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T-cells "à la CAR-T(e)" – Genetically engineering T-cell response against cancer

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ABSTRACT

The last decade will be remembered as the dawn of the immunotherapy era during which we have witnessed the approval by regulatory agencies of genetically engineered CAR T-cells and of checkpoint inhibitors for cancer treatment. Understandably, T-lymphocytes represent the essential player in these approaches. These cells can mediate impressive tumor regression in terminally-ill cancer patients. Moreover, they are amenable to genetic engineering to improve their function and specificity. In the present review, we will give an overview of the most recent developments in the field of T-cell genetic engineering including TCR-gene transfer and CAR T-cells strategies. We will also elaborate on the development of other types of genetic modifications to enhance their anti-tumor immune response such as the use of co-stimulatory chimeric receptors (CCRs) and unconventional CARs built on non-antibody molecules. Finally, we will discuss recent advances in genome editing and synthetic biology applied to T-cell engineering and comment on the next challenges ahead.

Contents

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1. Introduction

In the past decade, we have been witnessing a tremendous progress in cancer treatment due to the development of immunotherapeutic approaches that can target efficiently tumor cells. While checkpoint inhibitors can clearly lead to unprecedented clinical responses in advanced cancer patients [1], their efficacy is based in part on the preexistence of tumor-specific T cells. However, to remedy to the absence of the latter, a broad effort that started in the 80's, has been deployed in the form of T-cell engineering approaches. It was first geared to endow T cells with novel specificities. In subsequent studies, strategies were (and are currently) elaborated to qualitatively enhance lymphocyte functions such as resistance to inhibitory signals, persistence or homing capabilities. As we shall see, challenges remain and substantial efforts are invested to safely harness the curative potential of an engineered immune system.

2. T-lymphocytes: a potent and malleable platform

The immune system has been attributed several protective capabilities directed against pathogens and lately, against neoplastic cells. Though "self" by origin, cancer cells can express tumor antigens that discriminate them from normal tissues and which can be targeted by the adaptive immune system. These can be classified into tumor associated antigens (TAA) which are also expressed in normal tissues, but are differentially or overexpressed in tumor cells, and tumor specific antigens (TSA) which are expressed specifically in tumor tissues, often as a result of mutations in their coding region [2]. T-lymphocytes are amongst the most potent immune components that can eradicate cancer cells. In this regard, the use of checkpoint inhibitors to block inhibitory pathways in T cells can lead to tumor regression [1]. Moreover, the adoptive cell transfer (ACT) of tumor infiltrating lymphocytes (TIL) [3] isolated from the tumor itself, can mediate impressive tumor regression in advanced melanoma patients, with nearly a quarter of the treated individuals achieving durable complete responses [4]. Recent findings show also the successful application of TIL therapy to other types of cancer including cholangiocarcinoma [5], cervical cancer [6], colorectal cancer [7] and lately, breast cancer [8].

Whereas TIL therapy exemplifies the strong curative potential of T cells, it is relying on the preexistence, correct isolation and expansion of these cells. These laborious tasks are not always easily undertaken due to the poor presence of specific anti-tumor T cells (if they even exist) and the relatively long period of time required to grow and expand the T cells from patients (who often have limited life expectancy). Therefore, two main strategies have been developed to *de novo* genetically engineer the specificity of the immune response against cancer, using either T-cell receptors (TCR) or an antibody-based chimeric antigen receptors (CAR) [9].

3. Engineering T cells to express an antigen specific TCR

3.1. TCR structure

T lymphocytes recognize specific peptides (or epitopes) presented by the MHC complex, *via* their TCR. Following recombination and selection in the thymus, T cells clonally express a fixed TCR with defined specificity. The TCR is a heterodimer complex mostly comprised of α/β chains (only 5% approximately of the T cells display a TCR that consists of γ/δ chains). Both chains are formed by 2 Ig-like domains, namely a variable and a constant region (VR and CR). The variable portions interact with the MHC/antigen complex mainly *via* 6 protruding loops (3 on each chain) known as CDRs (Complementarity-determining regions). The role of the constant region is to promote the pairing between the alpha and beta chain as well as to facilitate the interaction with the CD3 signaling complex. Indeed, since the α and β chains have a short cytoplasmic region, they cannot signal by themselves when binding to their cognate antigen. Therefore, the TCR α/β dimer is associated with three CD3 dimers (a dimer of CD3γ and CD3ε, of CD3δ and CD3ε and of CD3ζ) which can provide activation signals *via* their ITAMs (immunoreceptor tyrosine-based activation motifs) contained in the cytoplasmic domains. For naïve T cells, an additional co-stimulatory signal is usually required to properly activate the T cells and induce cell survival and proliferation, such as that mediated by the binding of CD28 receptor to its ligand CD80/CD86. A third signal (destined to promote differentiation) may be provided by cytokines [3].

3.2. TCR-gene transfer

As depicted below, the TCR determine T-cell specificity and an underlying consequence to this is that it may be feasible to endow T cells with new specificities using TCR-gene transfer approaches. Dembic et al. first demonstrated the successful redirection of T-cell specificity using TCR-gene transfer in a murine system [10]. Though the original intent of their study was to define TCR dynamics, it led to a new area of translational research geared at reprogramming T-cell specificity. This strategy was then applied to provide T cells with melanoma [11] and viral specificity [12].

Within two decades, this approach was applied clinically by Morgan et al. [13] for the treatment of metastatic melanoma. In this first pioneering TCR-gene transfer clinical trial, a MelanA/MART1-HLA-A*0201 restricted specific TCR termed DMF4 was isolated from a TIL clone and expressed in T cells isolated from metastatic melanoma patients. MART1 is a TAA expressed in close to 90% of melanomas [14] and the fact that 40–50% of the Caucasian population express the HLA-A*02 allele hypothetically made this approach clinically relevant to more than a third of melanoma patients. While only 2 patients out 15 displayed had an objective response, this trial demonstrated the feasibility of TCR-gene transfer. To achieve a better response, a second clinical trial was conducted with another high affinity TCR for MART1 (derived from the same patient) designed DMF5 [15]. In this trial, 30% of the patients demonstrated an objective response, but five days post infusion, patients also developed severe skin rash and oculo-vestibular side effects. Toxicity was found to be linked to off-target effects since MART1 is also expressed in the skin, ears, eyes and other pigmented tissues [16]. In parallel, Johnson et al. also made use of another melanoma-specific high-affinity TCR specific for $gp100_{154–162}$ and derived from an HLA-A2 transgenic mice and this led to a response rate of 19% [16]. To extend this approach to other malignancies, the use of TCR for non-melanoma tumors was also investigated and several studies made use of TCRs directed against overexpressed or germline antigens. For example, 10 patients underwent TCR-gene transfer using a wt p53-specific TCR isolated also from HLA-A2 transgenic mice [17] and although a non-mutated ubiquitous epitope was targeted, no major toxicity was noted and a single cholangiocarcinoma patient experiencing a partial regression [18]. While no obvious relationship was found between levels of p53 measured in tumors and p53-specific T-cell reactivity to these tumors [19], further studies revealed that T-cell reactivity seems associated with p53 protein stability induced by different mutations [20]. It is important to bear in mind that the choice of the targeted antigen is crucial to the success of- and reduced toxicity by gene-engineered T-cell therapy [21]; targeting tumor associated antigens could bear serious and even fatal consequences. Patients treated with a CEA-specific TCR experienced tumor regression accompanied with severe colitis [22] while the use of MAGE-A3 specific-TCR led to coma, leuko-encephalopathy and lethal cardiac toxicity [23,24]. Thus, strategies should be elaborated to help determining the potential cross-reactivities displayed by TCRs [25,26]. In sharp contrast, when targeting the cancer testis antigen NY-ESO1 using derivatives of the 1G4 TCR, impressive results (the best to date for TCR-gene transfer treatments) were obtained with objective responses ranging between 50 and 90%: two studies show the successful treatment of synovial cell sarcoma, melanoma and multiple myeloma with no immune-related adverse effects reported [27,28]. Viral antigens expressed by cancer cells can also

