

REVIEW

Development of adaptive immune effector therapies in solid tumors

P. Comoli¹, C. Chabannon², U. Koehl^{3,4}, F. Lanza⁵, A. Urbano-Ispizua⁶, M. Hudecek⁷, A. Ruggeri⁸, S. Secondino⁹, C. Bonini¹⁰ & P. Pedrazzoli^{9*}, on behalf of the European Society for Blood and Marrow Transplantation, Cellular Therapy & Immunobiology Working Party – Solid Tumor Sub-committee

¹Cell Factory and Pediatric Hematology Oncology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; ²Institut Paoli-Calmettes, Aix-Marseille University, INSERM CBT 1409, Centre for Clinical Investigation in Biotherapy, Marseille, France; ³Institute of Clinical Immunology, University of Leipzig and Fraunhofer Institute for Cell Therapy and Immunology, Leipzig; ⁴Institute of Cellular Therapeutics, Hannover Medical School, Hannover, Germany; ⁵Hematology and Stem Cell Transplant, Romagna Transplant Network, Ravenna, Italy; ⁶Department of Hematology, IDIBAPS, Institute of Research Josep Carreras, Hospital Clínic, University of Barcelona, Barcelona, Spain; ⁷Department of Medicine II, University Hospital Würzburg, Würzburg, Germany; ⁸Department of Pediatric Hematology and Oncology, Bambino Gesù Children's Hospital, Rome; ⁹Oncology Unit, Fondazione IRCCS Policlinico San Matteo, Department of Internal Medicine and Medical Therapy, University of Pavia, Pavia; ¹⁰Experimental Hematology Unit, Division of Immunology, Transplantation and Infectious Diseases, University Vita-Salute San Raffaele and Ospedale San Raffaele Scientific Institute, Milan, Italy

*Correspondence to: Prof. Paolo Pedrazzoli, UOC Oncology, Fondazione IRCCS Policlinico S. Matteo, Piazzale Golgi, 19-27100 Pavia, Italy. Tel: +39-0382-501659; E-mail: p.pedrazzoli@smatteo.pv.it

State-of-the-art treatment strategies have drastically ameliorated the outcome of patients affected by cancer. However, resistant and recurrent solid tumors are generally nonresponsive to conventional therapies. A central factor in the sequence of events that lead to cancer is an alteration in antitumor immune surveillance, which results in failure to recognize and eliminate the transformed tumor cell. A greater understanding of the dysregulation and evasion of the immune system in the evolution and progression of cancer provides the basis for improved therapies. Targeted strategies, such as T-cell therapy, not only generally spare normal tissues, but also use alternative antineoplastic mechanisms that synergize with other therapeutics. Despite encouraging success in hematologic malignancies, adaptive cellular therapies for solid tumors face unique challenges because of the immunosuppressive tumor microenvironment, and the hurdle of T-cell trafficking within scarcely accessible tumor sites. This review provides a brief overview of current cellular therapeutic strategies for solid tumors, research carried out to increase efficacy and safety, and results from ongoing clinical trials.

Key words: immunotherapy, solid tumors, T cells, CAR-T, checkpoint inhibitors

Introduction

Resistant, metastatic, or recurrent solid tumors represent unmet clinical challenges, since they are seldom surgically resectable, and largely nonresponsive to radiation and chemotherapy. Relapse and chemoresistance are generally due to cancer cells endowed with stem-like features (CSC) [1], which persist in complex cellular niches that provide a unique microenvironment to protect and promote CSC survival, self-renewal, maintenance, and migratory ability [2].

Modern medical innovations simultaneously try to address tumor heterogeneity in space and over time—including cancer stem cell recognition and eradication—and to initiate, stimulate or amplify a clinically meaningful antitumor immune response.

Tumor cells develop multiple mechanisms to evade immune recognition, including downregulation of tumor antigens, generation of an immunosuppressive microenvironment through secretion of anti-inflammatory cytokines and expression of negative immune regulators able to silence immune effectors [3]. ATCT was introduced and tested in the field of solid tumors; however, it has produced only sporadic responses. This standstill is attributable to limitations in the understanding of tumor biology and interactions with its microenvironment. In addition, techniques used to identify, isolate, and amplify immune effector cells have not been amenable to manufacturing processes that comply with industry standards. Considerable progress has been made along these two avenues: improved knowledge of cancer immune evasion mechanisms and host–tumor interactions have

led to the development of immune checkpoint inhibitors, which are lead products for immune modulation *in vivo*. Simultaneously, considerable technical refinements have opened new prospects for the development of immune cell-based medicinal products [4–6], as recently exemplified by the unprecedented success with chimeric antigen receptor (CAR)-T cells targeting B-cell hematologic malignancies [7–9].

Replicating these results in solid tumors is a major scientific challenge, and may not be feasible to the extent observed with CD19 positive malignancies. Hurdles include the difficulty of identifying target antigens that are homogeneously expressed by all tumor cells while absent on normal tissues, impairment of T-cell trafficking to the tumor site, and limited T-cell persistence and proliferation in a hostile tumor microenvironment that favors immune escape. Emerging evidence suggests that control of resistant or metastatic cancers will not be achieved with a single therapeutic agent, but rather with combinations of conventional and immunotherapeutic cancer treatments [10]. In this perspective, drugs that target immune checkpoints, such as the CTLA4-B7 and the PD1-PDL1 pathways, have led to clinical benefits across a number of different tumor types and may well represent a sort of adjuvant backbone facilitating response to ATCT [11].

Here, we focus on the description of ATCT starting from early experiments and extending to more recent approaches based on gene modification, with the aim of overcoming immune evasion to pave the way for effective solid tumor control.

T cells and tumor recognition

To understand how ATCT contributes to cancer immune surveillance, it is important to get insight into the basics of tumor recognition. Maturation of T cells occurs within the thymus, where the T-cell receptor (TCR) repertoire is shaped by somatic gene rearrangement and selection processes, resulting in a T-cell pool characterized by limited reactivity to self but strong reactivity to foreign antigens. The TCR does not bind directly to its antigen, but recognizes a limited number of short peptides derived from the antigen (epitopes), bound to major histocompatibility complex class I (MHC I) or class II (MHC II) on the target cell surface. Most cells express MHC I, which presents epitopes to CD8+ cytotoxic T lymphocytes (CTLs): lysis of cells expressing cognate epitopes in the context of MHC I is considered the main mechanism responsible for antitumor immune surveillance. However, in recent years, the role of CD4+ T cells, which recognize epitopes complexed with MHC II on the surface of professional antigen-presenting cells, has gained increasing attention, not only for their helper function in sustaining CD8+ T-cell responses and activating innate immunity, but also for direct killing of tumor cells [12].

Adaptive cell therapy with natural T cells endowed with antitumor activity

The modern era of ATCT began with the use of recombinant human interleukin-2 (rhIL-2) in the treatment of melanoma and renal cell cancers, and the observation that this cytokine was able to favor human T-cell growth *in vitro*. This led to the first clinical

applications of cytokine-induced immune effector cells, generated from patients' lymphocytes in the presence of high-dose rhIL-2. Objective clinical responses were reported [13], and although these could not be dissociated from the effects of the co-infusion of high-dose rhIL-2, they provided the basis for clinical evaluation of tumor-specific T cells obtained by *in vitro* culture (Figure 1).

Tumor-infiltrating lymphocytes

T cells directed to tumor-associated antigens (TAA) generally infiltrate tumor tissue, represent a biomarker associated with improved prognosis in some instances and may be extracted, expanded *ex vivo* in the presence of homeostatic cytokines, and reinfused into patients. Evidence that transferred T cells can eradicate cancer in humans comes from the success of ATCT with cultured lymphocytes isolated from tumor biopsies (TILs) in patients with melanoma [14–17]. The first trial, conducted at the National Cancer Institute (NCI) on 20 patients with metastatic disease, showed that transfer of expanded TILs and rh-IL2, preceded by a single dose of cyclophosphamide, could induce more than 50% objective responses including a complete response (CR) with minimal toxicity [13]. Since this preliminary report, results in melanoma have further improved through the optimization of TIL expansion protocols and preparative regimens, combining fludarabine with cyclophosphamide chemotherapy, and testing different doses of total body irradiation, successfully doubling the CR rate to 24% [15, 18]. The importance of lymphodepleting chemotherapy, highlighted in the melanoma setting, has also been crucial for CAR-T-cell therapy protocols [7, 19]. Although TILs could be demonstrated in other solid tumors, their expansion and *in vivo* efficacy did not reach the success of melanoma treatment, experienced in hundreds of patients treated at the NCI [5] and recently duplicated by other groups [20, 21], and by a multicenter phase II study that is currently being expanded for registration purposes [22]. Recently, preliminary data showing objective responses and CRs were reported for cholangiocarcinoma (CCA) [23] and cervical cancer [24], and a number of clinical trials are currently ongoing to test ATCT with TILs in gastrointestinal, gynecological, head and neck, breast and lung cancers.

