies

The earliest inkling of clinical activity of an engineered TCR T cell therapy was provided by a trial in which 2 of 13 patients with melanoma had tumor regression upon infusion of autologous T cells engineered to express a specific MART-1 TCR [11]. The first TCR T cell trial in a cancer different from melanoma was launched in 2008 and induced responses in 4 of 6 patients with synovial sarcoma expressing the cancer germline antigen NY-ESO-1 [15]. Subsequently, an improved MART-1 TCR induced responses in 30% of patients and a gp100 TCR T in 19% [34]. However, several instances of severe toxicity cast a shadow on the viability of TCR T cell therapy as a safe approach for the treatment of cancer. In 2009, three patients developed severe inflammatory colitis upon infusion of a TCR T cell product targeting CEA, which led to the discontinuation of the trial [14]. In 2011, another NCI study treated patients with solid tumors with a MAGE-A3 TCR T product and 3 of the first 9 rapidly developed severe neurotoxicity and 2 of them died due to cross-reactivity against MAGE-A12, which is expressed in the brain [23]. A similar study at the University of Pennsylvania using a MAGE-A3 TCR developed by Adaptimmune resulted in the death of 2 patients due to cardiogenic shock within one week from infusion. This phenomenon resulted from the recognition by the MAGE-A3 TCR of an off-target peptide derived from the protein titin [24], which is highly expressed on cardiac muscle [31]. Improvements in cross-reactivity testing have allowed the safe evaluation in recent years of novel TCR T cell therapies in multiple human cancers. The most promising data have been generated with TCRs directed against NY-ESO-1.

6.1. NY-ESO-1 TCR T

NY-ESO-1 is a cancer germline antigen that is found overexpressed in a variety of human cancers, including in 70–80% of patients with synovial sarcoma and myxoid round cell liposarcoma and in 10–50% of metastatic melanomas, NSCLC, and ovarian cancers [35,36]. The first clinical trial with an NY-ESO-1 targeted TCR T cell approach in patients with synovial sarcoma or metastatic melanoma was launched at the NCI in 2008 and results were published in 2011. This study employed a retrovirally transduced high-affinity HLA-A*02-restricted TCR engineered to recognize a peptide flanked by amino acids 157 to 165 of the NY-ESO-1 protein [37,38]. Treated patients had tumors that intensely expressed NY-ESO-1 (2+ to 4) in >50% of their cells. Lymphodepletion consisted of fludarabine (25 mg/m^2/d for 5 days) and cyclophosphamide (60 mg/kg/d for 2 days), which was then followed by infusion of autologous NY-ESO-1 TCR T cells at a total median dose of 5×10^{10} (range, 1.6 to 130×10^9) as well as systemic administration of IL-2. Initially 17 patients (6 with synovial cell sarcoma and 11 with melanoma) underwent treatment and 9 (53%) achieved a response. A more recent update of the study included longer follow-up data as well as efficacy data on 21 additional patients (12 with synovial sarcoma and 9 with melanoma). The ORR was 61% in synovial sarcoma, and the estimated 3-year and 5-year survival rates were 38% and 14%, respectively. The ORR among patients with metastatic melanoma was 55% and the estimated survival rate was 33% both at 3 and at 5 years [37]. Most toxicities observed in the trial were attributed to the lymphodepleting regimen and/or the systemic administration of IL-2. This study established NY-ESO-1 as a *bona fide* cancer antigen for adoptive T cell therapies but more importantly, it solidified the notion that engineered T cells that activate the full TCR upon antigen engagement (as opposed to just the zeta chain engineered into CAR T cells) can consistently render responses in patients with solid tumors.

Adaptimmune Therapeutics, through their proprietary SPEAR® (Specific Peptide Engineered Affinity Receptor) technology is currently developing a human-derived affinity-enhanced lentivirally transduced TCR that recognizes the NY-ESO-1/LAGE-1-derived SLLMWITQC peptide in complex with HLA-A*0201 (NY-ESO^{c259}). NY-ESO-1^{c259}SPEAR® cells are being tested in patients expressing HLA-A*02 with a variety of NY-ESO-1-expressing tumors such as multiple myeloma, synovial sarcoma, myxoid round cell liposarcoma, melanoma, NSCLC, or ovarian cancer. In a multiple myeloma study, 20 patients received NY-ESO-1^{c259}SPEAR® two days after having undergone autologous stem cell transplant (SCT). Engineered T cells were safe, trafficked to the bone marrow and displayed extended persistence, which correlated with clinical anti-myeloma activity [39]. Of the 20 patients, 14/20 (70%) patients had a near CR or CR, which compares favorably with outcomes post autologous SCT where response rates are typically <40%. The median progression free survival (PFS) was 19.1 months.