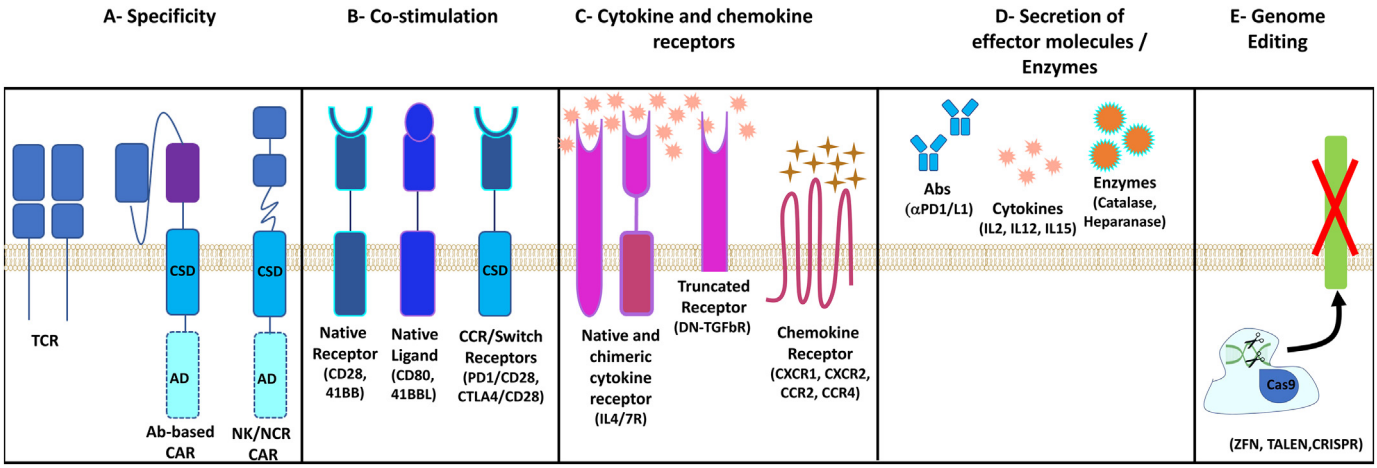

Fig. 1. Schematic representation of diverse strategies used in genetic engineering of T-cells. In brackets are indicated some examples of relevant molecules/approaches. Ab – Antibody; NCR – Natural Cytotoxicity Receptor; CCR – Co-stimulatory Chimeric Receptor; AD – Activation domain (*e.g.* CD3z IC); CSD – Co-stimulatory domain (*e.g.*, CD28 IC, 41BB IC); ZFN – Zinc Finger.

be targeted as described in a current phase I/II clinical trial (NCT02858310) using a HPV-E7 specific TCR for the treatment of metastatic HPV+ carcinoma.

3.3. Isolation and structural modifications of TCRs

A central challenge in TCR-gene therapy is to isolate the genes encoding an anti-tumor TCR restricted to a defined MHC-peptide complex. TCR sequences can be isolated from a reactive TIL, *in vitro* stimulated peripheral T cells, frozen tumor samples, yeast display libraries, humanized TCR-transgenic or HLA-transgenic animals [15,17,29–34]. From there, as the 5′ region of the TCR α and β chain is highly polymorphic, one can perform a 5'RACE (rapid amplification of cDNA ends) procedure or otherwise elaborated PCRs and screening of reconstituted TCR library to isolate the desired sequences [34,35]. Single cell PCR has also been performed successfully to isolate functional TCRs [36,37].

As aforementioned, the TCR is composed of 2 chains (α/β) which dimerize through their respective constant regions. When expressing an additional (exogenous) TCR into a T-cell, mispairing can occur with the endogenous TCR chains, that is, the exogenous α can pair with the endogenous β through their CR and *vice versa* [38]. Additionally, since TCR surface expression is dependent on the association with (the limited pool of) CD3 chains, competition with endogenous TCR chains can lead to decreased exogenous (therapeutic) TCR expression. Moreover, it is possible that these TCRs mixed heterodimers bear undefined self-reactivity [39,40].

Therefore, several strategies have been employed to enhance exogenous TCR expression and/or reduce mispairing [31,41]. For example, we showed that the replacement of TCR human constant regions with murine ones can lead to preferential pairing between the "murinized" exogenous chain [38]. Furthermore, in order to reduce the potential immunogenicity of murine constant regions [18], it was possible to identify key amino acid residues that mediate the preferential pairing and to use minimally murinized TCR chains that retained most of the activity displayed by the fully murinized one [42,43]. The use of murinized receptor was successfully extended to other TCRs [44–46]. Another possibility to promote chain pairing we and others explored, was to engineer an additional di-sulfide bond in the constant region of the TCR inserted chains [47,48]. One can also swap residues [49] or entire domains [50] between the α and β chains to promote exquisite pairing. To overcome the competition for the CD3ζ chains between endogenous and exogenous TCRs, other strategies were elaborated such as the generation of chimeric TCR fused to the intra cellular region of CD3ζ [51] or of a single-chain TCR in which the variable α and β portions are linked (similarly to scFv) [52]. Alternatively, one can also knock down the expression of the endogenous TCR using shRNA or RNAi [53,54] zing fingers [55],TALEN [56] or more recently CRISPR/Cas9 systems [57,58] (Fig. 1).

Another general approach to increase TCR potency, and thus T-cell avidity, is to increase the relative expression of the chains [59,60]. Codon optimization [61,62] or introduction of "strong" TCRs [63] have often proven an promising options to increase receptor expression. It also possible to boost the level of CD3 molecules (a bottleneck for TCR expression) [64] or to mutate glycosylated residues [65] to increase TCR expression. We have devised an original approach to selectively improve exogenous TCR stability by increasing the hydrophobic nature of the TCRα transmembrane region. Incorporation of hydrophobic residues at evolutionary-permissive positions resulted in an enhanced surface expression of the TCR chains, leading to an improved cellular avidity and anti-tumor TCR activity [66]. Since then, this approach was successfully applied to a number of TCRs [8,67–70].

4. Chimeric antigen receptors (CARs): From design to commercialization

The idea of "reprogramming" T-cell specificity by expressing a targeting receptors was exemplified in the early works of Dembic et al. [10] using TCR. However, around the same time Kuwana et al. [71] and Gross et al. [72] demonstrated the feasibility of using chimeric receptors based on antibodies. In parallel, several groups showed in the early nineties the possibility to generate and express chimeric receptors in T cells that incorporated a signaling moiety [73–75]. The latter led to the design and successful implementation of a first antibody-based chimeric antigen receptor (CAR) that incorporated a signaling moiety [76]. Since then, the development of three generations of CARs [77], which differ in their basic structure, took place and this led to CAR therapy approval by the FDA in mid-2017 [78]. We shall explore herein various aspects of CAR design and development.

4.1. CAR basic structural principal and determinant of function

First and foremost, we should emphasize the fact that empirical studies and functional testing are crucial to ascertain the potential and function of CARs [79]. That being said, several guidelines can be enunciated to ease the design process [80]. CAR primary structure includes a targeting moiety (often derived from an antibody), a hinge region, a transmembrane portion and fused to signaling endodomain (s) (Fig. 1). In addition, 3 "generations" of CARs have been studied; originally, first generation CAR included only an activating / signaling moiety in the form of CD3ζ or FcγRIII endodomains. Second generation

CARs include a co-stimulatory domain (usually CD28 or 4-1BB domains) before the activating domain. Third generation CARs make use of multiple co-stimulatory domains in tandem [77]. CARs bear several advantages: they combine in a single molecule activating and costimulatory domains and they work in an MHC-independent way (and thus are not restricted to a particular population bearing a suitable HLA allele) [60,81,82]. However, unlike TCRs which can recognize proteosomally degraded proteins presented by MHC molecules, CARs are limited to surface antigens only, which constitute only approximately 1/5 of the proteome [83]. One way to bridge between the flexibility of CAR and the broad recognition pattern of intracellular antigens mediated by TCRs, is to use TCR-like antibody fragments [84,85] or CAR/TCR hybrids [86]. These can be usually isolated from phage display libraries [84,87–92] and further converted into CARs [93]. Nevertheless, some affinity adjustments may be needed in order to achieve optimal function of these hybrid molecules when expressed as membrane-bound CARs [94,95].

As aforementioned, the ectodomain of a classical CAR consists of a scFv domain (derived from an antibody) proceeded by a signal peptide (SP). Both scFv chains, *i.e.* variable heavy (VH) and (VL) light chains, are interconnected by a flexible linker (usually $(Gly_4Ser)_3$ - Gly for flexibility and Ser for solubility, but other linkers such as GSTSGSGKPGSGEGS can be successfully used). To date, more than 100 CAR specificities are being investigated in the different pre-clinical and clinical studies [96] with almost 300 CAR T-cell clinical trials taking place [82]. Additionally, bi-specific CARs can also be generated by fusing two scFv's together [97,98]. As for TCRs, the choice of antigen is crucial to prevent off-target effects, which can even be fatal [99,100]. This undesirable behavior may be mitigated for example by modulating the antibody affinity [101,102], by pairing high affinity heavy chains derived from a defined antibody to various light chains [103] or by mutating the signaling moiety [104].

The hinge or spacer region linking the targeting moiety to the transmembrane region of the CAR and can be derived from CD28, CD8a or Fc IgG molecules. It has been shown that its nature may influence CAR function [105,106]. For example, to reach antigens that are short and proximal to the target cell membrane, one should use lengthier hinges [107]. Hence, the hinge region can modulate the antigen recognition and, as it was shown for an anti-CD22 CAR [108] or for a ROR1 specific CAR [109], could facilitate CAR dimerization. Moreover, the hinge region can influence CAR flexibility, making for example heavily glycosylated antigens, such as MUC1, more accessible [110]. Interestingly, hinge/spacer domains can impact on a variety of cellular effects such as tonic signaling [111] and they are amenable to mutagenesis to modulate the "off-target" activity of CAR-T cells [112]. Finally, hinge domains can also bear a functional purpose and can include Strep-Tag to facilitate transduced cell isolation, sorting and function [113].

