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Unleash the power of the mighty T cells-basis of adoptive cellular therapy

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ABSTRACT

Adoptive cellular therapy (ACT) is an immunotherapy which involves the passive transfer of lymphocytes into a lymphodepleted host after ex vivo stimulation and expansion. Tumor-infiltrating lymphocytes (TILs) have shown objective tumor responses mainly restricted to melanoma and rely on a laborious manufacturing process. These limitations led to emergence of engineered cells, where normal peripheral blood lymphocytes are modified to express T cell receptors (TCRs) or chimeric antigen receptors (CARs) specific for tumor-associated antigens (TAAs). To date, CD19-targeted chimeric antigen receptor T (CAR T) cells have been the most extensively studied, showing complete and durable responses in B-cell malignancies. Antitumor responses with engineered T cells have often been accompanied by undesired toxicities in clinical trials including cytokine release syndrome (CRS) and neurotoxicity. In this review, we provide an overview of adoptive cellular strategies, early and ongoing clinical trials, adverse events and strategies to mitigate side effects and overcome limitations.

1. Introduction

Despite breakthrough in advanced cancer treatment, cancer-related mortality remains high. In recent years, cancer immunotherapy has emerged as the fifth pillar of cancer therapy alongside surgery, chemotherapy,radiotherapy and targeted therapy. Current immunotherapeutic strategies are designed to overcome the limitations of host immune responses and include cancer vaccines, checkpoint inhibitors and adoptive cellular therapy. Adoptive cellular therapy (ACT) is based on the transfer of lymphocytes into the human tumor host after their ex vivo stimulation and expansion. ACT will be the focus of this review.

2. History of cellular therapy

The notion of injecting cellular material into a human body dates to 1889, when Brown-Séquard injected himself with animal-derived testicular extracts in attempts for rejuvenation (Brown-Séquard, 1889). In the 1930s, Paul Niehans performed intramuscular injections of live cells harvested from different species including cow and sheep embryos (Stambler, 2014). In 1968, the first successful non-twin human bone marrow transplantation (BMT) was performed in Minnesota and this remains an integral part of hematologic malignancy treatment at present. The success of this application has fueled stem cell and cellular therapy research in the last few decades giving birth to ACT.

Abbreviations: Anti-CEA, anti-carcinoembryonic antigen; APCs, antigen-presenting cells; Axi-cel, axicabtagene ciloleucel; B-ALL, B-cell acute lymphoblastic leukemia; BiTE, bispecific T cell engager; BMT, bone marrow transplantation; CAIX, carboxy-anhydrase IX; CAR, chimeric antigen receptor; CIK, cytokine-induced killer; CLL, chronic lymphocytic leukemia; CR, complete remission; CRP, C-reactive protein; CRS, cytokine release syndrome; CTL, cytotoxic T lymphocyte; CTLA-4, cytotoxic T-lymphocyte associated protein-4; DLBCL, diffuse large B-cell lymphoma; DLI, donor lymphocyte infusion; FL, follicular lymphoma; GM-CSF, granulocyte-macrophage colony-stimulating factor; GVHD, graft-versus-host disease; GVL, graft versus leukemia; HLA, human leukocyte antigen; HPSCT, hematopoietic stem cell transplantation; HSCs, hematopoietic stem cells; HSV-TK, herpes simplex virus thymidine kinase; iC9, inducible caspase-9; iCARs, inhibitory CARs; IL, interleukin; IL-6R, interleukin-6 receptor; INF, interferon; LDH, lactate dehydrogenase; MAS, macrophage activation syndrome; MCL, mantle cell lymphoma; MDSCs, myeloid-derived suppressor cells; MHC, major histocompatibility complex; MRD, minimal residual disease; NHL, non-Hodgkin's lymphoma; NK, natural killer; NMA, non-myeloablative; ORR, objective response rate; OS, overall survival; PD-1, programmed cell death-1; PR, partial response; R/R, relapsed/refractory; RCC, renal cell carcinoma; REMS, Risk Evaluation and Mitigation Strategy; ScFv, single chain variable segment; synNotch, synthetic Notch; T regs, regulatory T cells; TAA, tumor-associated antigen; TBI, total-body irradiation; Tcm, central memory T cells; TCR, T cell receptor; Teff, effector T cells; Tem, effector memory T cells; TIL, tumor-infiltrating lymphocyte; Tn, naïve T lymphocytes; TNF, tumor necrosis factor; Tscm, stem cell memory

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3. Cancer immunity and immunotherapy

The development of cancer immunotherapy is based on the central role that the adaptive immune system plays in antitumor immunity. In 2002, the "cancer immunoediting" model was introduced by Dunn et al, which is a process composed of three stages: elimination, equilibrium and escape (Dunn et al., 2002). The elimination phase involves the destruction of neoplastic cells by effector cells. The equilibrium phase refers to an equilibrium state between the immune system and tumor cells which escaped elimination, where selection pressure is exerted leading to emergence of mutations and immune escape. These cells proliferate to form tumors in the escape phase (Dunn et al., 2002). At the cellular level, the steps leading to immune destruction are described by the "cancer immunity cycle" by (Chen and Mellman (2013)). Tumor antigens released into the microenvironment are picked by antigen-presenting cells (APCs) and presented on major histocompatibility complex (MHC) molecules after intracellular processing. APCs traffic to lymph nodes and prime tumor-specific T cells, which differentiate into effector T cells. Effector cells migrate to the tumor bed and bind to tumor antigens specifically, leading to tumor cell destruction and release of new antigens thus perpetuating the cycle (Chen and Mellman (2013)). In ideal conditions, these steps are expected to result in tumor eradication. However, there are several tumor and host-driven factors which enable immune escape. These include immunosuppressive cells like regulatory T cells (T regs) and myeloid-derived suppressor cells (MDSCs), expression of inhibitory molecules and release of inhibitory cytokines, defective antigen processing or presentation and T cell exhaustion (Vinay et al., 2015). In addition, central tolerance mechanisms cause deletion of high affinity antitumor T cells, leaving behind lower affinity T cells. This understanding has fueled the development of immunotherapeutic approaches targeting different steps of the immune cycle. Among the earliest forms were bacterial injections by William Cooley (Nauts et al., 1946). More recent forms include the intravesical Bacillus Calmette–Guérin (BCG) injection for bladder cancer (Morales et al., 1976), immune-stimulatory cytokines like interleukin (IL)-2 (Yang et al., 2003; Atkins et al., 1999), interferon (INF)-α (Fossa, 2000), and IL-15 (Conlon et al., 2015) for metastatic renal cell carcinoma and melanoma, dendritic cell vaccines, among which Sipuleucel-T is approved for prostate cancer (Tagliamonte et al., 2014), and checkpoint inhibitors. Finally, a new era of cancer immunotherapy started with use of specific antitumor T cells which are injected directly into the tumor host.

4. Adoptive cellular therapy

4.1. Introduction to adoptive cellular therapy

ACT relies on the intravenous infusion of lymphocytes into a human host, after their stimulation and expansion in vitro, to achieve an antitumor effect. While dendritic cell-based vaccine rely on triggering endogenous host defenses, ACT involves the passive transfer of antigen-specific effector cells. Allogeneic hematopoietic stem cell transplantation (HPSCT) and donor lymphocyte infusions (DLIs) are among the earliest applications of ACT, where infused hematopoietic stem cells (HSCs) and mature donor lymphocytes mediate a graft versus leukemia (GVL) effect (Weiden et al., 1979; Porter et al., 1999). However, both forms lack tumor-antigen specificity and are associated with graft-versus-host disease (GVHD). ACT currently encompasses a wide range of approaches mainly in the form of cytokine-induced killer (CIK) cells, tumor-infiltrating lymphocytes (TILs), T cell receptors (TCRs) and chimeric antigen receptor (CAR) T cells (Fig. 1A). TILs are naturally occurring intratumor T lymphocytes isolated from resected tumor fragments and reinfused into the host, along with IL-2, after pretreatment with a lymphodepleting regimen. Alternatively, peripheral blood mononuclear cells (PBMCs) can be engineered to express antigen-specific TCRs or CARs. The CAR construct is a hybrid molecule composed of an immunoglobulin-derived extracellular domain and TCR-derived intracellular domain (Feldman et al., 2015).

4.2. Effector cells for adoptive cellular therapy

ACT approaches differ in the effector cells employed, and include CD4 + T cells, CD8 + T cells (Hunder et al., 2008; Mackensen et al., 2006; Yee et al., 2002), CIK cells and natural killer (NK) cells. The differentiation state of engineered T cells has been shown to influence antitumor responses. Resting T cells exist in several forms including naïve (Tn), stem cell memory (Tscm), central memory (Tcm) and effector memory T (Tem) cells. These subsets proliferate and differentiate into effector T (Teff) cells upon stimulation (Hinrichs et al., 2009; Chang et al., 2014). Use of the less differentiated Tn, Tscm and Tcm cells, has shown superior outcomes in vivo through enhanced proliferation, delayed senescence and prolonged cytokine production (Bonini and Mondino, 2015). These observations led to efforts in manipulating molecular pathways, mainly PI3K-Akt-mTOR and wnt-β catenin pathways to generate these less differentiated forms (Kim et al., 2012; Gattinoni et al., 2010).

4.3. Antigen targets in adoptive cellular therapy

Many tumor-associated antigens (TAAs) have been identified as targets for ACT. In general, target antigens include non-mutated self-proteins which are overexpressed on tumors, cancer testis (CT) antigens, and mutated proteins selectively expressed on tumors. CT antigens are restricted to germ line cells and trophoblastic tissue in adults. CT genes have been classified into 3 categories: testis-restricted, testis/brain-restricted, and testis-selective which are expressed on normal adult tissue as well (Hofmann et al., 2008). CT antigen expression has been found in many tumors including melanoma, synovial cell sarcoma, lung, hepatocellular, germ cell and gastric cancers (Hofmann et al., 2008). Their restriction to immune-privileged sites makes them attractive targets in ACT. NY-ESO-1 and MAGE-A are two examples of testis-restricted antigens. Mutated gene products in melanoma have also been found to be targets for TILs. In addition, cancer-associated viruses like EBV and HPV can serve as targets for engineered T cells (Houot et al., 2015). To date, CD19, a surface molecule expressed on precursor and mature B cells, has been the most extensively studied target for B-cell malignancies. Its expression on normal B cells accounts for B-cell aplasia as a toxicity of CD19-CAR T cells observed in clinical trials (Park et al., 2016).