To date, most data with NY-ESO-1^{c259}SPEAR® have been generated in patients with unresectable, metastatic or recurrent synovial sarcoma. In a pilot trial, 39 patients were enrolled to 4 distinct cohorts, of whom 28 were infused engineered TCR T cells (Table 1). Cohort 1 explores the efficacy and safety of NY-ESO-1^{c259}SPEAR® and has completed accrual. The ORR was 50% (6/12) and the projected median OS was 159 weeks. This is clinically significant as the OS with pazopanib, the standard of care in patients with metastatic soft-tissue sarcoma, is only 12.5 months. All responders had peak expansion >75,000 transgene copies/μg gDNA. Five of 6 responders had detectable circulating NY-ESO-1^{c259}SPEAR® cells 1-year post-infusion. Expansion was less robust in the non-responding subjects, typically with expansion below 50,000 copies/μg gDNA. None of the non-responders had detectable NY-ESO-1^{c259}SPEAR® cells 1-year post-infusion. The most common adverse events among 53 patients treated with NY-ESO-1^{c259} SPEAR® in

Adaptimmune sponsored studies in synovial sarcoma, melanoma, ovarian cancer and multiple myeloma were rash (49%), diarrhea (40%), fatigue and pyrexia (36% each), nausea (26%), anemia and lymphopenia (25% each) and neutropenia (23%) [40]. Most of these toxicities are likely related to the fludarabine/cyclophosphamide lymphodepleting regimen. CRS was reported in only 8% of patients and no grade 3–4 neurotoxicity events were observed.

6.2. Next generation of TCR T cell clinical trials

Multiple antigens overexpressed on cancer cells with little to no expression on normal healthy tissues are being targeted by TCR T cell approaches in clinical trials, including cancer germline antigens (*e.g.* MAGE-A3, MAGE-A3, MAGE-A10) and tumor differentiation antigens such as MART-1, gp100, CEA, Her2, and p53 (Table 2). Clinical data from this next wave of TCR T cells will become available in the near future, but one critical question is to what extent tissue liabilities will limit the clinical applicability of these therapies. Because of the severe toxicities observed in prior studies with MAGE-A3 TCR T cell therapies due to cross-reactivity against MAGE-A12 and titin in the brain and heart muscle, respectively, the NCI developed a novel approach to therapeutically address this cancer germline antigen. Instead of using genes that encode HLA class I-restricted TCRs, they engineered an HLA-DPB1*0401 (class II)-restricted TCR that recognized MAGE-A3 [41]. These TCRs were retrovirally transduced into a purified CD4+ T cell population and given to patients with metastatic solid tumors in a dose escalation clinical trial in combination with systemically administered IL-2. Patients received an initial dose of 10^7 total cells, which was escalated at half-log increments up to the highest dose level of 0.78 to 1.23×10^{11} cells. Seventeen patients were treated. One patient had an objective response during dose escalation, still ongoing after >29 months. Of the 9 patients who received MAGE TCR T cells at the highest dose level, a partial response was observed in a patient with esophageal cancer lasting 4 months, in a patient with urothelial cancer still ongoing after >19 months, and in a patient with osteosarcoma, lasting 4 months. While most patients experience fever post-infusion, the toxicity profile was manageable, and no patient died due to TCR T cell therapy or experienced cross-reactivity related toxicity.

6.3. Are neoantigens clinically targetable? And is it practical?

The NCI and Kite Pharma are devoting efforts to the development of truly personalized TCR T cell therapies by targeting peptides resulting from the processing of proteins generated by somatic mutations borne by cancer cells. These so called neoantigens are virtually present in every tumor at varying frequencies and they are different from tumor to tumor. Furthermore, only a fraction of neoantigens are candidates to become TCR T cell targets as they need to be processed into small

Table 2
Selected TCR-T cell clinical trials. *HLA-A*02 restricted unless otherwise stated.