The transmembrane region (TM) can also impact on CAR expression and function and it is generally derived from CD28, CD3ζ, CD8a, CD4, or FcεRIγ molecules. It was suggested the role in CAR signal transduction *via* TM domain that comes into interaction with different membrane co-receptors [114]. We also recently showed that CD28 TM domain maybe superior to the native one in certain types of chimeric receptors [105,115]. Still, more research is required to obtain a more reliable understanding of TM domain impact on CAR stability and function [116].

The intracellular domain (IC) of CARs is certainly of paramount importance to the receptor function. Historically, 1st gen. CARs possessed either a CD3ζ or FcγRIII endodomain [76], though CD3ζ functional superiority was demonstrated in a mouse model [117]. The functional long-term benefits of CD3ζ over Fc signaling domain were attributed to the lower activation threshold provided by the three ITAMs in the CD3ζ signaling domain *vs.* one in the FcRγ chain [118]. Though several works depicted promising *in vitro* functionality of first generation CARs [119,120] as well as notable *in vivo* function [121], their persistence in the host could be variable, from quite limited [122] to extensive [123]. Nonetheless, a 2nd generation of CARs was developed to incorporate both signal 1 (activation *via* CD3-zeta) and signal 2 (co-stimulation) which are essential for the full activation and survival of naïve T cells. The advantage of including a costimulatory domain [124–126] over first generation CARs was undoubtedly demonstrated in a comparative clinical study a few years ago [127].

Both CD28 and 4-1BB (CD137) are commonly used, like in the case of the two FDA approved CARs, Axicabtagene ciloleucel and Tisagenlecleucel respectively. Recent comparative studies [128,129] showed that the 4-1BB could promote the outgrowth of $CD8^+$ central memory T cells with enhanced mitochondrial biogenesis, oxidative phosphorylation, respiratory capacity and fatty acid oxidation. On the other hand, CD28 domain contributed to enhanced glycolysis and effector memory phenotype and functionality. Moreover, anti-CD19 CAR constructs containing either CD28 or 41BB IC domains were compared in a recent clinical trial - although the response was quite similar for both groups (3 CRs of 5 patients in each), some differences were observed in terms of peak reaction time and adverse events [130]. Indeed, it seems that CD28 would be preferable when fast reactivity is needed while 4-1BB is more indicated when more prolonged function is desirable [131]. CAR antigen-independent tonic signaling may also represent a problematic issue as it was shown that the CD28 domain *via* CAR clustering mediates CD3ζ tonic signaling in αGD2, αHER2/Neu, αCD22 CAR-T cells but not in αCD19 CAR, leading to the CAR-T cell exhaustion and to reduced viability, while the incorporation of the 4-1BB signaling domain could mitigate this effect and prevent exhaustion [132]. Nonetheless, another recent study proposes that 4-1BB tonic signaling may activate the LTR in certain viral vectors in leading to some toxicity and reduced anti-tumor activity [128]. Additionally, it was shown that 4-1BB signaling domain produces antigen-independent tonic signaling in α-CD19 and α-GD2 CAR and is vector dependent [133]. In another comparative study, it was found that CD28 and 4-1BB signaling domains produce the same level of tonic signaling in α-CD5 second-generation CAR [134]. Thus, one should take into account that tonic signaling, produced either by CD28 or 4-1BB domains, may limit CAR-T cell persistence and lead to the T cell exhaustion and possible off-targets effects. Due to the modular character of these receptors, third generation CARs were designed to include more than one co-stimulatory domain (for example CD28 and 4-1BB) [77,135,136]. At this point, it is difficult to say if 3rd generation CARs are really advantageous compared to 2nd generation CARs in the clinics as they are also associated with toxicities and tumor relapse [99,137]. Nonetheless, a recent study by Ramos et al. reported that in a CD19-CAR clinical trials comparing 2nd generation CD28-based CAR to 3rd generation CD28/41BB CAR, the latter was able to facilitate enhanced CART expansion and seems to be more adapted for the treatment of low disease burden [138].

As both the B7 and TNFR co-stimulatory receptor families include multiple members, studies are not limited to CARs containing either CD28 and/or 4-1BB [96]. For example, ICOS co-stimulatory IC domain was demonstrated superior to that of CD28 or 4-1BB in an α-Mesothelin 2nd gen. [139] or 3rd gen. CAR [140], especially when expressed in Th17 differentiated cells. ICOS signaling was shown to enhance CAR-T cells persistence and ICOS-based CAR expressing CD4+ T-cell subset can provide support to 4-1BB or CD28 equipped CAR-CD8+ T cells [140]. OX-40 (CD134) is another T cell co-stimulatory molecule, belonging to the TNFR superfamily and expressed in CD4+ activated T cells. Its utilization in CARs was shown to lessen the amount of secreted IL10 [141]. CD27, also of the TNFR family, is implicated in the generation of long-term T-cell immunity [142] and its addition to 2nd-gen. CAR mediated superior immune response, better persistence and induction of the anti-apoptotic molecule Bcl_{XL} [143].

4.2. Beyond CD19: New CAR-T Cells for Solid Tumors – In search of the grail

The paradigm of anti-CD19 CAR is a striking example of the complexity related to finding an adequate target. Despite CD19

being a tumor associated (*i.e.* non-mutated) antigen and thereby expressed on normal cells, several characteristics made it a suitable antigen for CAR-T cell therapy: 1) Its expression is constant and up-regulated on tumor cells, 2) Normal cells expressing CD19 are of a self – renewable single cellular lineage (B cells) and 3) CD19 is expressed on non-vital tissues (even after total B-cell aplasia, patients treated with CD19-CAR T cells may eventually reach immune reconstitution) [82].

Additional antigens are now being evaluated as promising targets for T-cell based immunotherapy. BCMA is a normal plasma cells marker, falls in to the near-ideal antigen for multiple myeloma with no evidence of expression on the normal non-hematological tissue [144]. Latest multi-center BCMA-CAR trials results indicate a relatively safe profile (although cytokine release syndrome - CRS and transient neurotoxicity were observed) with an overall response rate over 90% [145,146]. Other antigens currently being targeted to treat hematological malignancies include CD20 for NHL, CD138 for MM [144], CD33 [147], CD123 for AML [148,149] and CD7 for certain leukemias and lymphomas [150]. Lately, using a very elegant approach to treat T-cell malignancies, Pule and colleagues isolated an antibody specific for the TCRβ1 constant region. A CAR based on this TCRβ1 specific antibody was efficient in mediating cytotoxicity against leukemic and healthy TCRβ1 cells while preserving the TCRβ2 population and thus potentially, full immunity [151].

Adoptive transfer of engineered T cells is an effective treatment for certain hematological malignancies [152] but it faces many challenges when targeting solid tumors [153,154]. A large number of CAR clinical sutides are attempting to replicate the success of α-CD19 and α-BCMA CARs against the solid neoplasms, but the preliminary outcome of more than 100 trials are less encouraging. Still, many antigens are being the focus of CAR-T cell therapy for solid tumors and these include Folate receptor (FR)α [122] in metastatic ovarian cancer, CAIX in metastatic RCC [155] and CD171 in neuroblastoma patients [156] though CAR therapy against those did not show any significant therapeutic effect. Other antigens of interest tested in clinical trials include mesothelin, targeted for example in the context of pancreatic cancer [157], HER2/Neu in various cancers [99,158,159], CEA [160], EGFRvIII [161] and PSMA [162]. Of note, Disialoganglioside GD2, which is mainly associated with neuroblastoma, was also shown to be overexpressed in glioma, retinoblastoma, Ewing's family of tumors, fibrosarcoma, rhabdomyosarcoma, osteosarcoma, small cell lung cancer, leiomyosarcoma, liposarcoma and melanoma [163]. In a first in-human clinical trial with α-GD2-ζ 1st gen. CAR-T cells, 1 out of 11 young neuroblastoma patients achieved CR [121] and in a long-term follow-up study of these 11 patients, 3 subsequent CRs were registered, making α-GD2-ζ 1st gen, CAR the first one able to mediate CR in solid tumor patients.

4.3. CAR immunogenicity as a limiting factor

Traditionally, scFv's used as CAR targeting moieties (for example the anti-CD19 FMC63 antibody [164]) have been isolated from (mouse) hybridoma. Human-Anti-Mouse Ab's (HAMA) production [122] and anti-transgene xenoreactivity [18] were linked to impaired T cells trafficking and transplanted T cells rejection, resulting in poor persistence and shortening of response. Beyond this, a humoral response could also be directed against residual vector-derived epitopes [165], immunogenic epitopes derived from unique fusion sites between CAR domains [166] or against selection markers or suicide genes expressed by the therapeutic vector [167]. Clinical data support these claims – for example in the long-term follow-up of 8 RCC patients treated with α-CAIX CAR, no clinical response was observed. In 3 of 8 patients, anti-transgene antibodies were detectable and patients' sera effectively blocked the CAR-T cells function [165]. Likewise, in a CD19/CD20-based CAR-T cell clinical trial, Jensen and colleagues showed that a central hurdle to therapeutic efficacy is linked to engineered T-cell limited persistence associated with humoral and cellular xenoreactivity [168]. Similarly, in 5 out of 29 ALL patients treated with αCD19-BBz who were found to be complete responders, CAR-T cells failed to persist after a second transplant infusion. Further examination revealed that all 5 patients developed anti-CAR immune response leading presumably to a $CD19^{+}$ leukemia relapse [169]. Anti-transgene xenoreactivity was also associated with serious adverse effects. Of the 3 mesothelioma patients treated with a murine SS1-based a-Mesothelin CAR, one individual developed a severe anaphylactic event as a symptom of anti-scFv xenoreactivity. In these patients, HAMAs were detectable, even though CAR expression was achieved using a transient system [170].