5. Cytokine-induced killer (CIK) cells

CIK cells were first introduced in 1991 by (Schmidt-Wolf et al. (1991)). These are CD3 + CD56 + cytotoxic effector cells derived from in vitro culturing of PBMCs with INF-γ, an anti-CD3 monoclonal antibody (OKT-3) and human recombinant IL-2 (Introna et al., 2006). They represent a distinct population of cells with mixed T cell and NK cell phenotypes, possessing both antigen-specific binding and NK-like cytotoxicity. They have several advantages including, ease of preparation, non-MHC restricted killing, direct tumor homing, and sparing of normal tissue (Introna, 2017). CIK cells have shown objective tumor responses in both hematological and solid tumors with minimal side effects (Schmeel et al., 2015). Clinical trials using CIK, as monotherapy or in combination with other immunotherapies, are ongoing to further delineate their efficacy (Supplementary Table 1).

6. Tumor-infiltrating lymphocytes (TILs)

6.1. Tumor-infiltrating lymphocytes therapy

TILs are lymphocytes, including B and T lymphocytes, found in the tumor microenvironment and exert antitumor activity. T cells express

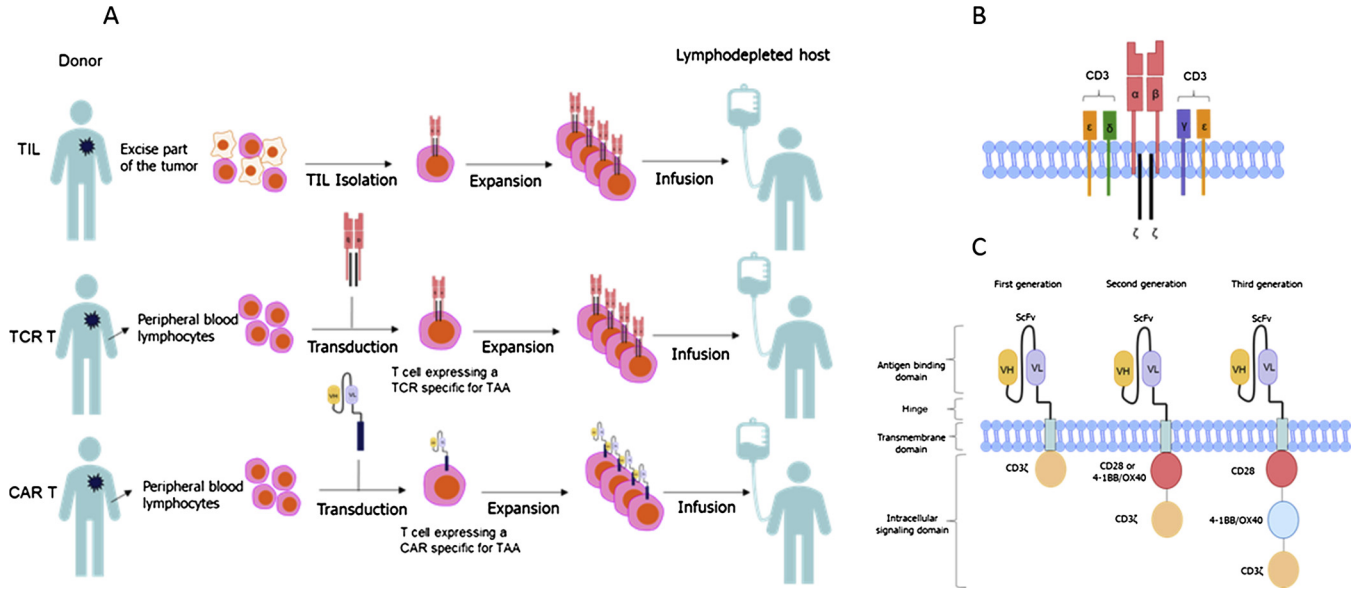

Fig. 1. A:Adoptive cellular therapy strategies: The main ACT approaches are TIL, TCR T and CAR T cells. TIL: TILs are isolated from tumor fragments, then expanded in vitro and re-infused into a tumor host after lymphodepletion. TCR T and CAR T cells: lymphocytes are isolated from the patient's peripheral blood, and genetically engineered to express a tumor-specific TCR or CAR using viral or non-viral transduction. TCR/CAR T cells are then expanded in vitro and infused into a tumor host after lymphodepletion. Abbreviations: ACT: adoptive cellular therapy, TIL: Tumor-infiltrating lymphocytes, TCR: T-cell receptor, CAR: Chimeric antigen receptor. B:T-cell receptor complex: The TCR complex is made of TCR alpha and beta chains, zeta chain accessory molecules, and CD3γ chain, CD3δ chain, and two CD3ε chains. Abbreviation: TCR: T cell receptor. CD3: cluster of differentiation 3. C:CAR T cell generations: CAR generations are distinguished by their intracellular signaling domains. First-generation CARs are made of a TCR-derived CD3ζ intracellular signaling domain. Second-generations CARs are composed of CD3 and one costimulatory domain like CD28, 4-1BB (CD137) or OX40 (CD134). Third-generation CARS are composed of CD3 and 2 costimulatory domains. Abbreviations: CAR: chimeric antigen receptor. CD3: cluster of differentiation 3.

tumor antigen-specific TCRs and exert cytotoxic activity through interactions between the TCR and tumor antigens bound to MHC class I. TILs are often "exhausted" or functionally impaired due to chronic antigenic stimulation. Therefore, techniques were developed to reactivate them. This was pioneered by Rosenberg et al. in 1988 at the National Cancer Institute (NCI) surgery branch, where autologous TIL were isolated from resected tumor fragments, expanded several weeks in culture and re-infused, in combination with IL-2, in 20 patients with metastatic melanoma after pretreatment with cyclophosphamide. Tumor regression was observed in 40–60% of cases and lasted from 2 months to more than 13 months (Rosenberg et al., 1988). Subsequent trials manipulated the host environment through pretreatment with lymphodepleting regimens. In a study by Dudley et al., lymphodepletion with cyclophosphamide and fludarabine prior to TIL transfer led to objective clinical responses in 18 of 35 (51%) patients with metastatic melanoma, including 3 complete responses. Treatment resulted in autoimmune attack on normal melanocytes, manifesting as vitiligo and anterior uveitis in 17 patients (Dudley et al., 2005). This was followed by 2 sequential phase II clinical trials showing that addition of total-body irradiation (TBI) was superior to non-myeloablative (NMA) chemotherapy alone. The objective response rate (ORR) increased from 49% with NMA chemotherapy with cyclophosphamide and fludarabine, to 52% and 72% with addition of 2 and 12 Gy of TBI respectively (Dudley et al., 2008). Of the 93 patients treated, 20 had complete tumor regression (22%), ongoing beyond 3 years in 19 of them (Rosenberg et al., 2011). Clinical response strongly correlated with increased telomere length of infused TILs, which reflected higher replicative potential. In another study of TILs in patients with metastatic melanoma, no differences in telomere length were found between responders and non-responders. However, clinical response was associated with higher infused TILs, increased number and percentage of CD8 + T cells in the infused product, and Teff and BTLA phenotypes (Radvanyi et al., 2012). Enhanced responses seen with intensifying preparative regimens has been explained by several factors including: 1) Decreased levels of host immune suppressive cells like Tregs (Yao et al., 2012), 2) increased availability of homeostatic cytokines, by depleting endogenous lymphocytes which otherwise act as cytokine sinks (Dudley et al., 2008), and 3) activation of the innate immune system which augments adoptively transferred T cell activity. In attempts to simplify the TIL preparation and decrease culture time, a minimally manipulated, CD8+ enriched "young TIL" product was studied. This product was made of bulk lymphocytes unselected for tumor reactivity. Objective tumor regression was seen in 48% to 58% of patients. Table 1 shows ongoing clinical trials utilizing TILs. TILs have mostly been studied in melanoma patients (NCT 00513604, NCT 01369875, NCT 01814046, NCT 01,005,745, NCT 00096382, NCT 00314106, NCT 0118091, NCT 01,701,674). A more comprehensive list of ongoing TIL studies are found in Supplementary Table 2.

6.2. Obstacles to TIL therapy

This approach is limited by several tumor and host related factors. It requires the presence of an immunogenic tumor which is amenable for resection and a suitable host able to tolerate lymphodepletion and withstand the delay associated with TIL preparation. In addition, the process is expensive, laborious, and relies on specialized laboratories. Most importantly, this approach requires the presence of preexisting TIL in the tumor microenvironment (Ruella and Kalos, 2014). Although TILs have been cultured from several tumors, only those derived from melanoma specimens have displayed cytolytic activity (Yannelli et al., 1996). This likely explains why the success of TILs has been mostly restricted to melanoma. Furthermore, endogenous antitumor T cells are usually of lower affinities, due to thymic deletion of higher affinity cells (Oates and Jakobsen, 2013).

7. Engineered t cells: TCRs and CAR T cells

The limitations of TILs have led to the introduction of engineered T cells, where normal PBMCs are genetically modified to express TCRs or CARs specific for TAAs. Transduction, the process by which foreign

Table 1
Ongoing trials for cytokine induced killer (CIK) cells. Abbreviations: CIK: cytokine-induced killer, NK: natural killer, MDS: myelodysplastic syndrome, AML: acute myeloid leukemia, HPSCT: hematopoietic stem cell transplant, DLT: dose-limiting toxicity, CBR: clinical benefit rate, ORR: objective response rate, OS: overall survival, PFS: progression-free survival, TTR: time to response, pts: patients, GVHD: graft-versus-host disease, EFS: event-free survival, FFP: free from progression, RR: response rate, MTD: maximum tolerated dose, IWG: international working group, DoR: duration of response, TTP: time to progression, DFS: disease-free survival, CR: complete remission.