Virus-specific cytotoxic T lymphocytes

Among T cells targeting antigens through their native receptors, CTLs directed to viral antigens, obtained by *in vitro* culture in the presence of virus-derived TAA, have also been extensively used [25]. Epstein-Barr virus (EBV)-specific T cells expanded from the hematopoietic stem cell donor have been demonstrated to prevent and treat virus-associated lymphoproliferative diseases [26–28]. Autologous EBV-specific CTLs, which have shown antitumor activity in patients with less immunogenic hematologic malignancies, such as Hodgkin or non-Hodgkin lymphoma [29, 30], have also been successfully employed in the treatment of EBV-associated solid tumors, such as nasopharyngeal carcinomas (NPC) [31–35]. More than 80 NPC patients treated with autologous EBV-targeted ATCT for recurrent, metastatic disease, have so far been reported, with a promising 20% objective response rate, including 10% CR. Attempts to enhance T-cell survival,

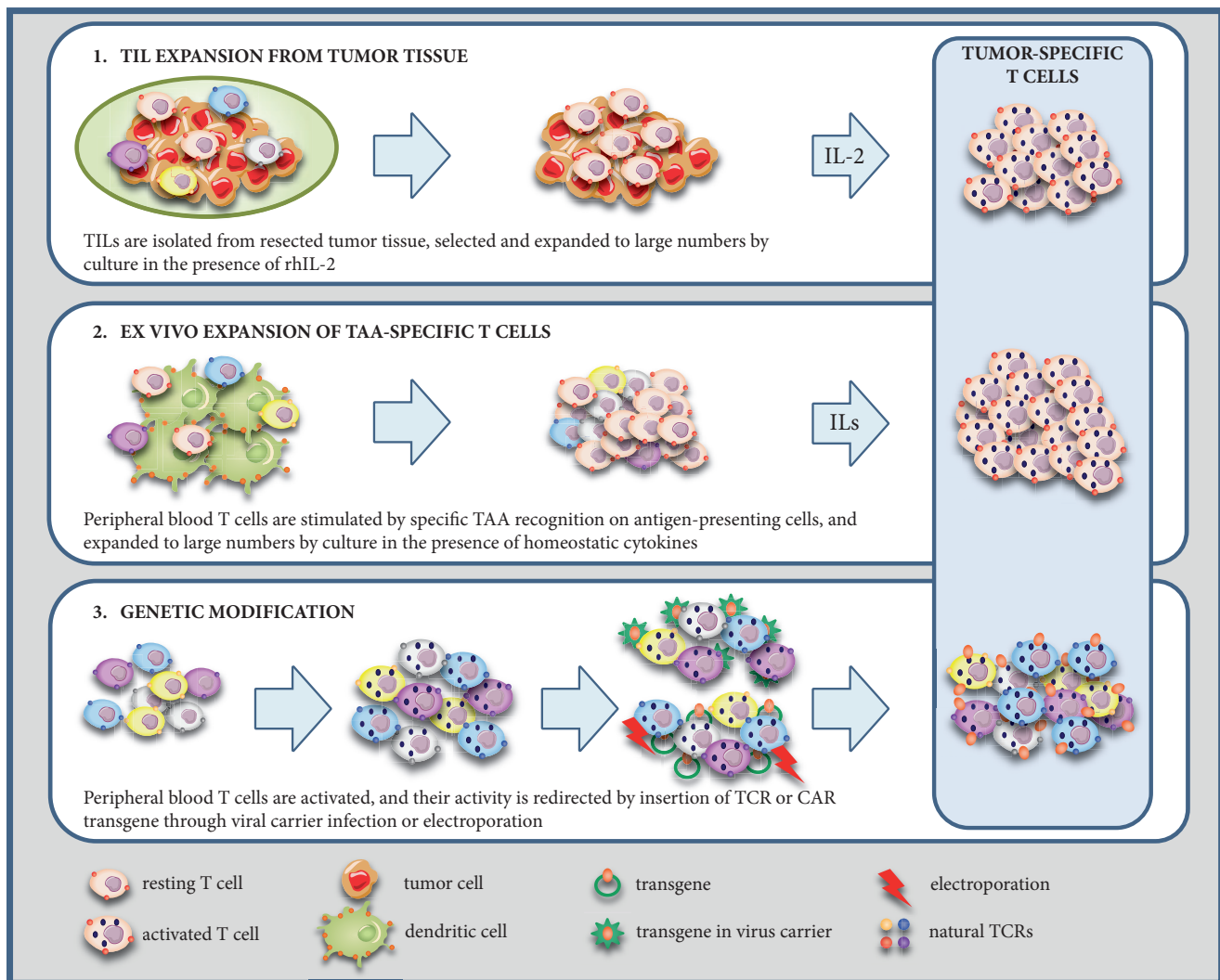


Figure 1. ATCT for solid tumors: manufacturing strategies. T cells derived from tumor resection tissue or peripheral blood are manipulated according to the illustrated approaches, in order to manufacture tumor-directed cellular products for ATMP. IL-2, recombinant human interleukin-2; ILs, interleukins; TAA, tumor-associated antigens; CAR, chimeric antigen receptors.

trafficking and effector function by administering a lymphodepleting preparative regimen, based on the melanoma TIL therapy experience, did not improve outcomes in this clinical setting [33, 34]. In line with EBV ATCT, cytomegalovirus-specific CTLs have been explored in clinical trials for glioblastoma [36], and human papilloma virus (HPV)-directed TILs have been successfully employed in HPV-associated malignancies [24].

T cells targeting other TAA

For tumors not associated with viruses, several classes of TAAs have been explored as potential targets [37]. These include antigens that are mostly non-tumor-selective, as they are found in normal tissues but overexpressed on tumors, or expressed only during fetal development or in immune-privileged sites such as testes. T cells targeting these TAAs may potentially attack healthy tissues expressing even low-level antigen, causing severe adverse events, referred to as on-target off-tumor toxicities, when an essential organ is involved. With recent advances in genomic

technologies, a quest for mutational and transcriptional tumor profiles was embraced, in order to identify optimal personalized targeted therapies. While the majority of mutant gene products are not targetable with the currently available pharmaceuticals, the genetic sequencing approach has allowed the identification of neoantigens generated by gene rearrangements or mutations [38]. Genomic studies suggest how the latter may be the main target antigens underlying the success of therapy with immune checkpoint inhibitors or TILs [39, 40]. Indeed, when compared with non-mutated self-antigens, the T-cell pool available for these antigens should not be affected by central T-cell tolerance. Shared hot-spot mutations of driver oncogenes are more likely to be expressed by all cells within a tumor as well as across tumor samples and histologies, and identification of TCRs targeting these neoantigens may be used as off-the-shelf reagents for TCR gene therapy. Moreover, their use is likely to limit the risk of on-target off-tumor toxicity. A proof of principle for the potential *in vivo* efficacy of T cells that target tumor neoantigens arising from cancer mutations has been provided in the setting of solid tumors [41].