A possible solution to scFv xeno-immunogenicity is to humanize the framework portions of antibody fragments or to use fully-human targeting moieties. While one may surmise that humanization could harm CAR structure and function, the function of selected humanized or fully-human scFv was shown to be on par or even superior to their murine counterparts [171]. Johnson et al., successfully targeted EGFRvIII with a humanized scFv which was shown functionally superior to the murine 3C10 scFv [172]. Similarly, a panel of fully-human scFv CARs against CD19 was generated using Ab/DNA libraries [166] and these demonstrated similar or superior activity compared to the well-characterized murine anti-CD19 FMC63 antibody.

4.4. Non scFv-based CARs

The recent success obtained with anti-CD19 CAR T cells for the treatment of several hematological malignancies [152] have motivated the immunotherapeutic community to look for additional targeting moieties specific for other antigens. Beyond specificity, the use of non-scFv based CAR EC domains may help solving the CAR immunogenicity issue we just described as well as problems of tonic signaling and exhaustion induction observed with certain scFv's due to their predisposition to oligomerization [132,152].

Several non-scFv targeting moieties were described in the recent years – for example, nanobodies [173,174] which are single Ig-like domain binding molecules can serve as surrogate for full scFv in CARs. Smaller moieties such as oligopeptides were also demonstrated to be adequate scaffold for the binding portion of CARs [175]. Additionally, synthetic proteins termed DARPins (designed ankyrin repeat proteins) can also be engineered to serve as CAR targeting moieties. Indeed, they contain ankyrin repeats (ARs) which are one of the most common protein binding motifs found in nature (usually stretches of 33 amino-acid forming a β-turn and two anti-parallel α-helices). These can be engineered to bind efficiently Her2 providing an alternative to the 4D5 murine scFv-based CAR [176,177]. Similarly, adnectins are affinity molecules derived from a fibronectin domain and can serve as an alternative to scFv in CAR configuration [178] though the complex structure of the two last examples may trigger immunogenicity.

Nevertheless, less synthetic and more “native” molecules can be utilized in CARs. This was first exemplified with the use of the CD4-zeta 1st gen. CAR for which persistence and *ex vivo* reactivity of the transduced cells was shown to last more than a decade (though no clinical response was observed) [123]. In another study, a membrane bound version of IL13 cytokine was developed to target glioblastoma tumors expressing the IL13Ra2 molecule. The latter is present on 80% of high-grade gliomas, including GBM, but much less on normal tissues [179]. A GBM patient with multiple foci in the brain and spine was treated with intracranial and intraventricular injections of IL13-BBz 2nd gen. CAR (bearing a E13Y for added specificity). All the intracranial and spinal tumors regressed though, unfortunately, soon after the treatment completion, 4 additional foci emerged and the patient relapsed probably due to escape variants [180] Since gliomas can express additional tumor associated antigens, bivalent CAR-T cells targeting HER2 *via* scFv and IL13Ra2 *via* IL13-mutein in tandem [181] or trivalent CAR-T cells specific for the HER2, IL13Ra2 and EphA2 [182] may represent a valuable treatment option. Lately,

Stepanov et al. showed it is feasible to isolate from peptide libraries ligands that bind to BCRs presented by lymphoma cells and to use these as targeting moieties in CARs [183].

4.5. Using NK receptors to bridge between innate and adaptive immunity

NK cells are a type of lymphocytes from the innate immunity arm that can eradicate viral-infected and malignant cells. Their function is dependent on the integrated balance of signals transduced by activating and inhibitory receptors [184–186]. As such, the recognition of cancer cells by NK cells is mediated by different activatory receptors such as DNAM-1, NKG2D and members of the natural cytotoxicity receptors (NCRs) family [187].

NKG2D is a type II TM receptor that can mediate NK-tumor recognition and its 8 cancer/stress ligands (*e.g.* MICA, MICB, ULBP1–6) [188] are attractive targets for immunotherapy as these are highly expressed on neoplasms while they are normally absent or minimally expressed by healthy tissues. Based on this, an NKG2D-based CAR was generated by fusing full-length NKG2D receptor to the CD3ζ signaling domains [189]. Promising results were achieved *in vitro* and *in vivo* with this CAR or its derivative in multiple myeloma [190], ovarian cancer [191], glioblastoma [192] and more. NKGD2-CARs are currently evaluated in clinical trials (NCT02203825, NCT03018405) and so far, no major toxicities were observed [193].

Other promising NK activating receptors are the members of the NCR family, known as NCR1 (NKp46), NCR2 (NKp44) and NCR3 (NKp30). Their ligands are often expressed by viral-infected and tumor cells and as such, NCR molecules can mediate the recognition of a wide range of tumors and their lysis by NK cells [194,195]. NCR ligands can also regulate the receptor function and may facilitate tumor escape from NK cells [195–197], which would support their extensive expression by tumor cells. Although usage of NK-based immunotherapies is currently limited by poor expansion and endogenous inhibitory mechanisms [198], one way to combine the tumor recognition capability of NK cells with T cell effectiveness and flexibility is to design CARs based on the EC domain of NCRs. Thus, we and others showed that NCR1 [199], NCR2 [105] and NCR3 [200] based chimeric receptors can mediate the recognition of several types of tumors (*e.g.* pancreatic, lung, cervical cancers) both *in vitro* and *in vivo*. Again, some major advantages to this kind of CARs are the possibility to target multiple cancers with the same molecule, their non-HLA restriction and of course the fact that these are native human receptor which should not trigger immunogenicity. An important task that will need to be accomplished in order to promote the implementation of NCR-based CAR-T cells in the clinic will be to precisely define the pattern of expression of NCR ligands.

5. Improving engineered T-cell function beyond specificity

The tumor microenvironment (TME) is a hostile milieu for immune cells, mainly NK and T cells [201]. Often, tumors can accumulate mutations that will facilitate their escape from immune surveillance and function [202]. 3rd party cells such as myeloid derived suppressor cells (MDSCs), tumor associated macrophages (TAMs) and Tregs may also be recruited to the tumor vicinity to suppress anti-tumor immune function. In addition, exposure of cancer cells to Th1-type pro-inflammatory cytokines (like IFNγ), may lead to the implementation of tolerance-inducing program such as the overexpression of PDL1 [203,204]. This suppressive environment can drive T cells into an exhausted/dysfunctional/anergic/tolerized phenotype [201,205], especially through the triggering of co-inhibitory receptors such as PD-1, CTLA-4, TIGIT, LAG3, VISTA or due to the exposure to immunosuppressive cytokines and enzymes such as TGFβ, IL10, IL4 and IDO [206–210]. Although these receptors are important for preventing autoimmunity [211,212], they induce dysfunctional and exhaustion phenotypes in tumor-specific T cell (*e.g.*, reduced cytokine secretion / effector function and apoptotic death). Some of the co-inhibitory ligands such as PD-L1 are known to be upregulated by solid tumors including myeloma, ovarian, breast and renal cancers [213]. Thus, blocking such pathways using antibodies (immune checkpoint inhibitors) may lead to "rejuvenation" of existing T cells and impressive anti-tumor functions [1,214] and to the clinical implementation of novel T-cell engineering treatments (reviewed in [215]).

5.1. Use of chimeric co-stimulation and/or switch receptors

As with chimeric antigen receptors, additional co-stimulatory signals could be provided to engineered cells to enhance their function, cytokine secretion and survival; for example restoration of CD28 expression [216] or simultaneous expression of CD80 and 4-1BBL (respective ligands for CD28 and 4-1BB; Fig. 1) [217] by T cells can promote cell proliferation both *in vitro* and *in vivo*. Moreover, we recently showed that the constitutive expression of 4-1BB/CD137 in engineered T cells can lead to improved cytokine secretion, proliferation and cytotoxicity *in vitro* an *in ovo* model we developed [218].

However, as co-inhibitory ligands are abundant at tumor sites and their co-stimulatory counterparts often rare [208,219], one way to take advantage of this setting is to engineer T cells to express chimeric co-stimulatory (switch) receptors (CCRs or CSRs). These consist of two fundamental portions: an extracellular domain that can bind to an inhibitory ligand either on tumor surface or soluble protein secreted in the tumor vicinity, fused to the intracellular domain of a stimulatory molecule (Fig. 1). The latter can be derived from a wide variety of co-stimulatory molecules and other activatory receptors such as primarily CD28 but also ICOS, 4-1BB, OX40, CD27, IL-7R, IL-2R [220]. Therefore, CCRs (also known as immune converters or switch receptors) can bind inhibitory ligands but should activate a stimulatory signaling pathway, thereby converting the negative signal into a positive one. CCRs can be used in combination with TCRs [115] or CARs [221] and provide another layer of reactivity or safety (when combined with 1st generation CAR).