NCT#	Phase	Intervention	Condition	Outcome Measures	Number to be enrolled
03360708	1	Vaccine and CIK	Glioblastoma	DLT, CBR, DoR, feasibility, ORR, OS, PFS, TTR	20
01392989	2	Post-transplant allogenic CIK	MDS, Myeloproliferative disorders	Proportion of pts achieving full donor T cell chimerism by day 90, OS, acute GVHD, EFS	44
00477035	1	Post-transplant auto CIK	Leukemia, Myeloma	Toxicities, FFP, EFS, OS, RR	22
01898793	1 and 2	Cytokine-induced memory-like NK cells	AML, MDS	MTD, CR, IWG RR, DoR, TTP, DFS, OS, toxicities	133
03068819	1	Cytokine-induced memory-like NK cells	Acute AML in children, relapsed post-transplant	Feasibility, safety, GVHD, CR, DFS, OS	24
02782546	2	Cytokine-induced memory-like NK cells	AML post haplo HPSCT	DFS, OS, incidence of relapse in CR pts	60

DNA is introduced into a cell, includes viral and non-viral based strategies. Gamma retroviruses and lentiviruses are the main viruses used for transduction, with lentiviral vectors having the added capacity of integrating into non-dividing cells and delivering larger DNA sequences (Kalos and June, 2013). Foamy viral vectors were also introduced as an attractive approach with enhanced integration capacity and lack of pathogenicity in humans (Williams, 2008). On the other hand, non-viral approaches include transposons such as sleeping beauty and piggyBac, and RNA electroporation (Zhao et al., 2010).

7.1. T cell receptors (TCRs)

TCRs are T cells engineered to express a tumor antigen-specific T cell receptor (Fig. 1B). The TCR gene is usually cloned from TAA-specific TCR genes found in TILs or PBMC or cloned from TAA-specific TCR genes generated by immunizing human leukocyte antigen (HLA)-I/II transgenic mice. These genes are then transduced into PBMCs, which are expanded in vitro and infused into a cancer host pretreated with a lymphodepleting regimen (Feldman et al., 2015). TCRs bind to antigens in an MHC-restricted manner, and thus require intact antigen processing mechanisms. However, this allows the targeting of intracellular antigens. One of the earliest reports on the use of TCRs was provided by Morgan et al (Morgan et al., 2006). Engineered T cells expressing a TCR targeting MART-1 antigen, were generated by cloning genes from TILs of melanoma patients and transducing them into autologous PBMCs using a retroviral vector. Adoptive transfer of this product into 15 HLA-A*0201 lymphodepleted patients with melanoma resulted in objective tumor regression in 2 patients, with circulating cells persisting 1 year after infusion (Morgan et al., 2006). In another study, greater responses were achieved by using higher avidity TCRs. 36 patients with metastatic melanoma were treated with either human TCRs targeting the MART-1:27-35 epitope (20 patients) or TCRs targeting the gp100:154-162 epitope generated by immunizing HLA-A2 transgenic mice (16 patients). Cancer regression was seen in 30% and 19% of cases respectively. Rates of ocular toxicity and ototoxicity were higher than those seen with TILs (41.7% vs 1.1–6.5%), hinting that these antigens may not be the main targets for TIL based therapies (Johnson et al., 2009). TCRs specific for TAAs in other tumors have also been studied. In a report by Parkhurst et al, 3 patients with refractory metastatic colorectal cancer were treated with autologous lymphocytes expressing murine anti-carcinoembryonic antigen (anti-CEA) TCRs (Parkhurst et al., 2011). Only 1 patient had tumor regression and all 3 patients experienced a dose-limiting severe transient inflammatory colitis (Parkhurst et al., 2011). In both these studies, toxicities related to shared antigen expression on normal tissue was seen, highlighting the need to target antigens not found on normal tissue or restricted to non-vital organs. This makes CT antigens attractive targets. In a study by Robbins et al, TCRs targeting the CT antigen NY-ESO6 yielded objective clinical responses in 11 of 18 patients with metastatic synovial cell sarcoma and in 11 of 20 melanoma patients. No TCR-related toxicities were seen (Robbins et al., 2015). Results of early phase studies on TCRs have just started to come out. A phase I/II study which enrolled patients with HPV-16 positive (oropharyngeal, vaginal cervical, anal and penile) cancers utilizing TCR targeting HPV-16 E6 has reported an ORR of 17% (NCT 02,280,811). Table 2 shows select ongoing clinical trials utilizing TCR. A more comprehensive list of ongoing TCR studies are found in Supplementary Table 3.

7.2. Chimeric antigen receptor (CAR) T cells

CAR T cells are T lymphocytes engineered to express a CAR which targets a tumor antigen specifically. The concept of CARs was first introduced in 1989, where a chimeric-T cell receptor was generated by fusion of the TCR constant domain and an antibody's variable domain (Gross et al., 1989). CAR T cells are composed of an antigen-binding extracellular domain which is derived from a single chain variable

Table 2
Ongoing trials for tumor-infiltrating lymphocytes (TILs). Abbreviations: TIL: tumor-infiltrating lymphocyte, CP: cyclophosphamide, fludara: fludarabine, IL-2: interleukin-2, pembro: pembrolizumab, RT: radiation therapy, ipi: ipilimumab, ACT: adoptive cellular therapy, TBI: total brain irradiation, Nivo: nivolumab, durva: durvalumab, NSCLC: non-small cell lung cancer, HNSCC: head and neck squamous cell carcinoma, HCC: hepatocellular carcinoma, AE: adverse events, RR: response rate, OS: overall survival, ORR: objective response rate, CRR: continuous complete remission, DoR: duration of response.

NCT#	Phase	Intervention	Condition	Outcome Measures	Number to be enrolled
Solid tumors					
01369875*	2	TIL	Melanoma	Clinical tumor regression, AE	2
01814046*	2	TIL	Ocular melanoma	ORR, AE	24
00091104	1	CP + fludara followed by vaccine, gene-modified WBC, aldesleukin	Melanoma	Safety, tumor regression, in vivo survival of transplanted cells, clinical response	136 (NCI)
02621021	2	TIL + IL-2 either alone or following pembro	Melanoma	RR, AE, OS	170 (NIH)
01,005,745*	NA	Lymphodepletion + ACT w high dose IL-2	Melanoma	TIL growth, ORR	19
00096382*	2	Aldesleukin, filgrastim, TIL, CP, fludara, RT	Melanoma	Clinical tumor regression, safety	34
00314106*	2	Chemotherapy, RT, cell infusion and IL-2	Melanoma	CRR, AE	26
01118091*	2	Aldesleukin, CD8+ young TIL	Melanoma	RR, PFS, toxicity	12
01,701,674*	NA	Ipi, lymphodepletion, ACT, high dose IL-2	Melanoma	DLT, feasibility, ORR, PFS	13
00513604*	2	Aldesleukin, autologous lymphocytes, CP, fludara, TBI	Melanoma	Clinical response, toxicity	158 (NIH)
03215810	1	Nivo and TIL	NSCLC	DLT, ORR	18
03419559	2	LN-145, autologous TIL +/- durva	NSCLC	ORR, AE, DoR	24
03083873	2	LN-145, autologous TIL	HNSCC	ORR, AE, CRR	47
01585428*	2	TIL for HPV cancers	Oropharyngeal, cervical, vaginal, anal, penile cancer	ORR, AE	29
01174121	2	TIL for metastatic cancer	Colorectal, gastric, pancreatic, HCC, glioblastoma	Tumor regression rate, AE, safety and efficacy of pembro following TIL	332 (NIH)

segment (ScFv) of an immunoglobulin, a hinge or spacer domain, a transmembrane domain and an intracellular domain. The composition of the intracellular domain distinguishes the different CAR generations (Fig. 1C). First-generation CARs include the TCR-derived CD3ζ intracellular signaling domain only. To amplify in vivo antitumor responses, the intracellular domain was modified to include one costimulatory domain in second-generation CARs, mainly CD28 (Savoldo et al., 2011), 4-1BB (CD137) (Song et al., 2011), or OX40 (CD134) (Pule et al., 2005), and 2 costimulatory domains in third-generation CARs (Li and Zhao, 2017; Till et al., 2012). Fourth-generation CARs also incorporate a promotor that leads to cytokine production upon CAR T cell activation (Wang et al., 2014). In addition to T cells, NK cells have also been engineered to express CARs. Unlike TILs and TCRs, CAR T cells recognize antigens independent of MHC presentation, bypassing the need for HLA matching. In addition, this enables them to overcome tumor resistance mechanisms such as MHC downregulation and defective antigen processing (Ruella and Kalos, 2014). CAR T cell targets can be proteins, carbohydrates or gangliosides. Unlike TCRs, CAR T cells only target cell-surface antigens. Although their antibody-derived extracellular domains protects them from central tolerance mechanisms, these same domains may subject them to host immune responses (Kalos and June, 2013).

7.2.1. CAR T cell therapy for hematologic malignancies

One of the earliest reports on the safety of CAR T cells in hematologic malignancies was provided in a proof of concept trial by Till et al. in 2008 (Till et al., 2008). First-generation CD20-specific CAR T cells were generated by transfection of PBMCs with a CAR-encoding DNA plasmid. This product was transferred with IL-2 to 7 patients with relapsed/refractory (R/R) B-cell non-Hodgkin's lymphoma (NHL) and mantle cell lymphoma (MCL). One of five evaluable patients had partial responses (PRs) lasting 3 months. Other than IL-2 related grade 1–2 toxicities, no toxicities related to the infusions were seen (Till et al., 2008). The same group subsequently tested a third-generation construct targeting CD20. 1 of 3 treated lymphoma patients had evaluable disease and had PR lasting 12 months. Treatment was well tolerated (Till et al., 2012). To date, the most promising results have been with anti-CD19 CAR T cells in B-cell malignancies. The earliest reports were by Kochenderfer et al., where dramatic partial remission lasting 32 weeks was achieved in a patient with advanced stage follicular lymphoma (FL) pretreated with cyclophosphamide and fludarabine (Kochenderfer et al., 2010). A subsequent study by Kochenderfer et al showed efficacy of anti-CD19 CAR T cells in diffuse large B-cell lymphoma (DLBCL). 15 Patients with B-cell malignancies received a conditioning chemotherapy regimen of cyclophosphamide and fludarabine followed by a single infusion of anti-CD19 CAR T cells. 8 patients achieved complete remissions (CRs) and 4 achieved PRs. CRs were obtained by 4 of 7 evaluable patients with chemotherapy-refractory DLBCL and the responses persisted 9–22 months (Kochenderfer et al., 2015). Anti-CD19 CAR T cell therapy also showed promising results in chronic lymphocytic leukemia (CLL) (Kalos et al., 2011; Porter et al., 2015). The success of anti-CD19 CAR T cell therapy was extrapolated to B-cell acute lymphoblastic leukemia (B-ALL). In a study by Brentjens et al, 5 patients with relapsed B-ALL with morphologic disease or minimal residual disease (MRD), were infused with second-generation anti-CD19 CAR T cells following cyclophosphamide conditioning. All patients achieved MRD-negative CRs. Eligible patients underwent transplant, thus hindering evaluation of response duration. Cytokine-induced toxicity was associated with tumor burden upon infusion (Brentjens et al., 2013). In another study, Maude et al evaluated a CD19-directed CAR T cell (CTL019) generated using a lentiviral vector. This study also showed unprecedented promising results. CR was attained in 90% (27 patients) with an overall survival (OS) of 78% in 30 pediatric and adult patients with heavily pretreated relapsed or refractory acute lymphoblastic leukemia (ALL). All patients developed cytokine release syndrome (CRS), which correlated with tumor burden. Severe CRS (27% of the patients) was managed effectively with tocilizumab, a humanized monoclonal antibody against the interleukin-6 receptor (IL-6R) (Maude et al., 2014). Subsequently, results from the multicenter, single-arm phase II ELIANA trial were reported. This was the first pediatric and young adults' global CAR T cell therapy registration trial examining anti-CD19 CAR Ts with CD3ζ and 4-1BB domains (tisagenlecleucel). In this study, 68 patients were infused and 63 were evaluable for efficacy. Results showed CR of 83%. 49% of patients experienced grade 3 or 4 CRS. Therefore, tisagenlecleucel was granted FDA approval for relapsed or refractory B-cell precursor ALL and DLBCL through the Risk Evaluation and Mitigation Strategy (REMS), only at facilities that have achieved special certification with staff trained to recognize and manage adverse events and with immediate availability of tocilizumab (NCT 02,228,096) (KYMRIAH, 2019). An updated analysis of the