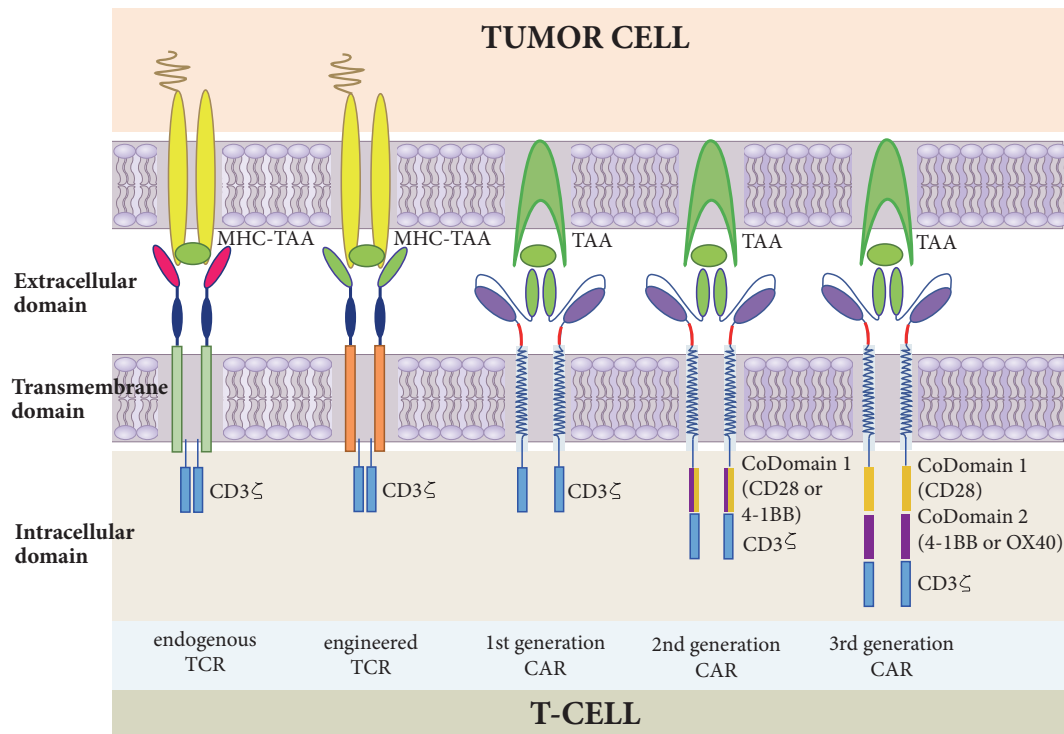


Figure 2. Structure of endogenous TCR, engineered TCR and CAR receptors. In first generation CARs, the variable heavy and light single-chains (antigen-binding moiety) are linked by a spacer to the transmembrane region, usually derived from CD28. The intracellular domain includes the CD3 ζ signaling pathway machinery, which activates the T cell in response to the specific tumor antigen binding. In second and third generation CARs, co-stimulatory domains, such as CD28 and/or 4-1BB, are added to improve antitumor potency, cytokine production, and persistence of the T cell. MHC, major histocompatibility complex; TAA, tumor-associated antigen; CoDomain, costimulatory domain.

Adaptive cell therapy with engineered T cells: the era of CARs

ATCT with TILs/CTLs has been mostly successful in cancers characterized by strong immunogenicity due to high frequency of mutational events [38]. The targeting of cancers such as pediatric solid malignancies or other frequent tumors, such as breast or prostate cancer, for which neoantigens generated by non-synonymous mutations are limited, is a more daunting task. Indeed, TAA are mostly overexpressed self-antigens, and, as such, subject to tolerance. In addition, TAA recognition by T cells is MHC-restricted, and the majority of solid tumors downregulate MHC expression as an immune escape mechanism. In order to overcome these hurdles, genetically engineered T cells have emerged as an alternative to TILs and CTLs (Figure 1).

Genetic retargeting has been obtained by means of two different approaches: (i) transfer of natural tumor-specific TCRs isolated from high-avidity T cells that recognize cancer antigens [42] and (ii) transfer of synthetic TCRs, also referred to as CARs [43] (Figure 2).

T cells engineered with natural TCRs

TCR therapy trials have mostly targeted overexpressed self/tumor antigens also expressed by healthy adult cells, such as gp100 and Melan-A/MART-1, or oncofetal antigens, present on healthy cells exclusively during fetal development, and ectopically expressed in tumors, such as NY-ESO-1 and MAGE. Autologous T cells

retrovirally transduced with a TCR specific for NY-ESO-1 resulted in objective clinical responses in 61% of patients with synovial cell sarcoma and 55% of patients with melanoma [44, 45], and one of three patients treated with autologous T cells engineered to express a TCR against human carcinoembryonic antigen (CEA) had an objective regression of metastatic colorectal cancer (CRC) [46]. The rate of objective responses obtained in melanoma patients did not significantly differ from those obtained in clinical trials evaluating TILs, but fewer sustained CRs were observed. These data are likely due to inherent limitations of TCR biology. In particular, HLA-restricted specificity limits the fraction of potential patients to those expressing the relevant HLA allele recognized by T cells, and clinical efficacy is dependent upon TCR affinity and the expression of MHC-antigen complexes on the tumor cell surface [42]. The transferred and endogenous TCRs compete for CD3 binding, resulting in mutual receptor dilution and lower antitumor efficacy. Moreover, the α and β chains of the endogenous TCR may mispair with the respective chains of the transferred TCR, forming hybrid receptors with unpredictable and potentially detrimental specificities. Furthermore, targeting of antigenic specificities that are expressed at low level on normal tissues has led to severe on-target off-tumor toxicity [47, 48].

T cells engineered with CARs

In order to circumvent the challenges posed by antigen escape variants, researchers have focused on the delivery of synthetic

CARs, constructed by fusing an antigen-binding domain that is derived from a single-chain variable fragment of a monoclonal antibody against a tumor surface antigen, to a flexible spacer region, a trans-membrane domain and the TCR intracellular domain, traditionally consisting of the CD3 zeta (ζ) chain, capable of activating T cells. As the antigen-directed exodomain binds directly to target cell surface epitopes, CAR recognition of tumor target cells is HLA-unrestricted, thus resistant to tumor escape mechanisms related to HLA downmodulation and altered processing. Initial trials with CAR-T cells targeting solid and hematologic tumors showed limited clinical results, with poor *in vivo* expansion and duration [49–52]. In the setting of solid tumors, the unique exception was CAR-T-cell therapy targeting the disialo-ganglioside GD2, which induced complete remission in 3/11 pediatric patients with neuroblastoma [53].

CAR-T-cell therapy in hematologic malignancies. Progressive improvements in the design of CARs have led to second and third generation molecules (Figure 2), that incorporate one or two additional costimulatory domains, such as CD28, 4-1BB and OX40, which enhance killing activity and expansion potential (CD28), as well as *in vivo* persistence (4-1BB), resulting in dramatic results in hematologic malignancies [54–61], with 81% 3-month remission rate and 50% event-free survival at 12 months in a phase II global multicenter clinical trial with CD19-CAR-T cells in children and young adults treated for acute lymphoblastic leukemia [61].

In every trial, efficacy correlated with the expansion of CAR-T cells, and relapses were due to immune escape mechanisms, such as targeted antigen loss or development of immunity directed to the CAR molecule mouse portion [58, 59]. The increase in CAR-T-cell clinical efficacy, however, was paralleled by the potential to induce severe adverse events, such as cytokine release syndrome (CRS), neurological toxicity, and on-target off-tumor toxicities [57–62]. While still within the field of lymphoid malignancies, the fast-growing experience when treating multiple myeloma patients with CAR-T cells targeting BCMA suggests that the intensity of side-effects may vary depending on the targeted tumor antigen, and that side-effects may be manageable even in an older population [63–65]. To date, two autologous CAR-T cells targeting CD19 have been approved by FDA and EMA for the treatment of patients with refractory/relapsed ALL or high-grade B-NHL. Since the two medicinal products benefited from the fast track procedure, the number of patients that contributed data were relatively small; thus, additional data obtained in ‘real-life’ practice after long-term follow up are needed to fully appraise their clinical utility.