With this in mind, we and others designed and characterized PD1/CD28 fusion molecules (Fig. 1). By transducing a $CD4^{+}CD28^{-}$ T cell lymphoma line or $CD8^{+}$CD28- with a PD1/CD28 CCR, Prosser et al. showed increased p-Akt and p-ERK levels and cytokine secretion (IL-2, GM-CSF, TNF and IFNg) when co-cultured with an artificial stimulating target [222]. We concomitantly developed on a similar molecule using a different design and showed that a human PD1/CD28 can improve T-cell function against cancer cells by synergizing with cancer–specific TCRs [115]. Using a truncated version of PD1, Ankri et al. also showed the necessity of including the CD28 endodomain to achieve "co-stimulatory immune conversion" and thus, improved T-cell function. We also demonstrated for the first time the therapeutic potential of CCRs *in vivo* using a xenograft model of human melanoma and noted a that T cells equipped with PD1/CD28 could delay tumor growth and survive longer in the tumor than their control counterpart [223].

Following this, Kobold *et al* constructed fully murine PD1/CD28 chimera replicas of the two aforementioned designs and also a CCR incorporating the TM portion of PD1 rather than CD28 [224]. They demonstrated that the latter could mediate an increased cytokine production, better proliferation and binding to soluble PD-L1 using a murine pancreatic cancer model and lymphoma [225]. It would be interesting to test the validity of these results in human settings though our preliminary results did not show a significant improvement when using PD1 TM rather than CD28 TM in human chimeras (unpublished results). As mentioned before, CCR can also be combined with a CAR rather than with a TCR. Indeed, Liu *et al* electroporated human T cells with anti-CD19 and anti-Mesothelin CARs together with a PD1/CD28 CCR [221]. Consistent with previous results, no improvement in *in vitro* cytotoxicity was observed though this switch receptor did contribute to higher cytokine secretion and improved *in vivo* function in

comparison to CAR alone. Lastly, Noessner and colleagues [226] demonstrated that low-affinity TCR engineered T cells as well as TILs (*i.e.* not CAR or TCR engineered) could benefit from the expression of the PD1/CD28 chimera we previously designed [115]. Moreover, PD1/CD28 engineered T cells combined with checkpoint blockade secreted significantly more IFN-γ compared to control T cells without PD-1/CD28. As with CARs, CCR can include multiple signaling domains – we and others described also a PD1 CCR that included CD28 and 4-1BB endodomains [227].

Besides PD1, CCR with other exodomains were also described. For example, a murine CTLA4/CD28 molecule could improve signaling through Akt and T-cell function using the melanoma pmel model [228] or in donor lymphocyte infusion (DLI) settings for diverse hematological malignancies in mouse [229]. In this work, both transduced murine CD4 and CD8 T cells were found to be important for DLI success. Interestingly, a recent study reported the "natural" occurrence of CTLA4-CD28 gene fusion in diverse types of T-cell lymphoma which can promote cell growth [230]. Further characterization of such kind of fusion (or natural CCR) should be explored. A recent report also depicted an extensive characterization and testing of different designs of human and murine CD200/CD28 CCR (or immunomodulatory fusion proteins - IFPs) [231].

Another possibility is to use an antigen-specific antibody fragment as targeting moiety for CCRs. Antibody-based and antigen-specific CCRs are not intended "to convert a negative signal into positive one" but rather to supply co-stimulation in an antigen-dependent manner [232] and/or to improve on target specificity [233]. It is also possible to transduce T-cells with a CD3ζ molecule fused to CD28 or 41BB IC domains (termed activating adapter molecules - ATAM) to provide co-stimulation to antigen specific T-cells [234].

5.2. Engineering T cells with cytokines and their receptors

T cell activation and differentiation requires several signals from the TCR (signal 1), costimulatory molecules (signal 2) and often from cytokines (often termed signal 3). The latter can have profound effects on T-cell differentiation and function. T cells can be genetically engineered to constitutively express cytokines such as IL2 [235], IL12 [236], IL15 [237] or their receptor [238] to improve their function and persistence (Fig. 1). CAR could also be modified to incorporate cytokine receptor domain. Kagoya et al. showed that a second generation CAR that contained a truncated IL2Rb domain and a STAT3- binding tyrosine-X-X-glutamine (YXXQ) motif allowed specific antigen-dependent activation of the JAK/STAT3 signaling pathways which promoted T cell proliferation, reduced terminal differentiation and enhanced intratumoral persistence [239]. Anti-CD19–28ζ CAR T cells modified to constitutively secrete IL-12 can mediate tumor eradication without the need for cytotoxic chemotherapy preconditioning in a mouse model [240] and can recruit host immune cells such as NK and DCs [241]. Armored-CAR T cells or TRUCKs (T cells Redirected for Universal Cytokine Killing) were also designed to repolarize the tumor microenvironment by co-expressing single chain IL-12. It was shown that constitutive IL-12 signaling enhances T-cell cytotoxicity and cytokine secretion, promoting resistance against Treg immunosuppression [242,243]. As such, IL12 relevance to CAR T-cell treatment is being tested in a Phase I clinical trial using a novel IL-12 secreting MUC16(ecto) - CAR T cells for ovarian cancer patients [244].

T cells can be rendered "immune" to the effects of immunosuppressive cytokines such as TGFβ. The TGFβ-receptor complex is a tetramer of two TGFβR1 and two TGFβR2 subunits [245]. By expressing a truncated TGFβR2 acting as dominant negative receptor in T cells [246], it is possible to improve the anti-tumor activity of engineered T cells [247,248] and this approach is being evaluated in multiple clinical trials (*e.g.* NCT02065362, NCT01955460, NCT00368082, NCT03089203). Additionally, NK92 cells transduced with a chimeric cytokine receptor in the form of extracellular domain of TGFβR2 fused to the signaling portion of NKG2D displayed better cytotoxic capacity [249]. Moreover, it is also possible to use chimeric cytokine receptor to modify T-cell behavior when exposed to certain cytokines. For example, IL4 can limit Th1 differentiation which could ultimately hinder CTL function [250]. Thus, retargeting IL4 function by expressing a IL-4/7 receptor chimeric to activate IL-7 signaling pathway led to enhanced activity against pancreatic tumors when combined with a PSCA-specific CAR [251].

T-cell infiltration and presence in the tumor is vital for the success of immunotherapy. This applies also to T-cell engineering. To overcome the lack of trafficking of specific T cells to tumors, it is possible to engineer them to express chemokine receptors such as CXCR2 [252,253], CCR4 [253], CCR2 [254,255] or CXCR1 as we previously showed [256] (Fig.1).

Finally, T-cell function can be enhanced by engineering them to express/secrete selected enzymes and other therapeutic molecules. To provide some resistance to the oxidative stress and limit cellular damages due to the exposure to reactive oxygen species (ROS), Ligtenberg et al. engineered T cells to express catalase [257]. Tumor encapsulation by extra cellular matrix is considered a potent escape mechanism displayed by certain types of tumor. To remedy to this, Caruana et al. engineered T cells to secrete heparanase enabling the efficient degradation of ECM and targeting of neuroblastoma tumors in mouse [258]. Furthermore, it is feasible to engineer T cells to secrete checkpoint blockers [259–261] (Fig. 1) or HVEM [262] to lower the immunosuppressive pressure in the tumor vicinity.

6. Other Practical consideration in T-cell engineering

6.1. Gene engineering and editing platforms

Dysregulation of immune activity can lead to fatal consequences and this even truer when it comes to genetically manipulated cells [99,100,263]. Thus, when using engineered T cells, it is critical to not only consider the nature and distribution of the targeted antigen but also the type of expression platform and the possible incorporation of switch/suicide genes to control the transgene expression and function.

The most common viral platforms of expression are based on either γ-retroviral vectors such as the MSCV (Mouse stem cell virus) -based or MPSV (myeloproliferative sarcoma virus) [13,264] and lentiviral vectors [265–268]. The latter may represent a better alternative than γ-retroviral vectors due to their ability to transduce non-dividing cells, to be more resistant to gene silencing [269] and to their safer integration site profile [60,270]. Nonetheless, both viral vector types are efficient and currently used in the FDA approved therapeutic products Axicabtagene ciloleucel (retrovirus) and Tisagenlecleucel (lentivirus). Still, their use requires safety assessments and the production of GMP-grade viral supernatant for human trials. In the past few years, several prominent leaders in the field have called for easing the regulation as there is no evidence RCL replication-competent lentivirus (RCL) or retrovirus (RCR) was observed in patients [271–273].