TCR target (HLA, notes)	Cancer	Identifier	Sponsor
NY-ESO-1	Myxoid round cell liposarcoma	NCT02992743	Adaptimmune
	Synovial cell sarcoma	NCT01343043	Adaptimmune
	Metastatic melanoma	NCT01350401	Adaptimmune
	Multiple myeloma	NCT01892293	Adaptimmune
	Non-small cell lung cancer	NCT02588612	Adaptimmune
	Ovarian	NCT01567891	Adaptimmune
	Multiple myeloma (+pembrolizumab)	NCT03168438	Adaptimmune
NY-ESO-1 (HLA-A*02:01 & HLA-A*02:06)	Synovial sarcoma	NCT03250325	Takara
NY-ESO-1 (HLA-A*0201)	Multiple Myeloma/Melanoma/Synovial Sarcoma/Myxoid Round Cell Liposarcoma	NCT03399448	University of Pennsylvania
CRISPR edited endogenous TCR and PD-1			
NY-ESO-1 (expressing dominant negative TGF-β Receptor II)	Locally advanced or metastatic solid tumors	NCT02650986	Roswell Park Cancer Institute
NY-ESO-1 (aldesleukin + dendritic cell vaccine + ipilimumab)	Locally advanced or metastatic solid tumors	NCT02070406	Jonsson Cancer Center
NY-ESO-1 (+ vaccine)	Malignant neoplasm	NCT01697527	Jonsson Cancer Center
NY-ESO-1	Bladder/Breast/Esophagus/Lung/other solid tumors	NCT02457650	Shenzhen Second People's Hospital (China)
NY-ESO-1 (murine TCR)	Metastatic non-melanoma cancers	NCT01967823	NCI
NY-ESO-1	Solid tumors	NCT02366546	Mie University (Japan)
MAGE-A3 (HLA-DP4)	Esophageal/Melanoma/Urothelial/Cervical/other solid tumors	NCT02111850	NCI
MAGE-A3/A6 (HLA-DPB1*04:01)	Solid tumors	NCT03139370	Kite Pharma
MAGE-A3 (HLA-A*01)	Esophageal, Melanoma/Urothelial/Cervical/other solid tumors	NCT02153905	NCI
MAGE-A4 (HLA-A*24)	Solid tumors	NCT02096614	Japan
MAGE-A4	Urothelial, Melanoma, Head & Neck, Ovarian, NSCLC, Esophageal, Gastric, Synovial sarcoma, Myxoid round cell liposarcoma	NCT03132922	Adaptimmune
MAGE-A10	Urothelial, melanoma, head & neck	NCT02989064	Adaptimmune
MAGE-A10	Non-small cell lung cancer	NCT02592577	Adaptimmune
MART-1	Metastatic melanoma	NCT02654821	The Netherlands Cancer Institute
MART-1 (dendritic cell vaccine + IL-2)	Metastatic melanoma	NCT00910650	Jonsson Cancer Center
WT1	Acute myeloid leukemia	NCT02770820	Fred Hutchinson Cancer Research Center
WT1	Acute myeloid leukemia	NCT02550535	Cell Medica
WT1	Acute myeloid leukemia/Chronic myeloid leukemia	NCT01621724	Cell Medica
HA-1	Acute leukemia post stem cell transplantation	NCT03326921	Fred Hutchinson Cancer Research Center
PRAME	High risk myeloid or lymphoid neoplasms	NCT03503968	Medigene AG
Alpha-fetoprotein	Hepatocellular carcinoma	NCT03132792	Adaptimmune
p53 (dendritic cell vaccine + IL-2)	Kidney, melanoma, other solid tumors	NCT00704938	NCI
HBV (HLA allele not disclosed)	Recurrent hepatocellular carcinoma post- transplant	NCT02719782	Sun Yat-Sen University (China)
HPV-16/18 E6/E7	Relapsed HPV-associated cancers	NCT02379520	Baylor College of Medicine
HPV E6	Vaginal/Cervical/Anal/Penile/Oropharyngeal	NCT02280811	NCI
HPV E6	Vulvar high-grade squamous cancer	NCT03197025	NCI