CAR-T-cell therapy in solid tumors. On the basis of results obtained in hematologic malignancies, a number of trials have been started to test the efficacy of CAR-T cells in solid tumors (supplementary Table S1, available at *Annals of Oncology* online). To date, however, clinical results in this setting have been much less encouraging, with a general lack of therapeutic response and presence of on-target off-tumor toxicity. However, some studies have achieved promising outcomes that justify further exploration of this approach in solid tumors. The group at Baylor College of Medicine pioneered the use of GD2-specific CAR-T cells for neuroblastoma [53, 66]. In a trial of 19 patients with

high-risk neuroblastoma, 3 had a CR to CAR-T-cell infusion, with only local pain and slight fever as adverse events [53, 66]. The same group evaluated the safety and antitumor activity of second-generation CD28 ζ HER2-specific CAR-modified virus-specific T cells in patients with progressive glioblastoma multiforme (GBM). The results showed that CAR-T cells are well tolerated, with no dose-limiting toxic effects, and can produce objective responses, as one patient showed a PR for more than 9 months, and seven patients had SD lasting several months [67]. Other clinical trials have demonstrated the feasibility and safety [68] and clinical efficacy [69] of second-generation EGFRvIII-specific and IL13BB ζ -specific CAR-T cells, respectively, in patients with refractory GBM.

Investigators at the University of Pennsylvania explored an approach based on mRNA-transduced CAR-T cells that target mesothelin (CART-meso) in patients with advanced malignant pleural mesothelioma or advanced pancreatic cancer. In the first two patients reported, CART-meso cells showed some antitumor activity *in vivo*, in the absence of distinct toxicities [70].

Second-generation CAR-T cells specific for epidermal growth factor receptor (EGFR) were employed in a phase I study that treated 11 patients with advanced non-small-cell lung cancer, obtaining 2 PR and 5 SD lasting from 2 to 8 months, with limited adverse events including skin toxicity, nausea, vomiting, dyspnea and hypotension [71]. EGFR-CAR-T cells were also applied by the same investigators in the treatment of one patient with refractory, metastatic CCA, demonstrating a PR lasting more than 1 year. However, the objective response was accompanied by epidermal and endothelial toxicity [72]. A clinical trial of CEA CAR-T therapy in 10 patients with metastatic CRC achieved SD in 7 patients who were in progression after previous treatments, without severe adverse events related to CAR-T therapy [73]. Similar to these experiences, second-generation HER2-specific CAR-T cells, used in a phase I clinical trial conducted on 19 patients with refractory HER2-positive sarcoma, could induce SD lasting from 12 weeks to 14 months in 4 assessable patients [74].

Toxicity of CAR-T-cell therapy. Various toxicities were observed after CAR-T-cell infusion in solid tumors. In the setting of hematologic malignancies, CRS is a frequent, potentially severe adverse event of CAR-T-cell therapy. The release of pro-inflammatory cytokines by the infused cells induces monocyte and macrophage activation that can lead to multiple organ failure. Currently, this complication is managed by cytokine level assessment and early administration of the anti-IL6R monoclonal antibody tocilizumab. However, CRS, as well as the neurological toxicity sometimes observed in the hematologic setting, is not a common event after CAR-T-cell therapy for solid tumors, possibly since the tumor load is lower. Conversely, trials conducted in solid tumor cohorts with CAR-T cells produced critical, unexpected on-target off-tumor toxicities, resulting from the recognition by CAR-T cells of TAA expressed on healthy tissues [75, 76]. Some antigens specific to tumors have been identified that result in more limited off-tumor effects, but many of these targets have mediated poor clinical efficacy. Moreover, as for hematologic malignancies, solid tumors undergo antigen escape due to selection pressure favoring tumor cells lacking the targeted antigen. Strategies to increase tumor selectivity while sparing healthy tissues are being evaluated to control on-target off-tumor toxicity.

Improving safety and efficacy of T-cell therapy for solid tumors

A significant amount of research has focused on enhancing the activated T-cell response against tumor cells, promoting their *in vivo* expansion, and improving their persistence in the host. Interventions encompass genetic modifications to increase T-cell affinity or avidity [77, 78] or to induce cytokine production [79–81], and selection of specific T-cell subsets [82, 83]. Regarding the choice of genetic modification vehicle, so far retroviral gene transfer vectors have been mostly used in CAR-T-cell trials, as they have the advantage of inducing high-level stable transduction in stimulated human T cells. However, immunogenicity and insertional mutagenesis, together with the costs due to viral vector production and use, has led to explore alternative methods, such as lentiviral vectors, that do not require cell cycling induction and cause very limited immune and inflammatory responses, or the use of gene transfer forms that do not depend on costly viral vector production, such as transposons, or the non-integrating systems like designer nucleases, DNA plasmids or RNA transfer [52, 57, 84, 85]. Strategies to enhance affinity to self-antigens have increased efficacy, but also led to unexpected severe off-target toxicity, when previously unknown cross-reactivity was observed with epitopes derived from unrelated proteins expressed by normal tissues [48].

T-cell migration and intra-tumor trafficking

The first hurdle encountered by T cells when targeting solid malignancies is the difficulty in migrating to and adequately penetrating the tumor. Solid cancers may evade immune surveillance by secreting chemokines which inhibit T-cell migration into the tumor [86]. Several different chemotherapeutics have been shown to induce chemokines and chemokine ligands [87], and their incorporation into a combined treatment approach with T cells may enhance CD8+ T-cell recruitment and reduce tumor growth. Alternatively, CAR-T cells may be modified to express chemokines that enhance their intra-tumor trafficking [88]. It has been shown that the forced expression of chemokine CCR2 on CAR-T cells targeting GD2 in neuroblastoma enhances T-cell infiltration and augments the antitumor activity of the transferred cells [89]. In addition, T cells migrating to the tumor may be hindered by a physical barrier of fibrotic extracellular matrix produced by tumor fibroblasts and myeloid cells. As loss of heparanase, an enzyme that contributes to degradation of extracellular matrix, has been observed in T cells after *in vitro* culture, its overexpression in CAR-T cells can enhance T-cell infiltration [90]. Alternatively, targeting the surrounding non-malignant stroma using CAR-T cells may be an option [91].

Tumor microenvironment repolarization

The complex immunosuppressive tumor microenvironment, and the segregation of tumor cells in areas that may be difficult to access, are other major hurdles to the efficacy of ATCT. Factors that negatively regulate T-cell activity and facilitate tumor immune evasion include tumor-resident regulatory T cells and myeloid-derived suppressor cells, as well as the presence of cytokines such as IL-10, transforming growth

factor beta, vascular endothelial growth factor, and prostaglandin E2. Additionally, negative feedback is provided by signaling through the programmed death-1 (PD-1) and CTLA-1 pathways (Figure 3A).

T-cell arming. To repolarize the tumor microenvironment and fully restore the effector function of transferred T cells, preclinical studies have tested the feasibility of conferring resistance to immunosuppressive molecules [92], or the ability to deliver cytokines to either activate host effectors or hinder host suppressors [79–81, 93], or coupling CAR-T cells with switch receptors interfering with the inhibitory signal provided by immune checkpoints [94] (Figure 3B). The cytokine-producing ‘TRUCKs’ (T cells Redirected for Universal Cytokine Killing) [95] significantly enhanced efficacy of MUC-16^{ecto} CAR against a preclinical model of ovarian carcinoma [96], and are currently being tested in phase I clinical trials.

Combination of T-cell therapy with checkpoint inhibitors. In addition to T-cell arming, an approach that combines ATCT with immune checkpoint inhibitors, antitumor agents that act by releasing the ‘brakes’ on pre-existing tumor-reactive T cells and facilitate generation of new T-cell responses, may represent a strategy to improve the activity and tumor killing by T-cell generated *ex vivo* [97–99] (Figure 3B). Recent positive experiences have been observed in refractory hematologic malignancies with CD19 CAR-T cells combined with the PD-1 blocking agent pembrolizumab [100, 101]; similar strategies are being investigated in solid tumors [102].

Increasing T-cell therapy safety

Solid tumors typically exhibit a heterogeneous pattern of TAA, promoting antigen escape. Thus, identifying patient-specific, tumor-specific mutated antigens [41, 103], potentially present also in cancer types that exhibit low-level immunogenicity, may help develop treatments for multiple cancer types so far not amenable to ATCT, and limit toxicity. Despite these advances in targeting, toxicity may still ensue. In order to limit severe consequences, strategies aimed at hampering an unwanted off-target T-cell response are being investigated. Recently, a pharmacologic on/off switch based on the use of the tyrosine kinase inhibitor dasatinib has been investigated [104].