Non-viral methods for T-cell engineering such as using the *sleeping beauty* transposon system were developed to avoid the need for expensive large-scale production and safety testing [69,274]. In this approach, there is a need to introduce in the target cell both the transposase (originally identified in the genome of extinct salmonid) and the transposon (DNA) to be integrated in the host cell genome. While originally this approach achieved only low transgene expression levels [274], it has been constantly improved in the past years and this led to its evaluation in a CD19-CAR trial in which complete remissions were achieved [275]. But recently, another exciting approach has been described to engineer T cells using non-viral platforms. Roth et al. made use of a CRISPR-Cas9 genome-targeting system to insert large DNA sequences (greater than 1 kb) at precise genomic locations in primary human T cells to correct pathogenic mutations or to endow them with tumor-specificity using an NY-ESO TCR [276]. An additional strategy to transfect T cells that we and others developed in the past consists in electroporating

activated T cells with mRNA [277]. One of the main strengths of this approach is the relative ease and rapidity of the process. The transgene is expressed within a few hours of electroporation but only transiently (for several days), with its expression decreasing rapidly following cell division. This is in sharp contrast to viral methods that do require several processing days, but on the other hand, genome integration leads to constitutive transgene expression. Nonetheless, mRNA electroporation was shown to be implementable even in the context of a clinical trial [157]. Further clinical application will require process streamlining using adequate devices and frequent injection to reach a sufficient number of cells for treatment and compensate for transient transgene expression.

Multiple tools for genome editing have been developed in the past two decades [278] and these include zinc-finger, TALEN and of course CRISPR/Cas9 systems which have been implemented for T-cell engineering [56,279]. Indeed, these approaches were demonstrated to reliably knock down the expression of PD1 and other checkpoint inhibitors or key signaling molecules [280–283] and further optimization has been performed to delineate proper conditions for CRISPR editing in primary T cells [284] showing the successful silencing of several checkpoint targets. Unexpectedly, Fraietta et al. recently showed that in the case of a CLL patient that achieved complete remission when treated with anti-CD19 CAR T-cells, the insertion of the CAR transgene caused a disruption in the methylcytosine dioxygenase TET2 gene [285]. Further investigation revealed that TET2 deficient cells from clonal origin constituted at the peak of the response, 94% of the CAR T cells in the patient and displayed a central memory phenotype. Moreover, intentional knockdown of TET2 in normal T-cells could recapitulate the functional enhancement observed in the aforementioned patient T-cells.

To minimize GVHD and TCR mispairing, TCR components can also be targeted [58,279,286,287]. For example, Eyquem et al. directed the insertion of a CAR in the TCRα locus, knocking down the endogenous TCR expression [286]. Similarly, Mastaglio and colleagues proposed a novel TCR "single editing" (SE) approach, based on the disruption of only the endogenous TCRα chain followed by the transfer of NY-ESO TCR genes and this allowed the rapid production of high numbers of tumor-specific T cells with a stem memory and central memory phenotype [288]. One can also combine multiple editing/engineering steps simultaneously to generate the "optimal" cancer-specific T-cell [282,289]. It is also possible to use genome editing to abrogate the expression of the targeted antigen from the (targeting) T cells themselves. Rasaiyaah et al. used TALEN to eliminate the endogenous TCR complex while expressing simultaneously an anti-CD3 CAR to target leukemic T cells with minimal off-target effects [290]. Similarly, CD33 expression in progenitor cells was successfully abrogated using CRISPR/Cas9 gene editing [291]. Injection of CD33-knocked out cells could help replenishing myeloid lineages in mice and non-human primate even when using concomitant CD33-specific CAR T-cells treatments. Nonetheless, the long-term effects of these approaches on the patient immune system and its competency remain to be evaluated.

6.2. Control of transgene expression and T-cell function

To ensure safer T-cell engineering implementation and precise activation, several strategies have been developed [152]. As for other types of gene therapy approaches, a suicide gene could be included in the therapeutic vector to mediate the elimination of gene-engineered cells. A common suicide gene is the Herpes Simplex Virus – derived Thymidine Kinase (HSV-TK), that has substantially higher affinity to Ganciclovir (GCV) that its human counterpart. When HSV-TK transduced T cells are exposed to GCV, the latter is phosphorylated and it can interfere with DNA synthesis. This approach has been already implemented in clinical trials [292]. Another possibility to induce rapid ablation of the cells is to make use of an inducible Caspase9 (iCas9) safety switch that can be activated by dimerization using a small-molecule drug. This switch was shown to successfully ablate CAR-T cells *in vitro* and *in vivo* [293,294] and this strategy too is being tested clinically (NCT02107963, NCT01953900). Besides suicide genes, it is also possible to deplete transduced T cells *in vivo* using antibodies that target certain determinants or tags expressed in the transgene [295,296]. Additionally, small molecules can be used to induce the transcription of the transgene [297] or the dimerization and assembly of split CAR chains [298]. Induction of CAR function was also exemplified in the case of a HIF-CAR which was design to incorporate HIF1α domain making it sensitive to oxygen and degraded under normoxia conditions [299]. Also, receptor potentiation by proteolysis of a small masking tag degraded in the presence of specific proteases expressed in the tumor microenvironment was elegantly demonstrated for an EGFR-specific CAR [300]. Recently, Helsen et al. reported the use of a T-cell antigen coupler (TAC) chimeric receptor [301] which consists of a double targeting moiety: an antigen-specific scFv followed by a CD3-activating scFv built on a CD4 scaffold. Such TAC could recognize a defined tumor antigen on the target cell using the endogenous TCR signaling pathway rather than an artificial signal mediated by classical CD28/CD3z signaling moiety.

Another layer of control could be achieved using strategies akin to (receptor-mediated) Boolean gates. For example, utilizing AND-gates based on dual receptor expression (*i.e.* a 1st generation CAR combined with a CCR) could ensure more precise and on-target activation of T cells [233,302,303]. Another type of "AND" gate can be designed in a "sequential" manner using a synthetic Notch (synNotch) system based on the combinatorial recognition of two antigens. Due to the distinct signal transduction of Notch receptor, which cleaves its IC domain upon ligand binding [304], it is possible to substitute its native IC domain by the domain of interest. The latter can be replaced by the highly effective (although xenogeneic) Gal4-VP64 transcription factor. Upon cleavage, it can translocate to the nucleus and drives the effector CAR expression. Such synthetic Notch-Gal4-VP64 receptor can be fused downstream to scFv targeting moiety ensuring the antigen-directed Notch proteolytic cleavage [305]. This system was extensively characterized in the context of CAR expression but also of conditional secretion of checkpoint inhibitors, bi-specific T-cell engagers and alternatively, immunosuppressive molecule such as IL10 or PDL1 [306]. As aforementioned, an "OR" gate is represented by bi-specific CAR or dual-CAR expressing T cells [97,148].

Finally, the use of an inhibitory CAR (iCAR) directed against a normal antigen to inhibit T-cell function could be considered a "NOT" gate. Using a PSMA-scFv fused to either the IC domains of CTLA-4 or PD-1, Fedorov et al. demonstrated the potential reduction of T-cell function when T cells expressing the iCAR and a CD19-specific (conventional) CAR were challenged by CD19+ PSMA+ tumors while CD19+ PSMA- tumors were successfully eradicated [307]. Still, for this approach to be effective clinically, exclusive normal ligands (*i.e.*, not expressed by tumor cells) will need to be identified and validated.

6.3. Universal CAR systems

As described above, TCR/CAR-engineered T cells target defined and fixed antigens. Lately, major efforts are invested in developing strategies for a more flexible and adaptable antigen specificity. These "universal" CAR systems split the antigen recognition mechanism into two components: expression of a signaling adaptor on T cells and a soluble targeting moiety counterpart (Fig. 1). Using such approaches, one could easily regulate/tune T cell anti-tumor response by alternating the soluble targeting moiety "on demand". For example, using biotinylated anti-mesothelin scFv or antibodies, avidin-based [308] or streptavidin-based [309] CAR take advantage of the high-affinity avidin:biotin interaction. A similar concept was developed based on peptide tagging [310] or FITC-labeled therapeutic antibodies or ligands and anti-FITC CARs [311,312]. T cells can also be engineered to express a CAR based on 4-1BB and CD3z signaling domains linked to a high-affinity Fc binding molecule in the form of a CD16 variant. Upon

incubation with "naked" Abs, these T cells become "coated" with the selected antibodies and can function as regular CAR T cells [313]. This approach recently reached an additional level of flexibility with the "Split, Universal, and Programmable" (SUPRA) CAR system [314]. Using a leucine-zipper based approach, the authors engineered a system that enables the possibility to direct T cells against different targets without re-engineering the T cells, to precisely tune the strength of T cell activation and to logically respond to several antigens.