peptides that in turn need to be presented by tumor cells in the context of specific HLA molecules. Thus, targeting neoantigens requires a personalized approach, which requires a complex process to generate TCR T cells that starts with the identification of the target antigens. Such antigens can be identified by exome sequencing a patient's tumor to isolate cancer mutations followed by synthesis of DNA sequences encoding the mutations within 25-mer peptides flanked by patient's sequences. This strategy covers all possible peptides up to 11 amino acids that could include the mutation. Subsequently, DNA sequences are stitched together into a tandem minigene and introduced into the patients' dendritic cells, which are then tested to assess whether any mutant peptides are expressed on HLA molecules. If so, the peptides (*i.e.* the neoantigens) can be identified by mixing patient's T cells with the dendritic cells and isolating the lymphocytes that react [42]. Then autologous T cells are collected and enriched for those that react against the neoantigen and infused back into the patient. Targeting driver mutations with adoptive T cell therapies is a provocative proposition because such mutations are critical for tumor development and are present in most, if not all, cells within a cancer. Furthermore, it is an approach that could be applicable to malignancies that are largely resistant to TIL therapy such as breast cancer, colorectal carcinoma, or cholangiocarcinoma. Indeed, transfer of TILs containing 25% polyfunctional TH1 cells specific for the ERBB2IP mutation resulted in tumor shrinkage and disease control for 13 months in a patient with metastatic cholangiocarcinoma. Upon progression, the patient was reinfused with a > 95% pure population of mutation-reactive Th1 cells and again experienced tumor regression that was ongoing after 6 months of follow-up [43]. The same group demonstrated the efficacy of the adoptive transfer of neoantigen-reactive T cells in a different tumor type. They identified a polyclonal CD8+ T cell reactive clones against mutant $KRAS^{G12D}$ in TILs obtained from a patient with metastatic colorectal cancer. Infusion of 1.11×10^{11} HLA-C*08:02–restricted TILs induced regression of lung metastases for 9 months, when one of the lesions progressed due to loss of the haplotype encoding for the HLA-C*08:02 class I MHC molecule.

While these examples demonstrate the potential of targeting neoantigens, they also highlight the complexity of developing this highly personalized therapeutic approach, which in its current form it is not scalable. Faster and simpler methods will be required to generate neoantigen-reactive T cells and more enriched populations of the latter targeting more than one neoantigen should be employed. Non-viral vectors (*e.g.* plasmid DNA-based platforms) for the transfer of neoantigen-reactive TCRs into the patient's T cells may accelerate this process and make it cheaper, and therefore more scalable.

7. Next generation platforms that engage the full TCR complex

Study data collected to date are compelling and support the continued development of adoptive T cell therapy for the treatment of cancer. However, success in solid tumors has proven evasive to CAR T cell platforms and the development of TCR T cell approaches have been limited by and large to the targeting of a few cancer testis antigens and has progressed at a very slow pace due to their inherent HLA matching restrictions. Nonetheless, available data indicate that adoptive T cell therapies that engage the entire TCR complex have been the only ones to consistently deliver efficacy in solid tumors. Engineered T cells that are armed with highly specific tumor associated antigen binding domains, that cause little to no-target-off-tumor toxicities, and that combine resilience to the hostile tumor microenvironment with long-term persistence are necessary.

The TCR is a heterodimeric complex comprised of 6 subunits (αTCR, βTCR, CD3ε, CD3γ and CD3δ; Fig. 1; reviewed in [44]). There are numerous tyrosine kinase binding sites located within the cytoplasmic domains of the CD3 proteins. The best defined signal motifs contained within the cytoplasmic domains of the invariant CD3 subunits of the TCR complex include the Immune-receptor-Tyrosine-based-Activation-Motifs (ITAMs) [45]. Upon phosphorylation ITAMs initiate the complex TCR signaling cascade. Unlike CAR constructs, which contain 3 ITAM domains per ζ chain, the integrated TCR has a total of 10 ITAM motifs. Surprisingly, increasing the number of phosphorylated ITAMs in a T cell does not merely amplify the signal but also enhances the signal quality. More precisely, increasing the number of phosphorylated ITAMs results in synchronous signaling across a broad T cell population, which is not observed when few ITAMs are phosphorylated [46]. In addition to activating motifs, the cytoplasmic domains of the TCR CD3 proteins also contain a number of phosphatase domains that provide critical negative feedback signals. Successful T cell activity is the culmination of the complex interplay of these known, and perhaps yet to be discovered, TCR signaling cascades. Together they regulate most T cell activities including activation, survival, differentiation, chemotaxis, metabolic activity, effector function and persistence. Harnessing the TCR to strike the perfect balance in the context of adoptive T cell therapy is a serious challenge. It is not possible, to incorporate the complexity of TCR signaling into a CAR construct. Notwithstanding, successful adoptive T cell therapy in solid tumors has required the involvement of the entire TCR complex. Eliminating the constraints imposed by HLA matching required by current TCR T cell platforms will constitute a major step forward in the applicability of these therapeutic approach to a much larger patient population. A number of novel platform technologies that engage the full TCR complex are emerging, and some of them are in clinical development. One such approach is the ARTEMIS platform, which is being developed by Eureka Therapeutics. Antibody Redirected T Cells with Endogenous Modular Immune Signaling). ARTEMIS T cells replace the endogenous α/βTCR variable and joining regions with domains from tumor specific binders. More precisely, ARTEMIS T cells have a "T cell receptor module" (TCRM) in lieu of the endogenous TCR. The TCRM is comprised of two T cell receptor domains, a chimeric αTCR domain with endogenous variable and joining regions replaced with one half of a tumor specific binding domain and a βTCR domain, with its variable and joining regions replaced with the other half of the binding domain [47]. Together, with other elements of the TCR, these chimeric proteins are thought to dimerize and form a TCR-like structure. Early clinical data from patients treated with T cell generated using the ARTEMIS platform are limited. Whilst no significant safety issues were reported and some T cell expansion upon infusion has been described, this was not observed to correlate with clinical activity [48]. Further follow up is necessary to determine the long-term potential of the ARTEMIS platform. However, a number of questions surrounding this approach remain, the most important of which being whether the expression of the tumor binding domain on TCR components devoid of intracellular signaling domains will result in an adequate T cell response.