Suicide gene insertion. The introduction of suicide genes may help remove undesirable toxicity [105, 106]. The first attempt at introducing suicide genes into T cells involved the use of HSV-thymidine kinase, which converts ganciclovir into a toxic metabolite, and proved effective in controlling graft-versus-host disease (GvHD) in several trials [105, 107]. However, the lytic response observed was slow, and the viral proteins may stimulate immunogenicity. More recently, the inducible-caspase 9 system was designed and tested. It is based on the expression of a monomeric iCasp9 domain that dimerizes upon administration of a small molecule, leading to cleavage of caspase 3 and apoptosis of T cells [106]. Alternatively, in the case of CAR-T cells, incorporating epitopes like RQR8/CD20 into the CAR construct provides a target for their depletion with antibodies such as rituximab [108].

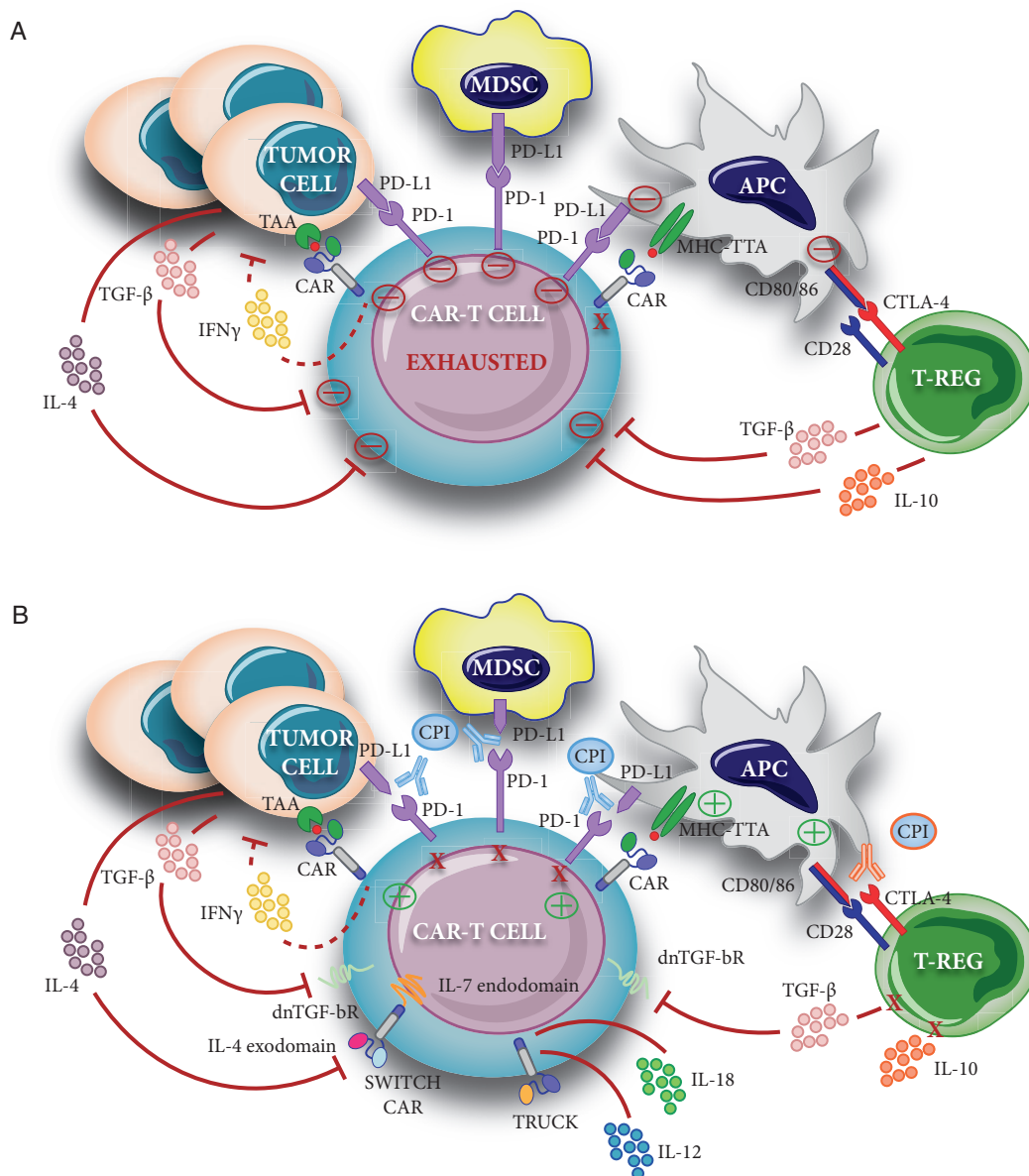


Figure 3. Summary of interventions to overcome the immunosuppressive tumor microenvironment and enhance T-cell efficacy. Tumor cells and suppressor cells (i.e. T-REGs; MDSCs) up-regulate inhibitory ligands, such as PD-L1 or CTLA-4, and secrete suppressive cytokines and factors (A). The use of checkpoint inhibitor agents, or modification of CAR T cells to induce cytokine secretion (TRUCKs), expression of a dominant negative (dn) TGFβ-receptor or conversion of a negative IL-4 signal to a positive IL-7 signal (SWITCH CARs) are examples of strategies able to help overcome tumor tolerance and restore tumor immunogenicity (B). CAR, chimeric antigen receptor; MDSC, myeloid-derived suppressor cells; APC, antigen-presenting cells; T-REG, regulatory T cell; MHC, major histocompatibility complex; TAA, tumor-associated antigen; CPI, checkpoint inhibitor; IL, interleukin; TGF-β, tumor growth factor-β; IFNγ, interferon-γ; dnTGF-βR, dominant negative TGFβ-receptor; SWITCH CAR, chimeric switch receptors; TRUCK, T cells redirected for universal cytokine killing.

Specificity enhancement. Other strategies have been devised to increase CAR-T-cell specificity, and, therefore, limit toxicity. One approach is based on T-cell modification with two different CARs, one containing the CD3ζ signaling domain to activate T-cell function, and the other providing the co-stimulation signal by CD28 and/or CD137 [109–111]. Full CAR-T-cell activation and function are only achieved in the context of the presence of both antigens, an unlikely occurrence in the case of cells from normal tissue. Another strategy to obtain differential recognition of malignant and normal cells is based on affinity-tuned CAR-T

cells that activate T cells based on the density of target antigen expression. Two independent studies demonstrated that a CAR-T cell with reduced affinity rendered T cells preferentially activated by a high, but not low, density of target antigen [112, 113].

Widening T-cell therapy access

Despite the therapeutic potential of ATCT, logistics and regulatory hurdles have limited translation into commercially available therapies. Regulatory demands for marketing authorization are

burdensome for academic institutions, although the new European clinical trial regulation favors accelerated novel drug evaluation and approval schemes to ensure early access to innovative therapies [114]. The majority of subjects treated to date with ATCT have received autologous or allogeneic dedicated T cells, but this approach may not be best suited for widespread cost-effective delivery of cellular therapy, since these are personalized medicines that are produced on-demand through a complex and costly supply chain, thus implying some delay in manufacturing of the final product and the risk that the disease will evolve and be fatal for candidate patients. Allogeneic ATCT, including CAR-T cells, represent potential off-the-shelf products, that could possibly be manufactured in small batches without the need for tailored products. However, they carry the risk of immune rejection by the host, and their short persistence may require additional therapies to consolidate responses, and could potentially cause GVHD, although in preliminary clinical trials *de novo* GVHD was not observed [115]. Gene editing offers the prospect of addressing human leukocyte (HLA) barriers and the development of universal T-cell therapies [113, 116, 117]. Recently, 'off-the-shelf' T cells modified using transcription activator-like effector nucleases and expressing CD19 or CD123 CAR are being used to treat refractory relapsed B-ALL [118] and myeloid malignancies, respectively.

Discussion

Conclusions

The major obstacle to further development of ATCT in solid tumors is the immunosuppressive environment. Strategies to counteract these tolerogenic mechanisms will be required to further enhance the efficacy of ATCT. In this scenario, T-cell therapy, alone or in combination with immune checkpoint inhibitors or other agents targeting either the cancer cell or the tumor environment, will likely play a role in improving cancer treatment outcomes. Designing and selecting the most appropriate clinical trials to rapidly identify combinatorial approaches that are efficient in the different patient populations, and devising new and sustainable reimbursement modalities or developing network models for ATMP production in academic centers will add to the many biological and medical challenges faced by the healthcare and patients' communities.