6.4. Looking for the best cell to engineer

Cumulative data obtained in recent years demonstrated that T cells with different functions and roles as memory or effector T cells may impact on CAR-T cell immunotherapy and that a more personalized therapy should be applied depending on the patient's T cell profiling [315]. T cell response depends on various parameters including TCR signal strength, the balance between stimulation and inhibitory signals as well as inflammatory and immune contexture [316]. Based on the type and strength of the signals received, T cells can differentiate into different subsets of cells with distinct phenotype and function [317]. A model of T cell differentiation was proposed in which cells progress from naïve T cells (T_N) to stem cell memory (T_{SCM}), central memory (T_{CM}) and effector memory (T_{EM}), defined by their phenotype and their functional ability. The effector function of $CD8^+$ T cells is increased upon T cell differentiation, while their memory function and proliferation are decreased [318]. Additional factors can affect the functional level and differentiation states of effector and memory T cells including the genetic signature of key effector genes, epigenetic states [319] as well as distinct metabolic pathway [320]. Nevertheless, it seems that T_{CM} could mediate increased persistence and growth leading to better anti-tumor activity compared to T_{EM} in xenograft models [321,322]. Moreover, the use of central memory–derived CD19 1st gen. CAR T–cells in patients with B-cell NHL has proven safe, though they did not persist longer than conventional bulk T cells expressing 2nd gen. CARs [323]. It was shown that a subset of T_{SCM} from a $CD45RA^+$ population, expressing high levels of CD62L, CD95, and CCR-7 were more persistent and more effective against tumors than T_{CM} [317]. Such cells can be generated artificially using tethered IL15 and mediate leukemia rejection in xenograft models [324]. Another possibility is to use hematopoietic stem cells and to differentiate them using the OP9/DL1 system as we and others previously showed [325,326] or iPSCs [327] though extra-care should be taken when genetically modifying such poorly differentiated cells [328]. Additionally, combination of $CD4^+$ and $CD8^+$ CD19-CAR T cells can result in a synergistic anti-tumor effect *in vivo* [169,329] and methodologies are being developed to ensure precise composition of CAR T-cell product [330]. To avoid potential off-target effects and reduce GVHD, viral-specific T cells can be engineered to express a CAR or TCR [121,331,332]. This could also provide a way to specifically stimulate engineered T cells after infusion using viral vaccines.

Not surprisingly, it has been shown that different cocktails of cytokines can affect the differentiation status of T cell phenotype, *in vivo* survival and function. For instance, pre-treatment of T cells with IL-7 and IL-15 or IL-15 and IL-21 was shown to increase T memory cell functions and anti-tumor activity of CAR-T cells [333,334]. CAR-T cells expanded with IL-7 and IL-15 displayed higher survival capacity *in vivo* when compared to CAR-T expanded with IL-2 [333]. IL-15 alone has also been shown to increase CD8 T cell function and hence its anti-tumor activity [335].

As aforementioned, NK cells can also exhibit potent anti-tumor function and their activity is modulated by an array of inhibitory and activating receptors [198]. Because of their cytotoxic capacity, a few studies have also examined the potential of NK lymphocytes to serve as a cellular platform for CARs (reviewed in [336]). This was done for example using primary NK cells [337] and the NK92 cell line with the intent of using it as a "off the shelf"/universal donor cell [338,339]. Incorporation of IL15 into the CAR construct increases NK cell persistence *in vivo* [340], whereas the addition of C-X-X motif chemokine receptor 4 (CXCR4) increased their mobility to the tumor site [341]. Clinical studies evaluating the efficacy of CAR-NK are scarce (half a dozen taking place mainly in China and one in the US [336]) and they mainly target CD19 for B cell malignancies, CD33 for CD33+ AML [147] or MUC1.

Ultimately, the possibility to use off-the-shelf CAR-T cells derived from universal donors would contribute to ease the treatment process and the logistics involved in engineered cell production and manufacturing, and thus, to make it more affordable. The utilization of allogenic donors could certainly address the aforementioned issues and could prove effective [342–344] but it bears with it the risk of GVHD or rejection of the transferred cells. Still, these concerns could be addressed by way of genome editing. Using a zinc-finger approach and subsequently a transposon strategy, it was possible to knock down the expression of the endogenous TCR and to express a CD19-specific CAR respectively [345]. In a pilot clinical trial, two ALL infant patients were treated with cells from an allogenic source in which the TCRα locus was deleted using TALEN [346], thereby preventing the expression of the TCR complex. The patients achieved remission, however, due to some contamination of unedited cells, they did experience GVHD. Alternatively, to prevent the rejection of the transferred cells, Ren et al. demonstrated the feasibility of knocking down β2-microglobulin, an essential component of all class I MHC molecules [347]. Though this approach might expose the HLA-knocked out engineered cells to NK activity, the latter may be neutralized if forcing the expression of regulatory HLA-E molecules [348].

7. Future applications beyond cancer

In the future, it is conceivable that engineered T cells may also be used to target also infectious diseases [41] or facilitate the induction of tolerance in transplanted recipient and individual suffering of autoimmune diseases [96,349]. It was shown that the engineering of natural Treg cells or co-transduction of T cells with specific TCRs and FoxP3 can generate Treg cells that were able to suppress arthritis in different models or mitigate autoimmunity [350,351]. On the other hand, CAR T cells expressing a chimeric autoantibody receptor (CAAR), consisting of the PV autoantigen, desmoglein (Dsg) 3 and signaling domains can be used to eliminate auto-reactive B cells in a mouse model of pemphigus vulgaris [352].

Infectious diseases are still the major cause of mortality in the developing world. Given the tremendous potential of T-cell engineering exemplified in cancer, one could easily adapt the aforementioned approached to target viruses such as HCV [353], CMV [354], EBV [355,356], HBV [357], and HPV [358,359] or even to treat opportunistic infections [360].

8. Conclusions

Modern medicine has been revolutionized by the discovery of antibiotics and the development of vaccines that turned most infectious diseases into a "solvable nuisance". However, cancer is a complex disease [202] that requires an equally elaborated fighting counterpart – if any, groundbreaking studies in the past 30 years convinced us that the immune system is up to this task. Nonetheless, several issues need to be solved to extend the success obtained in hematological malignancies to solid tumors. The identification of suitable targets will benefit from bioinformatics and precision medicine; we recently showed that personalized immunotherapy based on the neoantigen landscape is attainable, including in the context of T-cell engineering [361]. Another important step will be to combine therapies along with cell engineering such as checkpoint inhibitors, small molecules and chemotherapy [8,214]. Better biomarkers will also help custom-tailoring immunotherapy [362], hoping that regulatory approval will be given for treatment in the early stages of disease. As with every cancer therapy, resistance and

subsequent relapse are also relevant here and we need to implement approaches that would target simultaneously multiple targets/antigens to minimize tumor escape. Surprisingly, a recent report uncovered an unexpected resistance mechanism to CD19-CAR-T cell therapy: Ruella et al. showed that during the cell production process, a contaminating lymphoma B-cell underwent transduction leading to CD19-CAR expression. *Cis* interactions with surface CD19 resulted in its shielding by the CAR, making the antigen inaccessible to CAR-T cells [363]. While this phenomenon was not reported elsewhere, it does underscore the need to care for stringent production processes but more importantly, it demonstrates to what extent the development and mechanisms of resistance to engineered lymphocyte treatments may be unusal.

So far, most of the engineered T-cell related toxicities can be managed but the emergence of animal models to study CAR-mediated toxicities such as cytokine release syndrome (CRS) will certainly help devising safer treatments [364]. Another way to minimize adverse events will rely, with the help of synthetic biology, on the precise control and regulation of engineered T-cell function. Precise *in vivo* tracking of engineered cells using can also assist in this task by determining their biodistribution and homing to the tumor as well as evaluating their persistence [68,365]. Finally, one can hope that technological advances will simplify the manufacturing process of engineered T-cells and will even lead to the implementation of *in vivo* genetic engineering strategies [366]; preliminary exciting studies showed that DNA-carrying nanoparticle can selectively deliver CAR genes into host T cells facilitating tumor regression as conventional. These reprogrammed T cells remained functional as a 'living drug' and ultimately differentiated into long-lived memory T cells [367].

Oncotherapy itself has slowly gradually benefited from surgery, radiation therapy, chemotherapy and small-molecules. The present era is undoubtedly that of immunotherapy - many challenges remain ahead of us to turn neoplastic diseases into "a thing of the past", but it seems that we are "off to a good start".