Data recently generated from another emerging platform, referred to as T cell receptor fusion constructs (TRuCs), suggest that constructs engineered by fusing antibody-based binders to the invariant CD3 proteins in the TCR, and not the α or β TCR may provide enhanced potency. A critical advantage of this approach is that T cells generated from the TRuC platform (Fig. 1) target tumors in an HLA non-restricted fashion. Upon lentiviral transduction, the T cell naturally integrates TRuC constructs into the native TCR complex which is expressed on the cell surface, thus harnessing the full potential of T cell activation and effector function. Recent data show that TRuC T cells can effectively be reprogrammed for cancer therapy by introducing a construct that consists of an anti-tumor binding domain that is tethered to its TCRα, TCRβ CD3γ, CD3δ or CD3ε subunit. In these studies, the expression of the TRuC construct was dependent on the other TCR elements, with primary signaling events consistent with activation of the entire TCR, evident by phosphorylation of endogenous CD3ζ as well as CD3ε [49,50]. Whilst efficacy was observed using constructs derived from any of the TCR constituents, the most active constructs are those involving a binding domain tethered to the CD3ε protein. The precise reason for this is unknown, but maybe associated with TCR stoichiometry, with there

being two CD3ε chains within each TCR complex, *versus* one for the other TCR proteins. Using the CD3ε TRuC approach, binders to a number of hematological and solid tumor targets have been created and described to have good efficacy in preclinical models [49,50]. It is noteworthy that when compared to second generation CAR T cells (either 4-1BB or CD28) expressing an identical binder, TRuC-T cells released significantly less cytokines *in vitro* and in *in vivo* mouse models of solid and hematological malignancy while improving the efficacy of their CAR T cell counterparts.

The T cell antigen coupler (TAC) T cell is yet another platform to redirect T cells in a TCR-dependent, HLA-independent manner. The TAC chimeric proteins represent an alternative synthetic receptor which couples the TCR to antigen by linking a membrane-bound anti-CD3ε scFV to a second tumor-targeting domain [51]. This approach could be likened to cellular extension of the BiTE concept. TAC-engineered T cells displayed both enhanced *in vivo* anti-tumor efficacy and decreased off-tumor toxicity compared to first- and second-generation CARs in HER2-positive solid tumor mouse models [51].

These novel engineered T cell platforms are highly versatile and suggest that the necessary addition of costimulatory domains for second-generation CAR-T cell efficacy relates to the inability of CARs to properly utilize the endogenous T cell signaling circuitry. Unlike TCR-T cells, these novel platforms are not limited by HLA restriction as they utilize binding domains similar to those employed by CAR-T cells, but unlike the latter, they harness the endogenous TCR machinery for signaling purposes. Clinical trials will determine the potential of these novel approaches, but early data are encouraging.

8. Conclusions

CAR T cell therapies have been very successful for the treatment of hematological malignancies, (particularly those expressing either CD19 or BCMA) and two of them, tisagenlecleucel and axi-cel, have been approved by regulatory authorities. However, such level of success has not been replicated in solid tumors. Emerging data indicate that T cells engineered to express synthetic TCRs are promising therapeutics for the management of patients with solid tumors. While proof of concept has been achieved in a number of cancers, several obstacles will have to be solved in order to make these therapies widely available to broader number of patients. Some of these challenges include ameliorating the HLA restriction that penalizes a large proportion of patients not expressing the HLA against which the TCR T cells has been designed, a barrier potentially being overcome with next generation TCR platforms such as ARTEMIS, TRuC, and TAC platforms. Other elements that require addressing include improving the efficacy of TCR T cells in the context of immunosuppressive microenvironments, identifying novel targets whose expression is largely (if not totally) restricted to cancer cells, and improving manufacturing processes and supply chain logistics to decrease costs and maximize patient access.

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