Acknowledgements

The authors wish to thank all members of the CTIWP activities, the president of EBMT, and all members of EBMT Board of Directors, Executive Committee, Scientific Council and Central Registry Office for the precious and continuous support to the CTIWP.

Funding

This work was supported by the Italian Ministry of Health (grant Ricerca Finalizzata number RF-2011-02351315 to PP), by Associazione Italiana per la Ricerca sul Cancro (AIRC Ig 18458,

and AIRC 5 per Mille Rif. 22737, to CB), and by Fondazione IRCCS Policlinico San Matteo (grant Ricerca Corrente number 08067617 to PP, 08069113 and 08069119 to PC).

Disclosure

PC: Atara Therapeutics: advisory board; Bellicum Pharmaceuticals: advisory board. CC: Gilead: co-investigator, speaker, advisory board, hospitalities; Celgene: co-investigator, speaker. Novartis: co-investigator, speaker. Sanofi SA: research support, speaker, advisory board, hospitalities; Miltenyi: research support; Terumo BCT: speaker, advisory board; Fresenius: research support. CB: received a Research contract from Intellia Therapeutics and participated to the advisory boards of Molmed, Intellia Therapeutics, TxCell, Novartis, GSK, Allogene. She is inventor of patents in the field of adoptive T-cell therapy. The remaining authors have declared no conflicts of interest.

References

- Al-hajj M, Wicha MS, Benito-Hernandez A et al. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 2003; 100(7): 3983–3988.
- Mantovani A, Allavena P, Sica A et al. Cancer-related inflammation. *Nature* 2008; 454(7203): 436–444.
- Spranger S, Gajewski TF. Tumor-intrinsic oncogene pathways mediating immune avoidance. *Oncoimmunology* 2016; 5(3): e1086862.
- Topalian SL, Weiner GJ, Pardoll DM. Cancer immunotherapy comes of age. *J Clin Oncol* 2011; 29(36): 4828–4836.
- Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. *Science* 2015; 348(6230): 62–68.
- Bonini C, Mondino A. Adoptive T-cell therapy for cancer: the era of engineered T cells. *Eur J Immunol* 2015; 45(9): 2457–2469.
- Maude SL, Frey N, Shaw PA et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med* 2014; 371(16): 1507–1517.
- Park JH, Riviere I, Gonen M et al. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. *N Engl J Med* 2018; 378(5): 449–459.
- Kochenderfer JN, Somerville RPT, Lu T et al. Long-duration complete remissions of diffuse large B cell lymphoma after anti-CD19 chimeric antigen receptor T cell therapy. *Mol Ther* 2017; 25(10): 2245–2253.
- Apetoh L, Ladoire S, Coukos G et al. Combining immunotherapy and anticancer agents: the right path to achieve cancer cure? *Ann Oncol* 2015; 26(9): 1813–1823.
- Granier C, De Guellebon E, Blanc C et al. Mechanisms of action and rationale for the use of checkpoint inhibitors in cancer. *ESMO Open* 2017; 2(2): e000213.
- Melissen M, Slingluff CL Jr. Vaccines targeting helper T cells for cancer immunotherapy. *Curr Opin Immunol* 2017; 47: 85–92.
- Rosenberg SA, Lotze MT, Muul LM et al. A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. *N Engl J Med* 1987; 316(15): 889–897.
- Rosenberg SA, Packard BS, Aebersold PM et al. Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report. *N Engl J Med* 1988; 319(25): 1676–1680.
- Dudley ME, Yang JC, Sherry R et al. Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. *J Clin Oncol* 2008; 26(32): 5233–5239.

16. Rosenberg SA, Yang JC, Sherry RM et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. *Clin Cancer Res* 2011; 17(13): 4550–4557.
17. Gattinoni L, Finkelstein SE, Klebanoff CA et al. Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of adoptively transferred tumor-specific CD8⁺ T cells. *J Exp Med* 2005; 202(7): 907–912.
18. Goff SL, Dudley ME, Citrin DE et al. Randomized, prospective evaluation comparing intensity of lymphodepletion before adoptive transfer of tumor-infiltrating lymphocytes for patients with metastatic melanoma. *J Clin Oncol* 2016; 34(20): 2389–2397.
19. Turtle CJ, Hanafi LA, Berger C et al. Immunotherapy of non-Hodgkin's lymphoma with a defined ratio of CD8⁺ and CD4⁺ CD19-specific chimeric antigen receptor-modified T cells. *Sci Transl Med* 2016; 8(355): 355ra116.
20. Radvanyi LG, Bernatchez C, Zhang M et al. Specific lymphocyte subsets predict response to adoptive cell therapy using expanded autologous tumor-infiltrating lymphocytes in metastatic melanoma patients. *Clin Cancer Res* 2012; 18(24): 6758–6770.
21. Besser MJ, Shapira-Frommer R, Itzhaki O et al. Adoptive transfer of tumor-infiltrating lymphocytes in patients with metastatic melanoma: intent-to-treat analysis and efficacy after failure to prior immunotherapies. *Clin Cancer Res* 2013; 19(17): 4792–4800.
22. Sarnaik A, Khushalani NI, Chesney JA et al. Safety and efficacy of cryopreserved autologous tumor infiltrating lymphocyte therapy (LN-144, lifileucel) in advanced metastatic melanoma patients who progressed on multiple prior therapies including anti-PD-1. *J Clin Oncol* 2019; 37(Suppl 15); Abstr 2518.
23. Tran E, Turcotte S, Gros A et al. Cancer Immunotherapy based on mutation-specific CD4⁺ T cells in a patient with epithelial cancer. *Science* 2014; 344(6184): 641–645.
24. Stevanović S, Draper LM, Langan MM et al. Complete regression of metastatic cervical cancer after treatment with human papillomavirus-targeted tumor-infiltrating T cells. *J Clin Oncol* 2015; 33(14): 1543–1550.
25. Pedrazzoli P, Comoli P, Montagna D et al. Is adoptive T-cell therapy for solid tumors coming of age? *Bone Marrow Transplant* 2012; 47(8): 1013–1019.
26. Rooney CM, Smith CA, Ng CY et al. Use of gene-modified virus-specific T lymphocytes Epstein-Barr-virus-related lymphoproliferation. *Lancet* 1995; 345(8941): 9–13.
27. Heslop HE, Slobod KS, Pule MA et al. Plenary paper Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. *Blood* 2010; 115(5): 925–936.
28. Comoli P, Basso S, Zecca M et al. Preemptive therapy of EBV-related lymphoproliferative disease after pediatric haploidentical stem cell transplantation. *Am J Transplant* 2007; 7(6): 1648–1655.
29. Bollard CM, Aguilar L, Straathof KC et al. Cytotoxic T lymphocyte therapy for Epstein-Barr virus+ Hodgkin's disease. *J Exp Med* 2004; 200(12): 1623–1633.
30. Bollard CM, Gottschalk S, Torrano V et al. Sustained complete responses in patients with lymphoma receiving autologous cytotoxic T lymphocytes targeting Epstein-Barr virus latent membrane proteins. *J Clin Oncol* 2014; 32(8): 798–808.
31. Straathof KCM, Bollard CM, Papat U et al. Treatment of nasopharyngeal carcinoma with Epstein-Barr virus-specific T lymphocytes. *Blood* 2005; 105(5): 1898–1905.
32. Comoli P, Pedrazzoli P, Maccario R et al. Cell therapy of stage IV nasopharyngeal carcinoma with autologous Epstein-Barr virus-targeted cytotoxic T lymphocytes. *J Clin Oncol* 2005; 23(35): 8942–8949.
33. Louis CU, Straathof K, Bollard CM et al. Enhancing the in vivo expansion of adoptively transferred EBV-specific CTL with lymphodepleting CD45 monoclonal antibodies in NPC patients. *Blood* 2009; 113(11): 2442–2450.
34. Secondino S, Zecca M, Licitra L et al. T-cell therapy for EBV-associated nasopharyngeal carcinoma: preparative lymphodepleting chemotherapy does not improve clinical results. *Ann Oncol* 2012; 23(2): 435–441.
35. Huang J, Fogg M, Wirth LJ et al. Epstein-Barr virus-specific adoptive immunotherapy for recurrent, metastatic nasopharyngeal carcinoma. *Cancer* 2017; 123(14): 2642–2650.
36. Schuessler A, Smith C, Beagley L et al. Autologous T-cell therapy for cytomegalovirus as a consolidative treatment for recurrent glioblastoma. *Cancer Res* 2014; 74(13): 3466–3476.
37. Wurz GT, Kao C-J, DeGregorio MW. Novel cancer antigens for personalized immunotherapies: latest evidence and clinical potential. *Ther Adv Med Oncol* 2016; 8(1): 4–31.
38. Lawrence MS, Stojanov P, Polak P et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature* 2013; 499(7457): 214–218.
39. Tran E, Ahmadzadeh M, Lu Y et al. Immunogenicity of somatic mutations in human gastrointestinal cancers. *Science* 2015; 350(6266): 1387–1390.
40. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. *Science* 2015; 348(6230): 69–74.
41. Tran E, Robbins PF, Lu YC et al. T-cell transfer therapy targeting mutant KRAS in cancer. *N Engl J Med* 2016; 375(23): 2255–2262.
42. Morgan RA, Dudley ME, Wunderlich JR et al. Cancer regression in patients engineered lymphocytes. *Science* 2006; 314(5796): 126–129.
43. Eshhar Z, Waks T, Gross G et al. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. *Proc Natl Acad Sci USA* 1993; 90(2): 720–724.
44. Robbins PF, Morgan RA, Feldman SA et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. *J Clin Oncol* 2011; 29(7): 917–924.
45. Robbins PF, Kassim SH, Tran TLN et al. 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* 2015; 21(5): 1019–1027.
46. Parkhurst MR, Yang JC, Langan RC et al. T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. *Mol Ther* 2011; 19(3): 620–626.
47. Johnson LA, Morgan RA, Dudley ME et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. *Blood* 2009; 114(3): 535–547.
48. Linette GP, Stadtmauer EA, Maus MV et al. Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. *Blood* 2013; 122(6): 863–872.
49. Kershaw MH, Westwood JA, Parker LL et al. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. *Clin Cancer Res* 2006; 12(20): 6106–6115.
50. Park JR, Digiusto DL, Slovak M et al. Adoptive transfer of chimeric antigen receptor re-directed cytolytic T lymphocyte clones in patients with neuroblastoma. *Mol Ther* 2007; 15(4): 825–833.
51. Lamers CH, Sleijfer S, Vulto AG et al. Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. *J Clin Oncol* 2006; 24: 22–24.
52. Till BG, Jensen MC, Wang J et al. Adoptive immunotherapy for indolent non-Hodgkin lymphoma and mantle cell lymphoma using genetically modified autologous CD20-specific T cells. *Blood* 2008; 112(6): 2261–2271.
53. Pule M, Savoldo B, Myers GD et al. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. *Nat Med* 2008; 14(11): 1264–1270.
54. Brentjens RJ, Riviere I, Park JH et al. Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. *Blood* 2011; 118(18): 4817–4828.
55. Porter DL, Levine BL, Kalos M et al. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med* 2011; 365(8): 725–733.