Table 3
Ongoing trials for T cell receptor T cells (TCR T cells). Abbreviations: TCR: T cell receptor, CP: cyclophosphamide, fludara: fludarabine, mTCR: monoclonal T cell receptor, CT: cancer testis, PBL: peripheral blood lymphocyte, HLA: human leukocyte antigen, Tcm lymphocytes: central memory T lymphocytes, Tn lymphocytes: naïve T lymphocytes, pembro: pembrolizumab, NSCLC: non-small cell lung cancer, TIL: tumor-infiltrating lymphocyte, AE: adverse events, RR: response rate, MTD: maximum tolerated dose, DoR: duration of response, DLT: dose-limiting toxicity, DFS: disease-free survival, OS: overall survival, RP2D: recommended Phase II Dose.

NCT#	Phase	Intervention	Condition	Outcome Measures	Number to be enrolled
Solid tumors					
02133196	2	TCR, aldesleukin, CP, fludara	NSCLC	Tumor regression rate, TIL characteristics, AE, feasibility	85
00509288*	2	Lymphodepletion + anti-MART-1 F5 TCR gene-engineered lymphocytes	Melanoma	Tumor regression, toxicity	24
03190941	1 and 2	CP, fludara, aldesleukin, Anti-KRAS G12 V mTCR	Pancreatic, gastric, gastrointestinal, colon, rectal cancer	RR, MTD, in vivo survival of mTCR gene engineered cells	110
02858310	1 and 2	E7 TCR, aldesleukin, CP, fludara	Vulvar cancer	Safety, efficacy	180
02,280,811*	1 and 2	TCR targeting HPV-16 E6	Oropharyngeal, vaginal, cervical, anal, penile cancer	MTD, RR, DoR, DLT	12
01343043	1 and 2	CT antigen TCR-redirected T cells (NY-ESO-1c259 T cells)	Synovial sarcoma	RR, DLT, persistence of genetically modified T cells	65
00393029*	2	Anti-p53 TCR-gene engineered lymphocytes	Metastatic cancers that overexpress p53	Tumor regression, AE, in vivo survival of TCR gene-engineered cells	12
03412877	2	CP, fludara, aldesleukin, individual patient TCR-transduced PBL	Glioblastoma, NSCLC, ovarian, breast, gastrointestinal, genitourinary cancer	RR	210
02153905	1 and 2	Anti-MAGE-A3 HLAA*01-restricted TCR, aldesleukin, CP, fludara	Breast, cervical, renal, bladder cancer, melanoma	MTD, RR, AE, engineered cell survival	102
02111850	2	Anti-MAGE-A3 DP4 TCR, aldesleukin, CP, fludara	Cervical, renal, urothelial, breast cancer, melanoma	AE, RR	107
Hematologic Malignancies					
02770820	1 and 2	Auto-WT1-TCRc4 gene-transduced CD8 + Tcm/Tn lymphocytes, aldesleukin	AML	Toxicity, decrease in disease burden, DFS, OS	35
03326921	1	CD8+ and CD4+ donor memory T cells expressing HA1-specific TCR	Relapsed or refractory acute leukemia after donor SCT	Feasibility, DLT, reduction of leukemia	24
02030834	2	Redirected auto T cells engineered to contain anti-CD19 attached to TCRz and 4 signaling domains	NHL	AE	63
02776813	1	ACTR087 (antibody-coupled TCR)	Relapsed/Refractory B-cell lymphoma	DLT, MTD, RP2D, safety, ORR, DoR, PFS, OS	54
03168438	1	CT antigen TCR T cells (NY-ESO-1c259 T cells), pembro	Myeloma	AE, response	20

Table 4
Ongoing trials for chimeric antigen receptor T cells (CAR T cells). Abbreviations: CAR: chimeric antigen receptor, CP: cyclophosphamide, fludara: fludarabine, PBL: peripheral-blood lymphocyte, EGFRt: truncated epidermal growth factor receptor, pts: patients, allo: allogeneic, SCT: stem cell transplant, auto: autologous, Tcm lymphocytes: central memory T lymphocytes, durva: durvalumab, BCMA: B-cell maturation antigen, CTL019: tisagenlecleucel, NHL: non-Hodgkin's lymphoma, CLL: chronic lymphocytic leukemia, ALL: acute lymphoblastic leukemia, DLBL: diffuse large B-cell lymphoma, FL: follicular lymphoma, SLL: small lymphocytic lymphoma, ALCL: Anaplastic large cell lymphoma, HL: Hodgkin's lymphoma, NK-T cell: natural killer T cell, ALL: acute lymphoblastic leukemia, AML: acute myelogenous leukemia, AE: adverse events, DLT: dose-limiting toxicity, ORR: objective response rate, DoR: duration of response, PFS: progression-free survival, OS: overall survival, MTD: maximum tolerated dose, Cmax: maximum concentration, AUC: area under the curve, CR: complete remission, PR: partial response, QOL: quality of life, Tmax: time to maximum, EFS: event-free survival, PK: pharmacokinetics, CRS: cytokine release syndrome, RFS: relapse-free survival.

NCT#	Phase	Intervention	Condition	Outcome Measures	Number to be enrolled
01626495* (Maude, NEJM)	1	CART19	B-cell leukemia, B-cell lymphoma	AE, persistence and expansion of CAR T cells, impact of CAR T cells on cancer	76
01029366* (Maude, NEJM)	1	CART19	B-cell leukemia, B-cell lymphoma	Safety, feasibility, proof of mechanism, concept and bioactivity	26
00924326* (Kochenderfer, JCO)	1	CP, Fludara, Anti-CD19 CAR PBL	B-cell lymphoma	Safety and feasibility, in vivo survival of anti-CD19 CAR transduced cells, regression of disease	43
02348216* (ZUMA-1)	1 and 2	KTE-C19 (axicabtagene ciloleucel)	NHL	DLT, ORR, safety, DoR, PFS, OS	200
01865617	1 and 2	Auto anti-CD19 CAR-4-1BB-CD3ζ EGFRt-expressing T lymphocytes	CLL, NHL, ALL	Death within 8 weeks, persistence, migration, ORR, OS, PFS	189
02050347 (CARPASCIO)	1	CD19 CAR-CD28z T cells	Relapsed CD19+ malignancies post allo SCT	DLT, tumor response	40
01087294	1	Anti-CD19 CAR T cells from the original donor to pts with recurrence after allo SCT	B-cell NHL	Safety, response, persistence of CAR T cells	150
02706405	1	Auto Anti-CD19 CAR-4-1BB-CD3ζ-EGFRt-expressing CD4+/CD8 + Tcm lymphocytes (JCAR014), CP, fludara and durva	B-cell NHL	Toxicity, DLT, MTD of durva and JCAR014, Cmax, AUC, CR, PR	42
02631044 (TRANSCEND-NHL-001)	1	JCAR017 (lisocabtagene maraleucel)	B-cell NHL	AE, DLT, ORR, CR, DoR, PFS, OS, QOL, Cmax, Tmax, AUC of JCAR017	274
03310619	1 and 2	JCAR017 and durva	NHL, DLBCL, FL	DLT, CRR, AE, PFS, OS, ORR, DoR, EFS, PK	50
01747486* (Porter JCO)	2	CD19 re-directed auto T cells	CLL, SLL	AE	61
03049449	1	Anti-CD30 CAR T cells, CP, fludara	ALCL, HL, enteropathy associated T cell lymphoma, extranodal NK-T cell lymphoma	Safety and feasibility, immunogenicity, persistence, anti-lymphoma activity	76
01044069	1	CART19	Precursor B-cell ALL	Safety, anti-leukemic effect	93
02614066 (ZUMA-3)	1 and 2	KTE-C19 (axicabtagene ciloleucel)	ALL	DLT, CR, remission, MRD, allo SCT rate, OS	75
02625480	1 and 2	KTE-C19 (axicabtagene ciloleucel)	ALL	DLT, CR, DoR, MTD, allo SCT rate, OS	75
03190278	1	UCART123	AML	AE, anti-leukemic activity	156
03548207	1 and 2	CAR T cell against BCMA	Myeloma	AE, ORR, CAR T + cellular concentration	84
Pediatric					
02315612	1	Anti-CD22 CAR T cells	Pediatric and young adults with CD22 expressing B cell malignancies	Grade 3 or more CRS, CR, PR, patients with detectable CAR T cells	110
02435849* (ELIANA)	2	Single dose CTL019	Pediatric relapsed refractory B-cell ALL	ORR, DoR, RFS, EFS, OS	81

ELIANA trial showed durable remissions. Among 75 patients infused with tisagenlecleucel, OS was 90% and 76% at 6 and 12 months respectively. 77% of patients experienced CRS. 40% had neurologic events which were managed supportively (Maude et al., 2018). In the ZUMA-1 study, axicabtagene ciloleucel (axi-cel), another CD19-targeted CAR T was used to treat 101 DLBCL patients with refractory disease or relapse within one year after autologous HPSCT. This phase II study reported 82% ORR including 54% CR and 52% OS at 18 months. 3 patients died during treatment. CRS and neurologic events occurred in 13% and 28% of patients, respectively. Axi-cel was successfully manufactured for 99% of patients enrolled in the trial. This led to axicabtagene ciloleucel's FDA approval through REMS for R/R DLBCL (Neelapu et al., 2017). Table 3 shows select ongoing studies on CAR T cells. A more comprehensive list of ongoing CART studies is found in Table 4.