56. Till B, Jensen M, Wang J et al. CD20-specific adoptive immunotherapy for lymphoma using a chimeric antigen receptor with both CD28 and 4-1BB domains: pilot clinical trial results. *Blood* 2012; 119(17): 3940–3950.
57. Grupp SA, Kalos M, Barrett D et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med* 2013; 368(16): 1509–1518.
58. Cruz CR, Micklethwaite KP, Savoldo B et al. Infusion of donor-derived CD19-redirection virus-specific T cells for B-cell malignancies relapsed after allogeneic stem cell transplant: a phase 1 study. *Blood* 2013; 122(17): 2965–2973.
59. Lee DW, Kochenderfer JN, Stetler-Stevenson M et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. *Lancet* 2015; 385(9967): 517–528.
60. Kochenderfer JN, Dudley ME, Kassim SH et al. 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* 2015; 33(6): 540–549.
61. Maude SL, Laetsch TW, Buechner J et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med* 2018; 379: 439–448.
62. Brentjens R, Yeh R, Bernal Y et al. Treatment of chronic lymphocytic leukemia with genetically targeted autologous T cells: case report of an unforeseen adverse event in a phase I clinical trial. *Mol Ther* 2010; 18: 8–10.
63. Cornell RF, Lundry Locke F, Russell Bishop M et al. A phase 1 multicenter study evaluating KITE-585, an autologous anti-BCMA CAR T-cell therapy, in patients with relapsed/refractory multiple myeloma. *J Clin Oncol* 2018; 36(Suppl 15); doi.org/10.1200/JCO.2018.36.15_suppl.TPS31
64. Shah N, Alsina M, Siegel DS et al. Initial results from a phase 1 clinical study of bb21217, a next-generation anti BCMA CAR T therapy. *Blood* 2018; 132: 488.
65. Gregory T, Cohen AD, Costello CL et al. Efficacy and safety of P-BCMA-101 CAR-T cells in patients with relapsed/refractory (r/r) multiple myeloma (MM). *Blood* 2018; 132: 1012.
66. Louis CU, Savoldo B, Dotti G et al. Antitumor activity and long-term fate of chimeric antigen receptor – positive T cells in patients with neuroblastoma. *Blood* 2011; 118(23): 6050–6053.
67. Ahmed N, Brawley V, Hegde M et al. HER2-specific chimeric antigen receptor-modified virus-specific T cells for progressive glioblastoma: a phase 1 dose-escalation trial. *JAMA Oncol* 2017; 3(8): 1094–1101.
68. O'Rourke DM, Nasrallah MP, Desai A et al. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. *Sci Transl Med* 2017; 9(399); pii:eaaa0984. doi: 10.1126/scitranslmed.aaa0984.
69. Brown C, Alizadeh D, Starr R et al. Regression of glioblastoma after chimeric antigen receptor T-cell therapy. *N Engl J Med* 2016; 375(26): 2561–2569.
70. Beatty GL, Haas AR, Maus MV et al. Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells induce anti-tumor activity in solid malignancies. *Cancer Immunol Res* 2014; 2(2): 112–120.
71. Feng K, Guo Y, Dai H et al. Chimeric antigen receptor-modified T cells for the immunotherapy of patients with EGFR expressing advanced relapsed/refractory non-small cell lung cancer. *Sci China Life Sci* 2016; 59(5): 468–479.
72. Feng KC, Guo YL, Liu Y et al. Cocktail treatment with EGFR-specific and CD133-specific chimeric antigen receptor-T cells in a patient with advanced cholangiocarcinoma. *J Hematol Oncol* 2017; 10(1): 4.
73. Zhang C, Wang Z, Yang Z et al. Phase I escalating-dose trial of CAR-T therapy targeting CEA(+) metastatic colorectal cancers. *Mol Ther* 2017; 25(5): 1248–1258.
74. Ahmed N, Brawley VS, Hegde M et al. Human epidermal growth factor receptor 2 (HER2)-specific chimeric antigen receptor-modified T cells for the immunotherapy of HER2-positive sarcoma. *J Clin Oncol* 2015; 33(15): 1688–1696.
75. Morgan RA, Yang JC, Kitano M et al. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol Ther* 2010; 18(4): 843–851.
76. Lamers CH, Sleijfer S, van Steenbergen S et al. Treatment of metastatic renal cell carcinoma with CAIX CAR-engineered T cells: clinical evaluation and management of on-target toxicity. *Mol Ther* 2013; 21(4): 904–912.
78. Ahmadi M, King JW, Xue S et al. CD3 limits the efficacy of TCR gene therapy in vivo. *Blood* 2011; 118(13): 3528–3538.
79. Pegram HJ, Lee JC, Hayman EG et al. Tumor-targeted T cells modified to secrete IL-12 eradicate systemic tumors without need for prior conditioning. *Blood* 2012; 119(18): 4133–4142.
80. Hoyos V, Savoldo B, Quintarelli C et al. Engineering CD19-specific T lymphocytes with interleukin-15 and a suicide gene to enhance their anti-lymphoma/leukemia effects and safety. *Leukemia* 2010; 24(6): 1160–1170.
81. Kang TH, Mao C-P, He L et al. Tumor-targeted delivery of IL-2 by NKG2D leads to accumulation of antigen-specific CD8+ T cells in the tumor loci and enhanced anti-tumor effects. *PLoS One* 2012; 7(4): e35141.
82. Cieri N, Oliveira G, Greco R et al. Generation of human memory stem T cells after haploidentical T-replete hematopoietic stem cell transplantation. *Blood* 2015; 125(18): 2865–2875.
83. Sommermeyer D, Hudecek M, Kosasih PL et al. Chimeric antigen receptor-modified T cells derived from defined CD8(+) and CD4(+) subsets confer superior antitumor reactivity in vivo. *Leukemia* 2016; 30(2): 492–500.
84. Kebriaei P, Singh H, Huls MH et al. Phase I trials using sleeping beauty to generate CD19-specific CAR T cells. *J Clin Invest* 2016; 126(9): 3363–3376.
85. Monjezi R, Miskey C, Gogishvili T et al. Enhanced CAR T-cell engineering using non-viral Sleeping Beauty transposition from minicircle vectors. *Leukemia* 2017; 31(1): 186–194.
86. Harlin H, Meng Y, Peterson AC et al. Chemokine expression in melanoma metastases associated with CD8+ T-cell recruitment. *Cancer Res* 2009; 69(7): 3077–3085.
87. Hong M, Puaux AL, Huang C et al. Chemotherapy induces intratumoral expression of chemokines in cutaneous melanoma, favoring T-cell infiltration and tumor control. *Cancer Res* 2011; 71(22): 6997–7009.
88. Di Stasi A, De Angelis B, Rooney CM et al. T lymphocytes coexpressing CCR4 and a chimeric antigen receptor targeting CD30 have improved homing and antitumor activity in a Hodgkin tumor model. *Blood* 2009; 113(25): 6392–6394.
89. Craddock JA, Lu A, Bear A et al. Enhanced tumor trafficking of GD2 chimeric antigen receptor T cells by expression of the chemokine receptor CCR2b. *J Immunother* 2010; 33(8): 780–788.
90. Caruana I, Savoldo B, Hoyos V et al. Heparanase promotes tumor infiltration and antitumor activity of CAR-redirection T lymphocytes. *Nat Med* 2015; 21(5): 524–529.
91. Kakarla S, Chow KKH, Mata M et al. Antitumor effects of chimeric receptor engineered human T cells directed to tumor stroma. *Mol Ther* 2013; 21(8): 1611–1620.
92. Foster AE, Dotti G, Lu A et al. Antitumor activity of EBV-specific T lymphocytes transduced with a dominant negative TGF- β receptor. *J Immunother* 2008; 31(5): 500–505.
93. Zhang L, Morgan RA, Beane JD et al. Tumor-infiltrating lymphocytes genetically engineered with an inducible gene encoding interleukin-12 for the immunotherapy of metastatic melanoma. *Clin Cancer Res* 2015; 21(10): 2278–2288.
94. Liu X, Ranganathan R, Jiang S et al. A chimeric switch-receptor targeting PD1 augments the efficacy of second-generation CAR T cells in advanced solid tumors. *Cancer Res* 2016; 76(6): 1578–1590.
95. Chmielewski M, Abken H. CAR T cells transform to trucks: chimeric antigen receptor-redirection T cells engineered to deliver inducible IL-12