7.2.2. CAR T cell therapy for solid malignancies

The progress achieved with CAR T cells in hematologic malignancies has not been translated to solid malignancies. This road has been, at least in part, hindered by identifying suitable antigens to minimize on-target and off-target toxicities. For instance, CAR T cells targeting carboxy-anhydrase IX (CAIX) in patients with metastatic renal cell carcinoma (RCC) resulted in liver enzyme elevations attributed to antigen expression on bile duct epithelium. Tumor regression was not seen in any of the patients (Lamers et al., 2013). Other studies have also not reported significant responses. For example, in a phase I study, CAR T cells directed against a folate receptor showed no clinically significant responses in patients with ovarian cancer (Kershaw et al., 2006). CAR T cells have been studied in neuroblastoma, targeting two different antigens. In the phase 1 clinical trial by Park et al, 6 pediatric patients with R/R neuroblastoma were treated with CAR-engineered CD8 + T cells targeting the L1 cell-adhesion molecule CD171, which is overexpressed in neuroblastoma. Only one patient achieved PR (Park et al., 2007). In another study by Pule et al, EBV-specific cytotoxic T lymphocytes (CTLs) were engineered to express CAR T cells targeting the GD2 antigen. Viral-specific CTL had more prolonged survival compared to CAR T cells without viral specificity, likely explained by costimulatory signaling upon binding the EBV Antigen on APCs (Pule et al., 2008). Three of 11 patients had CR (Louis et al., 2011).

8. Toxicity of engineered t cells

8.1. On-target-off-tumor and off-target toxicity

Antitumor responses with tumor antigen-specific engineered T cells have often been accompanied by undesired toxicities in clinical trials. On-target toxicity refers to side effects caused by targeting tumor antigens shared by normal tissues. This has manifested as skin rash, uveitis, and hearing loss in patients treated with anti MART-1 and gp100 TCRs, which are melanoma differentiation antigens expressed in melanoma and melanocytes in the skin, eyes, and ears (Johnson et al., 2009; Yee et al., 2000). The severity of ocular toxicity has been shown to correlate strongly with antitumor responses and could be prevented with local steroid administration while preserving antitumor responses (Palmer et al., 2008). Severe transient inflammatory colitis was reported in all three patients with metastatic colorectal cancer treated with autologous T lymphocytes expressing a murine TCR against CEA, which was caused by normal expression of CEA on colonic epithelium (Parkhurst et al., 2011). In another study, a MAGE-A3 directed TCR was used to treat 9 patients with melanoma, esophageal cancer and synovial cell sarcoma. Neurologic toxicity was seen in 3 of 9 patients, manifesting as mental status changes leading to coma and death in 2 patients. This was explained by cross reactivity with an epitope on MAGE-A12, which is expressed in brain tissue (Morgan et al., 2013). On the other hand, off-target toxicity refers to toxicity related to organs not expressing the target antigen and occurs due to an unanticipated interaction with another antigen. This phenomenon was reported in a study using a TCR against HLA-A*01–restricted MAGE-A3 in patients with melanoma and multiple myeloma. The first 2 patients who received the treatment developed severe fatal cardiac caused by cross-reactivity with titin, a protein found in striated muscle tissue (Linette et al., 2013).

8.2. Cytokine release syndrome (CRS)

CRS has been the most common significant adverse effect accompanying CAR T cell therapy in clinical trials. CRS refers to a systemic inflammatory response caused by cytokine release, mainly INF-γ, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6, IL-8 and tumor necrosis factor (TNF)-α, upon CAR T cell activation and proliferation in vivo (Teachey et al., 2016). CRS manifestations range from mild to severe and life threatening and include constitutional symptoms, fever, malaise, myalgias, hypoxia, respiratory distress, cytopenia, capillary leak, consumptive coagulopathy, hypotension, hemodynamic instability and/or multiorgan toxicity (Wang and Han, 2018). Several grading systems are used to grade CSR from 1 to 4. These include: CTCAE v4.0, 2014 Lee et al scale and Penn grading scale (Porter et al., 2018). The underlying mechanisms of CRS have not been fully elucidated. However, the clinical and laboratory resemblance to macrophage activation syndrome (MAS) has identified macrophages as key mediators. Endothelial cells are also important players. Endothelial cell activation causes IL-6 release and leads to vascular leak and consumptive coagulopathy in severe cases (Wang and Han, 2018). Factors which increase CAR T cell levels in vivo have been shown to be predictive of CRS such as high tumor burden, lymphodepletion, and a higher dose of infused CAR T cells (Brentjens et al., 2013; Maude et al., 2014; Hay et al., 2017; Jin et al., 2018). Laboratory markers which are elevated in CRS include ferritin, C-reactive protein (CRP), liver enzymes, BUN, lactate dehydrogenase (LDH) and creatinine (Teachey et al., 2016). In a recent systematic review by Jin et al, the incidence of severe CRS in phase I clinical trials using CD19 CAR T cell therapy from 2010 and 2017 was found to be between 19.8% to 38.8% in B-cell malignancies (Jin et al., 2018). Mild to moderate forms of CRS are usually self-limited, while severe forms are managed with steroids and tocilizumab (Maude et al., 2014). CRS has also been observed with other therapies such as blinatumumab, a CD19/CD3 bispecific T cell engager (BiTE) antibody (Teachey et al., 2013), programmed cell death-1 (PD-1) inhibitors (Rotz et al., 2017) and rituximab (Winkler et al., 1999).

8.3. Neurotoxicity

Neurotoxicity is another toxicity frequently reported in clinical trials using CD19-directed CAR T cells (Kochenderfer et al., 2015; Porter et al., 2015; Maude et al., 2014, 2018; Neelapu et al., 2017). Its manifestations range from mild to severe, and include headaches, confusion, delirium, anxiety, tremors, seizures and cerebral edema. Symptoms typically manifest within 4–5 days from infusion. Mild cases are usually self-limited and managed with supportive care alone. Severe symptoms usually resolve with dexamethasone, but may be fatal (Wang and Han, 2018). The mechanisms underlying neurotoxicity have not been fully elucidated. Gust et al proposed that endothelial cell activation increases blood-brain barrier permeability, leading to passage of cytokines into the CSF (Gust et al., 2017). Interestingly, there was a high concordance between CRS and neurotoxicity suggesting a possibility of overlapping syndromes caused by cytokine release from antigen-specific CAR T cell activation (Wang and Han, 2018).

9. Strategies to overcome toxicities

Initiatives to avert toxicities associated with engineered T cells led to co-expression of suicide genes on engineered lymphocytes, which act

as safety switches by causing selective elimination of infused cells in vivo upon exposure to a prodrug. The herpes simplex virus thymidine kinase (HSV-TK) gene is a suicide gene which phosphorylates its prodrug ganciclovir leading to disruption of DNA replication and cell death (Sun et al., 2018). Caspases, on the other hand, are induced by an inert molecule and are less immunogenic (Jones et al., 2014). Specifically, the inducible caspase-9 (iC9) gene is composed of an FK binding protein linked to human caspase-9. Upon binding its prodrug AP1903, it dimerizes and leads to apoptosis of the infused cells. Suicides genes have shown to control GVHD by efficient T cell elimination, while preserving the GVL effect and promoting immune reconstitution (Ciceri et al., 2009; Di Stasi et al., 2011). iC9 has been tested in preclinical models using anti-CD19 and anti-CD20 CAR T cells, especially in constructs co-expressing IL genes, and has shown efficient T cell elimination (Hoyos et al., 2010; Quintarelli et al., 2007; Budde et al., 2013). mRNA electroporation is another strategy to achieve transient CAR expression (Beatty et al., 2014). Although T cell elimination can reverse adverse effects, this comes at the cost of eliminating their therapeutic effects. Therefore, other mechanisms were developed. Dual-targeted T cells selectively target cells expressing a combination of antigens, which increases their specificity. This is achieved by splitting the CAR activation signals and linking them to 2 separate antigens. The synthetic Notch (synNotch) receptor is a recently introduced dual targeting construct, where TAA1 binding results in release of a transcription factor, inducing expression of a CAR specific for another TAA2 (Roybal et al., 2016). Other mechanisms include the generation of constructs targeting non-tumor antigens and co-expressing inhibitory CARs (iCARs), which are made of PD-1 or cytotoxic T-lymphocyte associated protein-4 (CTLA-4) intracellular domains. Unlike suicide genes, iCARs lead to reversible inhibition of T cells; antitumor activity is restored upon encounter with the target antigen (Fedorov et al., 2013).

10. Obstacles and strategies to improve engineered T cells

Despite encouraging results in preclinical and clinical studies, a major limitation in ACT is the progressive loss of in vivo activity, which may be attributed, at least in part, to peripheral tolerance mechanisms. The same mechanisms which suppress endogenous antitumor immune responses can hinder the efficacy of adoptive cellular therapy. This includes an immunosuppressive microenvironment which is composed of immunosuppressive cells like regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), inhibitory cytokines and expression of immune checkpoints on tumor cells. The manipulation of the tumor microenvironment can be achieved by multiple ways to enhance the activity of ACT (reviewed by Beavis et al.) (Beavis et al., 2016). A lymphodepleting preparative regimen has previously been shown to enhance the efficacy of ACT possibly by means of depleting immunosuppressive cells and inhibitory cytokines. Fludarabine and cyclophosphamide have been found to downregulate the expression of indoleamine 2,3-dioxygenase (IDO) in tumor cells, thus inhibiting the degradation of tryptophan into metabolites that inhibit T cell activity (Ninomiya et al., 2015). The loss of function of engineered T cells has been shown to be associated with upregulation of inhibitory molecules like PD-1, Tim3 and Lag3 in mouse tumor models (Moon et al., 2016). Interference with the PD-1 pathway through PD-1 blockade reinstated the activity of transferred CAR T cells and was associated with a decrease in myeloid suppressor cells, in preclinical models (Cherkassky et al., 2016; John et al., 2013). Similarly, CTLA-4 blockade was shown to enhance the theraeutic efficacy of ACT (Mahvi et al., 2015). These findings suggest a potential role for combination with checkpoint inhibitors. In addition, the manipulation of intracellular signaling pathways has the potential to promote resistance to immunosuppressive signals. For example, the constitutive expression of Akt in adoptively transferred T cells provided resistance to Tregs and resulted in enhanced T cell proliferative capacity and survival (Sun et al., 2010). T cells have also been engineered to express pro-inflammatory cytokines, cytokine receptors and costimulatory molecules in efforts to augment in vivo inflammatory responses. Co-expression of IL-12 and IL-15 in CAR T cells has demonstrated enhanced expansion, persistence, and in vivo antitumor responses in preclinical models (Hoyos et al., 2010; Jaspers and Brentjens, 2017; Pegram et al., 2012; Koneru et al., 2015). Similar results were seen with co-expression of chimeric cytokine receptors (Wilkie et al., 2010; Leen et al., 2014). Safety genes have been included in some of these constructs to enhance their safety (Hoyos et al., 2010; Koneru et al., 2015). Another major limitation to CAR T cell therapy is antigenic escape, which accounts for a portion of relapse cases (Sotillo et al., 2015). Dual-targeting CAR-T cell have been proposed as a mechanism to overcome this. By targeting 2 antigens, antitumor activity is preserved when 1 antigen is lost. In a study by Ruella et al, combined targeting of CD19 and CD123 prevented CD-19 negative B-ALL relapses in xenograft models.(Ruella et al., 2016). Another major obstacle to the widespread use of ACT approach is the cost. The mean expected cost of treatment with CAR T cell therapy has been estimated at $402 647 for axicabtagene ciloleucel and $510 963 for tisagenlecleucel. This accounts for both drug and non-drug costs (Hernandez et al., 2018). Several tools have been developed to silence the expression of endogenous genes like the TCR and HLA in efforts towards creating a universal allogeneic “off the shelf” T cell product (Torikai et al., 2012; Poirot et al., 2015; Torikai et al., 2013; Singh et al., 2017). This may help reduce production time and costs and allow the treatment of patients in whom autologous T cell manufacturing cannot be achieved or are in need of urgent treatment.

11. Conclusion

ACT is a promising immunotherapeutic strategy capable of inducing complete and durable remissions in some cancers. To date, the most exciting results have been with CD19 CAR T cells in B-cell malignancies. Despite its success, there are multiple obstacles, which include identifying target tumor antigens while sparing normal tissue, enhancing vivo persistence, and overcoming peripheral tolerances. Trials are currently ongoing to find an application for ACT in other tumors, explore novel antigenic targets and study the role of combination with other immunotherapeutic strategies. As the different ACT approaches undergo further improvement, efforts should be geared towards increasing the efficacy, reducing the toxicities and increasing accessibility of this treatment to a wider patient population by reevaluating and reducing its costs.

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Authors’ contributions

AS was involved in the conceptualization, formal analysis, methodology, project administration, methodology, supervision and reviewing and editing the original draft. NA and MN were involved in the reference collection, formal analysis and writing of the original draft. All authors were involved in the interpretation of data and critical revision of the content. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

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Appendix A. Supplementary data

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References

Atkins, M.B., Lotze, M.T., Dutcher, J.P., Fisher, R.I., Weiss, G., Margolin, K., et al., 1999. High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. J. Clin. Oncol. 17 (7), 2105–2116.

Beatty, G.L., Haas, A.R., Maus, M.V., Torigian, D.A., Soulen, M.C., Plesa, G., et al., 2014. Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells induce anti-tumor activity in solid malignancies. Cancer Immunol. Res. 2 (2), 112–120.

Beavis, P.A., Slaney, C.Y., Kershaw, M.H., Gyorki, D., Neeson, P.J., Darcy, P.K., 2016. Reprogramming the tumor microenvironment to enhance adoptive cellular therapy. Semin. Immunol. 28 (1), 64–72.

Bonini, C., Mondino, A., 2015. Adoptive T-cell therapy for cancer: the era of engineered T cells. Eur. J. Immunol. 45 (9), 2457–2469.

Brentjens, R.J., Davila, M.L., Riviere, I., Park, J., Wang, X., Cowell, L.G., et al., 2013. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Sci. Transl. Med. 5 (177), 177ra38.

Brown-Séquard, C.E., 1889. The effects produced on man by subcutaneous injection of a liquid obtained from the testicles of animals. Lancet (137), 105–107.

Budde, L.E., Berger, C., Lin, Y., Wang, J., Lin, X., Frayo, S.E., et al., 2013. Combining a CD20 chimeric antigen receptor and an inducible caspase 9 suicide switch to improve the efficacy and safety of T cell adoptive immunotherapy for lymphoma. PLoS One 8 (12), e82742.

Chang, J.T., Wherry, E.J., Goldrath, A.W., 2014. Molecular regulation of effector and memory T cell differentiation. Nat. Immunol. 15 (12), 1104–1115.

Chen, D.S., Mellman, I., 2013. Oncology meets immunology: the cancer-immunity cycle. Immunity 39 (1), 1–10.

Cherkassky, L., Morello, A., Villena-Vargas, J., Feng, Y., Dimitrov, D.S., Jones, D.R., et al., 2016. Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. J. Clin. Invest. 126 (8), 3130–3144.

Ciceri, F., Bonini, C., Stanghellini, M.T., Bondanza, A., Traversari, C., Salomoni, M., et al., 2009. Infusion of suicide-gene-engineered donor lymphocytes after family haploidentical haemopoietic stem-cell transplantation for leukaemia (the TK007 trial): a non-randomised phase I-II study. Lancet Oncol. 10 (5), 489–500.

Conlon, K.C., Lugli, E., Welles, H.C., Rosenberg, S.A., Fojo, A.T., Morris, J.C., et al., 2015. Redistribution, Hyperproliferation, Activation of Natural Killer Cells and CD8 T Cells, and Cytokine Production During First-in-Human Clinical Trial of Recombinant Human Interleukin-15 in Patients With Cancer. J. Clin. Oncol. 33 (1), 74–82.

Di Stasi, A., Tey, S.K., Dotti, G., Fujita, Y., Kennedy-Nasser, A., Martinez, C., et al., 2011. Inducible apoptosis as a safety switch for adoptive cell therapy. N. Engl. J. Med. 365 (18), 1673–1683.

Dudley, M.E., Wunderlich, J.R., Yang, J.C., Sherry, R.M., Topalian, S.L., Restifo, N.P., et al., 2005. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. J. Clin. Oncol. 23 (10), 2346–2357.

Dudley, M.E., Yang, J.C., Sherry, R., Hughes, M.S., Royal, R., Kammula, U., et al., 2008. Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. J. Clin. Oncol. 26 (32), 5233–5239.

Dunn, G.P., Bruce, A.T., Ikeda, H., Old, L.J., Schreiber, R.D., 2002. Cancer immunoediting: from immunosurveillance to tumor escape. Nat. Immunol. 3 (11), 991–998.

Fedorov, V.D., Themeli, M., Sadelain, M., 2013. PD-1- and CTLA-4-based inhibitory chimeric antigen receptors (iCARs) divert off-target immunotherapy responses. Sci. Transl. Med. 5 (215), 215ra172.

Feldman, S.A., Assadipour, Y., Kriley, I., Goff, S.L., Rosenberg, S.A., 2015. Adoptive cell therapy–Tumor-Infiltrating lymphocytes, T-Cell receptors, and chimeric antigen receptors. Semin. Oncol. 42 (4), 626–639.

Fossa, S.D., 2000. Interferon in metastatic renal cell carcinoma. Semin. Oncol. 27 (2), 187–193.

Gattinoni, L., Ji, Y., Restifo, N.P., 2010. Wnt/beta-catenin signaling in T-cell immunity and cancer immunotherapy. Clin. Cancer Res. 16 (19), 4695–4701.

Gross, G., Waks, T., Eshhar, Z., 1989. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. Proc. Natl. Acad. Sci. U. S. A. 86 (24), 10024–10028.

Gust, J., Hay, K.A., Hanafi, L.A., Li, D., Myerson, D., Gonzalez-Cuyar, L.F., et al., 2017. Endothelial activation and blood-brain barrier disruption in neurotoxicity after adoptive immunotherapy with CD19 CAR-T cells. Cancer Discov. 7 (12), 1404–1419.

Hay, K.A., Hanafi, L.A., Li, D., Gust, J., Liles, W.C., Wurfel, M.M., et al., 2017. Kinetics and biomarkers of severe cytokine release syndrome after CD19 chimeric antigen receptor-modified T-cell therapy. Blood 130 (21), 2295–2306.

Hernandez, I., Prasad, V., Gellad, W.F., 2018. Total costs of chimeric antigen receptor T-Cell immunotherapy. JAMA Oncol. 4 (7), 994–996.

Hinrichs, C.S., Borman, Z.A., Cassard, L., Gattinoni, L., Spolski, R., Yu, Z., et al., 2009. Adoptively transferred effector cells derived from naive rather than central memory CD8+ T cells mediate superior antitumor immunity. Proc. Natl. Acad. Sci. U. S. A. 106 (41), 17469–17474.

Hofmann, O., Caballero, O.L., Stevenson, B.J., Chen, Y.T., Cohen, T., Chua, R., et al., 2008. Genome-wide analysis of cancer/testis gene expression. Proc. Natl. Acad. Sci. U. S. A. 105 (51), 20422–20427.

Houot, R., Schultz, L.M., Marabelle, A., Kohrt, H., 2015. T-cell-based immunotherapy: adoptive cell transfer and checkpoint inhibition. Cancer Immunol. Res. 3 (10), 1115–1122.