- modulate the tumour stroma to combat cancer. *Cancer Immunol Immunother* 2012; 61(8): 1269–1277.
96. Koneru M, O’Cearbhaill R, Pendharkar S et al. A phase I clinical trial of adoptive T cell therapy using IL-12 secreting MUC-16(ecto) directed chimeric antigen receptors for recurrent ovarian cancer. *J Transl Med* 2015; 13(1): 102.
 97. Naidoo J, Page DB, Wolchok JD. Immune modulation for cancer therapy. *Br J Cancer* 2014; 111(12): 2214–2219.
 98. John LB, Devaud C, Duong CP et al. Anti-PD-1 antibody therapy potentially enhances the eradication of established tumors by gene-modified T cells. *Clin Cancer Res* 2013; 19(20): 5636–5646.
 99. Cherkassky L, Morello A, Villena-Vargas J et al. Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. *J Clin Invest* 2016; 126(8): 3130–3144.
 100. Maude SL, Hucks GE, Seif AE et al. The effect of pembrolizumab in combination with CD19-targeted chimeric antigen receptor (CAR) T cells in relapsed acute lymphoblastic leukemia (ALL). *J Clin Oncol* 2017; 35(Suppl 15): 103.
 101. Chong EA, Melenhorst JJ, Lacey SF et al. PD-1 blockade modulates chimeric antigen receptor (CAR)-modified T cells: refueling the car. *Blood* 2017; 129(8): 1039–1041.
 102. Heczey A, Louis CU, Savoldo B et al. CAR T cells administered in combination with lymphodepletion and PD-1 inhibition to patients with neuroblastoma. *Mol Ther* 2017; 25(9): 2214–2224.
 103. Lu Y-C, Yao X, Crystal JS et al. Efficient identification of mutated cancer antigens recognized by T cells associated with durable tumor regressions. *Clin Cancer Res* 2014; 20(13): 3401–3410.
 104. Mestermann K, Giavridis T, Weber J et al. The tyrosine kinase inhibitor dasatinib acts as a pharmacologic on/off switch for CAR T cells. *Sci Transl Med* 2019; 11(499); pii:eaau5907.
 105. Ciceri F, Bonini C, Stanghellini MT et al. 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* 2009; 10(5): 489–500.
 106. Di Stasi A, Tey SK, Dotti G et al. Inducible apoptosis as a safety switch for adoptive cell therapy. *N Engl J Med* 2011; 365(18): 1673–1683.
 107. Chabannon C, Kuball J, Bondanza A et al. Hematopoietic stem cell transplantation in its 60s: a platform for cellular therapies. *Sci Transl Med* 2018; 10(436): pii:eaap9630.
 108. Philip B, Kokalaki E, Mekkaoui L et al. A highly compact epitope-based marker/suicide gene for easier and safer T-cell therapy. *Blood* 2014; 124(8): 1277.
 109. Wilkie S, van Schalkwyk MC, Hobbs S et al. Dual targeting of ErbB2 and MUC1 in breast cancer using chimeric antigen receptors engineered to provide complementary signaling. *J Clin Immunol* 2012; 32(5): 1059–1070.
 110. Kloss CC, Condomines M, Cartellieri M et al. Combinatorial antigen recognition with balanced signaling promotes selective tumor eradication by engineered T cells. *Nat Biotechnol* 2013; 31(1): 71–75.
 111. Lanitis E, Poussin M, Klattenhoff AW et al. Chimeric antigen receptor T Cells with dissociated signaling domains exhibit focused antitumor activity with reduced potential for toxicity in vivo. *Cancer Immunol Res* 2013; 1(1): 43–53.
 112. Caruso HG, Hurton LV, Najjar A et al. Tuning sensitivity of CAR to EGFR density limits recognition of normal tissue while maintaining potent antitumor activity. *Cancer Res* 2015; 75(17): 3505–3518.
 113. Liu X, Jiang S, Fang C et al. Affinity-tuned ErbB2 or EGFR chimeric antigen receptor T cells exhibit an increased therapeutic index against tumors in mice. *Cancer Res* 2015; 75(17): 3596–3607.
 114. Detela G, Lodge A. EU regulatory pathways for ATMPs: standard, accelerated and adaptive pathways to marketing authorisation. *Mol Ther Methods Clin Dev* 2019; 13: 205–232.
 115. Brudno JN, Somerville RP, Shi V et al. Allogeneic T cells that express an anti-CD19 chimeric antigen receptor induce remissions of B-cell malignancies that progress after allogeneic hematopoietic stem-cell transplantation without causing graft-versus-host disease. *J Clin Oncol* 2016; 34(10): 1112–1121.
 116. Torikai H, Reik A, Liu PQ et al. 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* 2012; 119(24): 5697–5705.
 117. Provasi E, Genovese P, Lombardo A et al. Editing T cell specificity towards leukemia by zinc finger nucleases and lentiviral gene transfer. *Nat Med* 2012; 18(5): 807–815.
 118. Qasim W, Zhan H, Samarasinghe S et al. Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. *Sci Transl Med* 2017; 9(374); pii:eaaj2013.