Hoyos, V., Savoldo, B., Quintarelli, C., Mahendravada, A., Zhang, M., Vera, J., et al., 2010. Engineering CD19-specific T lymphocytes with interleukin-15 and a suicide gene to enhance their anti-lymphoma/leukemia effects and safety. Leukemia 24 (6), 1160–1170.

Hunder, N.N., Wallen, H., Cao, J., Hendricks, D.W., Reilly, J.Z., Rodmyre, R., et al., 2008. Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1. N. Engl. J. Med. 358 (25), 2698–2703.

Introna, M., 2017. CIK as therapeutic agents against tumors. J. Autoimmun. 85, 32–44.

Introna, M., Franceschetti, M., Ciocca, A., Borleri, G., Conti, E., Golay, J., et al., 2006. Rapid and massive expansion of cord blood-derived cytokine-induced killer cells: an innovative proposal for the treatment of leukemia relapse after cord blood transplantation. Bone Marrow Transplant. 38 (9), 621–627.

Jaspers, J.E., Brentjens, R.J., 2017. Development of CAR T cells designed to improve antitumor efficacy and safety. Pharmacol. Ther. 178, 83–91.

Jin, Z., Xiang, R., Qing, K., Li, X., Zhang, Y., Wang, L., et al., 2018. The severe cytokine release syndrome in phase I trials of CD19-CAR-T cell therapy: a systematic review. Ann. Hematol.

John, L.B., Devaud, C., Duong, C.P., Yong, C.S., Beavis, P.A., Haynes, N.M., et al., 2013. Anti-PD-1 antibody therapy potently enhances the eradication of established tumors by gene-modified T cells. Clin. Cancer Res. 19 (20), 5636–5646.

Johnson, L.A., Morgan, R.A., Dudley, M.E., Cassard, L., Yang, J.C., Hughes, M.S., et al., 2009. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood 114 (3), 535–546.

Jones, B.S., Lamb, L.S., Goldman, F., Di Stasi, A., 2014. Improving the safety of cell therapy products by suicide gene transfer. Front. Pharmacol. 5, 254.

Kalos, M., June, C.H., 2013. Adoptive T cell transfer for cancer immunotherapy in the era of synthetic biology. Immunity 39 (1), 49–60.

Kalos, M., Levine, B.L., Porter, D.L., Katz, S., Grupp, S.A., Bagg, A., et al., 2011. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci. Transl. Med. 3 (95), 95ra73.

Kershaw, M.H., Westwood, J.A., Parker, L.L., Wang, G., Eshhar, Z., Mavroukakis, S.A., et al., 2006. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clin. Cancer Res. 12 (20 Pt 1), 6106–6115.

Kim, E.H., Sullivan, J.A., Plisch, E.H., Tejera, M.M., Jatzek, A., Choi, K.Y., et al., 2012. Signal integration by Akt regulates CD8 T cell effector and memory differentiation. J. Immunol. 188 (9), 4305–4314.

Kochenderfer, J.N., Wilson, W.H., Janik, J.E., Dudley, M.E., Stetler-Stevenson, M., Feldman, S.A., et al., 2010. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. Blood 116 (20), 4099–4102.

Kochenderfer, J.N., Dudley, M.E., Kassim, S.H., Somerville, R.P., Carpenter, R.O., Stetler-Stevenson, M., et al., 2015. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. J. Clin. Oncol. 33 (6), 540–549.

Koneru, M., Purdon, T.J., Spriggs, D., Koneru, S., Brentjens, R.J., 2015. IL-12 secreting tumor-targeted chimeric antigen receptor T cells eradicate ovarian tumors in vivo. Oncoimmunology 4 (3), e994446.

Package Insert - KYMRIAH Available from: https://www.fda.gov/downloads/UCM573941.pdf.

Lamers, C.H., Sleijfer, S., van Steenbergen, S., van Elzakker, P., van Krimpen, B., Groot, C., et al., 2013. Treatment of metastatic renal cell carcinoma with CAIX CAR-engineered T cells: clinical evaluation and management of on-target toxicity. Mol. Ther. 21 (4), 904–912.

Leen, A.M., Sukumaran, S., Watanabe, N., Mohammed, S., Keirnan, J., Yanagisawa, R., et al., 2014. Reversal of tumor immune inhibition using a chimeric cytokine receptor. Mol. Ther. 22 (6), 1211–1220.

Li, H., Zhao, Y., 2017. Increasing the safety and efficacy of chimeric antigen receptor T cell therapy. Protein Cell 8 (8), 573–589.

Linette, G.P., Stadtmauer, E.A., Maus, M.V., Rapoport, A.P., Levine, B.L., Emery, L., et al., 2013. Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. Blood 122 (6), 863–871.

Louis, C.U., Savoldo, B., Dotti, G., Pule, M., Yvon, E., Myers, G.D., et al., 2011. Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients

with neuroblastoma. Blood 118 (23), 6050–6056.
Mackensen, A., Meidenbauer, N., Vogl, S., Laumer, M., Berger, J., Andreesen, R., 2006. Phase I study of adoptive T-cell therapy using antigen-specific CD8+ T cells for the treatment of patients with metastatic melanoma. J. Clin. Oncol. 24 (31), 5060–5069.
Mahvi, D.A., Meyers, J.V., Tatar, A.J., Contreras, A., Suresh, M., Leverson, G.E., et al., 2015. Ctla-4 blockade plus adoptive T-cell transfer promotes optimal melanoma immunity in mice. J. Immunother. 38 (2), 54–61.
Maude, S.L., Frey, N., Shaw, P.A., Aplenc, R., Barrett, D.M., Bunin, N.J., et al., 2014. Chimeric antigen receptor T cells for sustained remissions in leukemia. N. Engl. J. Med. 371 (16), 1507–1517.
Maude, S.L., Laetsch, T.W., Buechner, J., Rives, S., Boyer, M., Bittencourt, H., et al., 2018. Tisagenlecleucel in children and young adults with B-Cell lymphoblastic leukemia. N. Engl. J. Med. 378 (5), 439–448.
Moon, E.K., Ranganathan, R., Eruslanov, E., Kim, S., Newick, K., O'Brien, S., et al., 2016. Blockade of programmed death 1 augments the ability of human t cells engineered to target NY-ESO-1 to control tumor growth after adoptive transfer. Clin. Cancer Res. 22 (2), 436–447.
Morales, A., Eidinger, D., Bruce, A.W., 1976. Intracavitary Bacillus Calmette-Guerin in the treatment of superficial bladder tumors. J. Urol. 116 (2), 180–183.
Morgan, R.A., Dudley, M.E., Wunderlich, J.R., Hughes, M.S., Yang, J.C., Sherry, R.M., et al., 2006. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science 314 (5796), 126–129.
Morgan, R.A., Chinnasamy, N., Abate-Daga, D., Gros, A., Robbins, P.F., Zheng, Z., et al., 2013. Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. J. Immunother. 36 (2), 133–151.
Nauts, H.C., Swift, W.E., Coley, B.L., 1946. The treatment of malignant tumors by bacterial toxins as developed by the late William B. Coley, M.D., reviewed in the light of modern research. Cancer Res. 6, 205–216.
Neelapu, S.S., Locke, F.L., Bartlett, N.L., Lekakis, L.J., Miklos, D.B., Jacobson, C.A., et al., 2017. Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. N. Engl. J. Med. 377 (26), 2531–2544.
Ninomiya, S., Narala, N., Huye, L., Yagyu, S., Savoldo, B., Dotti, G., et al., 2015. Tumor indoleamine 2,3-dioxygenase (IDO) inhibits CD19-CAR T cells and is downregulated by lymphodepleting drugs. Blood 125 (25), 3905–3916.
Oates, J., Jakobsen, B.K., 2013. ImmTACs: Novel bi-specific agents for targeted cancer therapy. Oncoimmunology 2 (2), e22891.
Palmer, D.C., Chan, C.C., Gattinoni, L., Wrzesinski, C., Paulos, C.M., Hinrichs, C.S., et al., 2008. Effective tumor treatment targeting a melanoma/melanocyte-associated antigen triggers severe ocular autoimmunity. Proc. Natl. Acad. Sci. U. S. A. 105 (23), 8061–8066.
Park, J.R., Digiusto, D.L., Slovak, M., Wright, C., Naranjo, A., Wagner, J., et al., 2007. Adoptive transfer of chimeric antigen receptor re-directed cytolytic T lymphocyte clones in patients with neuroblastoma. Mol. Ther. 15 (4), 825–833.
Park, J.H., Geyer, M.B., Brentjens, R.J., 2016. CD19-targeted CAR T-cell therapeutics for hematologic malignancies: interpreting clinical outcomes to date. Blood 127 (26), 3312–3320.
Parkhurst, M.R., Yang, J.C., Langan, R.C., Dudley, M.E., Nathan, D.A., Feldman, S.A., et al., 2011. T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. Mol. Ther. 19 (3), 620–626.
Pegram, H.J., Lee, J.C., Hayman, E.G., Imperato, G.H., Tedder, T.F., Sadelain, M., et al., 2012. Tumor-targeted T cells modified to secrete IL-12 eradicate systemic tumors without need for prior conditioning. Blood 119 (18), 4133–4141.
Poirot, L., Philip, B., Schiffer-Mannioui, C., Le Clerre, D., Chion-Sotinel, I., Derniame, S., et al., 2015. Multiplex genome-edited T-cell manufacturing platform for "Off-the-Shelf" adoptive T-cell immunotherapies. Cancer Res. 75 (18), 3853–3864.
Porter, D.L., Connors, J.M., Van Deerlin, V.M., Duffy, K.M., McGarigle, C., Saidman, S.L., et al., 1999. Graft-versus-tumor induction with donor leukocyte infusions as primary therapy for patients with malignancies. J. Clin. Oncol. 17 (4), 1234.
Porter, D.L., Hwang, W.T., Frey, N.V., Lacey, S.F., Shaw, P.A., Loren, A.W., et al., 2015. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. Sci. Transl. Med. 7 (303), 303ra139.
Porter, D., Frey, N., Wood, P.A., Weng, Y., Grupp, S.A., 2018. Grading of cytokine release syndrome associated with the CAR T cell therapy tisagenlecleucel. J. Hematol. Oncol. 11 (1), 35.
Pule, M.A., Straathof, K.C., Dotti, G., Heslop, H.E., Rooney, C.M., Brenner, M.K., 2005. A chimeric T cell antigen receptor that augments cytokine release and supports clonal expansion of primary human T cells. Mol. Ther. 12 (5), 933–941.
Pule, M.A., Savoldo, B., Myers, G.D., Rossig, C., Russell, H.V., Dotti, G., et al., 2008. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. Nat. Med. 14 (11), 1264–1270.
Quintarelli, C., Vera, J.F., Savoldo, B., Giordano Attianese, G.M., Pule, M., Foster, A.E., et al., 2007. Co-expression of cytokine and suicide genes to enhance the activity and safety of tumor-specific cytotoxic T lymphocytes. Blood 110 (8), 2793–2802.
Radvanyi, L.G., Bernatchez, C., Zhang, M., Fox, P.S., Miller, P., Chacon, J., et al., 2012. Specific lymphocyte subsets predict response to adoptive cell therapy using expanded autologous tumor-infiltrating lymphocytes in metastatic melanoma patients. Clin. Cancer Res. 18 (24), 6758–6770.
Robbins, P.F., Kassim, S.H., Tran, T.L., Crystal, J.S., Morgan, R.A., Feldman, S.A., et al., 2015. A pilot trial using lymphocytes genetically engineered with an NY-ESO-1-reactive T-cell receptor: long-term follow-up and correlates with response. Clin. Cancer Res. 21 (5), 1019–1027.
Rosenberg, S.A., Packard, B.S., Aebersold, P.M., Solomon, D., Topalian, S.L., Toy, S.T., et al., 1988. Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report. N. Engl. J. Med. 319 (25), 1676–1680.
Rosenberg, S.A., Yang, J.C., Sherry, R.M., Kammula, U.S., Hughes, M.S., Phan, G.Q., et al., 2011. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin. Cancer Res. 17 (13), 4550–4557.
Rotz, S.J., Leino, D., Szabo, S., Mangino, J.L., Turpin, B.K., Pressey, J.G., 2017. Severe cytokine release syndrome in a patient receiving PD-1-directed therapy. Pediatr. Blood Cancer 64 (12).
Roybal, K.T., Williams, J.Z., Morsut, L., Rupp, L.J., Kolinko, I., Choe, J.H., et al., 2016. Engineering T cells with customized therapeutic response programs using synthetic notch receptors. Cell 167 (2), 419–432 e16.
Ruella, M., Kalos, M., 2014. Adoptive immunotherapy for cancer. Immunol. Rev. 257 (1), 14–38.
Ruella, M., Barrett, D.M., Kenderian, S.S., Shestova, O., Hofmann, T.J., Perazzelli, J., et al., 2016. Dual CD19 and CD123 targeting prevents antigen-loss relapses after CD19-directed immunotherapies. J. Clin. Invest. 126 (10), 3814–3826.
Savoldo, B., Ramos, C.A., Liu, E., Mims, M.P., Keating, M.J., Carrum, G., et al., 2011. CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients. J. Clin. Invest. 121 (5), 1822–1826.
Schmeel, L.C., Schmeel, F.C., Coch, C., Schmidt-Wolf, I.G., 2015. Cytokine-induced killer (CIK) cells in cancer immunotherapy: report of the international registry on CIK cells (IRCC). J. Cancer Res. Clin. Oncol. 141 (5), 839–849.
Schmidt-Wolf, I.G., Negrin, R.S., Kiem, H.P., Blume, K.G., Weissman, I.L., 1991. Use of a SCID mouse/human lymphoma model to evaluate cytokine-induced killer cells with potent antitumor cell activity. J. Exp. Med. 174 (1), 139–149.
Singh, N., Shi, J., June, C.H., Ruella, M., 2017. Genome-editing technologies in adoptive t cell immunotherapy for Cancer. Curr. Hematol. Malig. Rep. 12 (6), 522–529.
Song, D.G., Ye, Q., Carpenito, C., Poussin, M., Wang, L.P., Ji, C., et al., 2011. In vivo persistence, tumor localization, and antitumor activity of CAR-engineered T cells is enhanced by costimulatory signaling through CD137 (4-1BB). Cancer Res. 71 (13), 4617–4627.
Sotillo, E., Barrett, D.M., Black, K.L., Bagashev, A., Oldridge, D., Wu, G., et al., 2015. Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. Cancer Discov. 5 (12), 1282–1295.
Stambler, I., 2014. The unexpected outcomes of anti-aging, rejuvenation, and life extension studies: an origin of modern therapies. Rejuvenation Res. 17 (3), 297–305.
Sun, J., Dotti, G., Huye, L.E., Foster, A.E., Savoldo, B., Gramatges, M.M., et al., 2010. T cells expressing constitutively active Akt resist multiple tumor-associated inhibitory mechanisms. Mol. Ther. 18 (11), 2006–2017.
Sun, S., Hao, H., Yang, G., Zhang, Y., Fu, Y., 2018. Immunotherapy with CAR-modified t cells: toxicities and overcoming strategies. J. Immunol. Res. 2018, 2386187.
Tagliamonte, M., Petrizzo, A., Tornesello, M.L., Buonaguro, F.M., Buonaguro, L., 2014. Antigen-specific vaccines for cancer treatment. Hum. Vaccin. Immunother. 10 (11), 3332–3346.
Teachey, D.T., Rheingold, S.R., Maude, S.L., Zugmaier, G., Barrett, D.M., Seif, A.E., et al., 2013. Cytokine release syndrome after blinatumomab treatment related to abnormal macrophage activation and ameliorated with cytokine-directed therapy. Blood 121 (26), 5154–5157.
Teachey, D.T., Lacey, S.F., Shaw, P.A., Melenhorst, J.J., Maude, S.L., Frey, N., et al., 2016. Identification of predictive biomarkers for cytokine release syndrome after chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. Cancer Discov. 6 (6), 664–679.
Till, B.G., Jensen, M.C., Wang, J., Chen, E.Y., Wood, B.L., Greisman, H.A., et al., 2008. Adoptive immunotherapy for indolent non-Hodgkin lymphoma and mantle cell lymphoma using genetically modified autologous CD20-specific T cells. Blood 112 (6), 2261–2271.
Till, B.G., Jensen, M.C., Wang, J., Qian, X., Gopal, A.K., Maloney, D.G., et al., 2012. CD20-specific adoptive immunotherapy for lymphoma using a chimeric antigen receptor with both CD28 and 4-1BB domains: pilot clinical trial results. Blood 119 (17), 3940–3950.
Torikai, H., Reik, A., Liu, P.Q., Zhou, Y., Zhang, L., Maiti, S., et al., 2012. A foundation for universal T-cell based immunotherapy: t cells engineered to express a CD19-specific chimeric-antigen-receptor and eliminate expression of endogenous TCR. Blood 119 (24), 5697–5705.
Torikai, H., Reik, A., Soldner, F., Warren, E.H., Yuen, C., Zhou, Y., et al., 2013. Toward eliminating HLA class I expression to generate universal cells from allogeneic donors. Blood 122 (8), 1341–1349.
Vinay, D.S., Ryan, E.P., Pawelec, G., Talib, W.H., Stagg, J., Elkord, E., et al., 2015. Immune evasion in cancer: mechanistic basis and therapeutic strategies. Semin. Cancer Biol. 35 (Suppl), S185–S198.
Wang, Z., Han, W., 2018. Biomarkers of cytokine release syndrome and neurotoxicity related to CAR-T cell therapy. Biomark. Res. 6, 4.
Wang, M., Yin, B., Wang, H.Y., Wang, R.F., 2014. Current advances in T-cell-based cancer immunotherapy. Immunotherapy. 6 (12), 1265–1278.
Weiden, P.L., Flournoy, N., Thomas, E.D., Prentice, R., Fefer, A., Buckner, C.D., et al., 1979. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. N. Engl. J. Med. 300 (19), 1068–1073.
Wilkie, S., Burbridge, S.E., Chiapero-Stanke, L., Pereira, A.C., Cleary, S., van der Stegen, S.J., et al., 2010. Selective expansion of chimeric antigen receptor-targeted T-cells with potent effector function using interleukin-4. J. Biol. Chem. 285 (33), 25538–25544.
Williams, D.A., 2008. Foamy virus vectors come of age. Mol. Ther. 16 (4), 635–636.
Winkler, U., Jensen, M., Manzke, O., Schulz, H., Diehl, V., Engert, A., 1999. Cytokine-release syndrome in patients with B-cell chronic lymphocytic leukemia and high lymphocyte counts after treatment with an anti-CD20 monoclonal antibody (rituximab, IDEC-C2B8). Blood 94 (7), 2217–2224.
Yang, J.C., Sherry, R.M., Steinberg, S.M., Topalian, S.L., Schwartzentruber, D.J., Hwu, P.,

et al., 2003. Randomized study of high-dose and low-dose interleukin-2 in patients with metastatic renal cancer. J. Clin. Oncol. 21 (16), 3127–3132.
Yannelli, J.R., Hyatt, C., McConnell, S., Hines, K., Jacknin, L., Parker, L., et al., 1996. Growth of tumor-infiltrating lymphocytes from human solid cancers: summary of a 5-year experience. Int. J. Cancer 65 (4), 413–421.
Yao, X., Ahmadzadeh, M., Lu, Y.C., Liewehr, D.J., Dudley, M.E., Liu, F., et al., 2012. Levels of peripheral CD4(+)FoxP3(+) regulatory T cells are negatively associated with clinical response to adoptive immunotherapy of human cancer. Blood 119 (24), 5688–5696.
Yee, C., Thompson, J.A., Roche, P., Byrd, D.R., Lee, P.P., Piepkorn, M., et al., 2000. Melanocyte destruction after antigen-specific immunotherapy of melanoma: direct evidence of t cell-mediated vitiligo. J. Exp. Med. 192 (11), 1637–1644.
Yee, C., Thompson, J.A., Byrd, D., Riddell, S.R., Roche, P., Celis, E., et al., 2002. Adoptive T cell therapy using antigen-specific CD8+ T cell clones for the treatment of patients with metastatic melanoma: in vivo persistence, migration, and antitumor effect of transferred T cells. Proc. Natl. Acad. Sci. U. S. A. 99 (25), 16168–16173.
Zhao, Y., Moon, E., Carpenito, C., Paulos, C.M., Liu, X., Brennan, A.L., et al., 2010. Multiple injections of electroporated autologous T cells expressing a chimeric antigen receptor mediate regression of human disseminated tumor. Cancer Res. 70 (22), 9053–9061.