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References

[1] A. Ribas, J.D. Wolchok, Cancer immunotherapy using checkpoint blockade, Science 359 (2018) 1350–1355.
[2] P.G. Coulie, B.J. Van den Eynde, P. van der Bruggen, T. Boon, Tumour antigens recognized by T lymphocytes: at the core of cancer immunotherapy, Nat. Rev. Cancer 14 (2014) 135–146.
[3] S.A. Rosenberg, Decade in review-cancer immunotherapy: Entering the mainstream of cancer treatment, Nat. Rev. Clin. Oncol. 11 (2014) 630–632.
[4] S.A. Rosenberg, J.C. Yang, R.M. Sherry, U.S. Kammula, M.S. Hughes, G.Q. Phan, D.E. Citrin, N.P. Restifo, P.F. Robbins, J.R. Wunderlich, K.E. Morton, C.M. Laurencot, S.M. Steinberg, D.E. White, M.E. Dudley, Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy, Clin. Cancer Res. 17 (2011) 4550–4557.
[5] E. Tran, S. Turcotte, A. Gros, P.F. Robbins, Y.C. Lu, M.E. Dudley, J.R. Wunderlich, R.P. Somerville, K. Hogan, C.S. Hinrichs, M.R. Parkhurst, J.C. Yang, S.A. Rosenberg, Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer, Science 344 (2014) 641–645.
[6] S. Stevanovic, L.M. Draper, M.M. Langhan, T.E. Campbell, M.L. Kwong, J.R. Wunderlich, M.E. Dudley, J.C. Yang, R.M. Sherry, U.S. Kammula, N.P. Restifo, S.A. Rosenberg, C.S. Hinrichs, Complete regression of metastatic cervical cancer after treatment with human papillomavirus-targeted tumor-infiltrating T cells, J. Clin. Oncol. 33 (2015) 1543–1550.
[7] E. Tran, P.F. Robbins, Y.C. Lu, T.D. Prickett, J.J. Gartner, L. Jia, A. Pasetto, Z. Zheng, S. Ray, E.M. Groh, I.R. Kriley, S.A. Rosenberg, T-Cell transfer Therapy Targeting Mutant KRAS in Cancer, N. Engl. J. Med. 375 (2016) 2255–2262.
[8] N. Zacharakis, H. Chinnasamy, M. Black, H. Xu, Y.C. Lu, Z. Zheng, A. Pasetto, M. Langhan, T. Shelton, T. Prickett, J. Gartner, L. Jia, K. Trebska-McGowan, R.P. Somerville, P.F. Robbins, S.A. Rosenberg, S.L. Goff, S.A. Feldman, Immune recognition of somatic mutations leading to complete durable regression in metastatic breast cancer, Nat. Med. 24 (2018) 724–730.
[9] M.H. Kershaw, J.A. Westwood, P.K. Darcy, Gene-engineered T cells for cancer therapy, Nat. Rev. Cancer 13 (2013) 525–541.
[10] Z. Dembic, W. Haas, S. Weiss, J. McCubrey, H. Kiefer, H. von Boehmer, M. Steinmetz, Transfer of specificity by murine alpha and beta T-cell receptor genes, Nature 320 (1986) 232–238.
[11] T.M. Clay, M.C. Custer, J. Sachs, P. Hwu, S.A. Rosenberg, M.I. Nishimura, Efficient transfer of a tumor antigen-reactive TCR to human peripheral blood lymphocytes confers anti-tumor reactivity, J. Immunol. 163 (1999) 507–513.
[12] H.W. Kessels, M.C. Wolkers, D.B. Van, M.A. van der Valk, T.N. Schumacher, Immunotherapy through TCR gene transfer, Nat. Immunol 2 (2001) 957–961.
[13] R.A. Morgan, M.E. Dudley, J.R. Wunderlich, M.S. Hughes, J.C. Yang, R.M. Sherry, R.E. Royal, S.L. Topalian, U.S. Kammula, N.P. Restifo, Z. Zheng, A. Nahvi, C.R. de Vries, L.J. Rogers-Freezer, S.A. Mavroukakis, S.A. Rosenberg, Cancer regression in patients after transfer of genetically engineered lymphocytes, Science 314 (2006) 126–129.
[14] A.I. Riker, U.S. Kammula, M.C. Panelli, E. Wang, G.A. Ohnmacht, S.M. Steinberg, S.A. Rosenberg, F.M. Marincola, Threshold levels of gene expression of the melanoma antigen gp100 correlate with tumor cell recognition by cytotoxic T lymphocytes, Int. J. Cancer 86 (2000) 818–826.
[15] L.A. Johnson, B. Heemskerk, D.J. Powell Jr., C.J. Cohen, R.A. Morgan, M.E. Dudley, P.F. Robbins, S.A. Rosenberg, Gene transfer of tumor-reactive TCR confers both high avidity and tumor reactivity to nonreactive peripheral blood mononuclear cells and tumor-infiltrating lymphocytes, J. Immunol. 177 (2006) 6548–6559.
[16] L.A. Johnson, R.A. Morgan, M.E. Dudley, L. Cassard, J.C. Yang, M.S. Hughes, U.S. Kammula, R.E. Royal, R.M. Sherry, J.R. Wunderlich, C.C. Lee, N.P. Restifo, S.L. Schwarz, A.P. Cogdill, R.J. Bishop, H. Kim, C.C. Brewer, S.F. Rudy, C. Vanwaes, J.L. Davis, A. Mathur, R.T. Ripley, D.A. Nathan, C.M. Laurencot, S.A. Rosenberg, Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen, Blood 114 (2009) 535–546.
[17] C.J. Cohen, Z. Zheng, R. Bray, Y. Zhao, L.A. Sherman, S.A. Rosenberg, R.A. Morgan, Recognition of fresh human tumor by human peripheral blood lymphocytes transduced with a bicistronic retroviral vector encoding a murine anti-p53 TCR, J. Immunol. 175 (2005) 5799–5808.
[18] J.L. Davis, M.R. Theoret, Z. Zheng, C.H. Lamers, S.A. Rosenberg, R.A. Morgan, Development of human anti-murine T-cell receptor antibodies in both responding and nonresponding patients enrolled in TCR gene therapy trials, Clin. Cancer Res. 16 (2010) 5852–5861.
[19] M.R. Theoret, C.J. Cohen, A.V. Nahvi, L.T. Ngo, K.B. Suri, D.J. Powell, M.E. Dudley, R.A. Morgan, S.A. Rosenberg, Relationship of p53 Overexpression on Cancers and Recognition by anti-p53 TCR Transduced T cells, Hum. Gene Ther. 19 (11) (2008 Nov) 1219–1232, https://doi.org/10.1089/hgt.2008.083.
[20] K. Shamalov, S.N. Levy, M. Horovitz-Fried, C.J. Cohen, The mutational status of p53 can influence its recognition by human T-cells, Oncoimmunology 6 (2017), e1285990. .
[21] E. Tran, P.F. Robbins, S.A. Rosenberg, 'Final common pathway' of human cancer immunotherapy: targeting random somatic mutations, Nat. Immunol. 18 (2017) 255–262.
[22] M.R. Parkhurst, J.C. Yang, R.C. Langan, M.E. Dudley, D.A. Nathan, S.A. Feldman, J.L. Davis, R.A. Morgan, M.J. Merino, R.M. Sherry, M.S. Hughes, U.S. Kammula, G.Q. Phan, R.M. Lim, S.A. Wank, N.P. Restifo, P.F. Robbins, C.M. Laurencot, S.A. Rosenberg, T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis, Mol. Ther. 19 (2011) 620–626.
[23] R.A. Morgan, N. Chinnasamy, D. Abate-Daga, A. Gros, P.F. Robbins, Z. Zheng, M.E. Dudley, S.A. Feldman, J.C. Yang, R.M. Sherry, G.Q. Phan, M.S. Hughes, U.S. Kammula, A.D. Miller, C.J. Hessman, A.A. Stewart, N.P. Restifo, M.M. Quezado, M. Alimchandani, A.Z. Rosenberg, A. Nath, T. Wang, B. Bielekova, S.C. Wuest, N. Akula, F.J. McMahon, S. Wilde, B. Mosetter, D.J. Schendel, C.M. Laurencot, S.A. Rosenberg, Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy, J Immunother. 36 (2013) 133–151.
[24] G.P. Linette, E.A. Stadtmauer, M.V. Maus, A.P. Rapoport, B.L. Levine, L. Emery, L. Litzky, A. Bagg, B.M. Carreno, P.J. Cimino, G.K. Binder-Scholl, D.P. Smethurst, A.B. Gerry, N.J. Pumphrey, A.D. Bennett, J.E. Brewer, J. Dukes, J. Harper, H.K. Tayton-Martin, B.K. Jakobsen, N.J. Hassan, M. Kalos, C.H. June, Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma, Blood 122 (2013) 863–871.
[25] J.D. Stone, D.T. Harris, D.M. Kranz, TCR affinity for p/MHC formed by tumor antigens that are self-proteins: impact on efficacy and toxicity, Curr. Opin. Immunol. 33 (2015) 16–22.
[26] E.S. Hickman, M.E. Lomax, B.K. Jakobsen, Antigen selection for Enhanced Affinity T-Cell Receptor-based Cancer Therapies, J. Biomol. Screen. 21 (2016) 769–785.
[27] P.F. Robbins, S.H. Kassim, T.L. Tran, J.S. Crystal, R.A. Morgan, S.A. Feldman, J.C. Yang, M.E. Dudley, J.R. Wunderlich, R.M. Sherry, U.S. Kammula, M.S. Hughes, N.P. Restifo, M. Raffeld, C.C. Lee, Y.F. Li, M. El-Gamil, S.A. Rosenberg, 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 (2015) 1019–1027.
[28] A.P. Rapoport, E.A. Stadtmauer, G.K. Binder-Scholl, O. Goloubeva, D.T. Vogl, S.F. Lacey, A.Z. Badros, A. Garfall, B. Weiss, J. Finklestein, I. Kulikovskaya, S.K. Sinha, S. Kronsberg, M. Gupta, S. Bond, L. Melchiori, J.E. Brewer, A.D. Bennett, A.B. Gerry, N.J. Pumphrey, D. Williams, H.K. Tayton-Martin, L. Ribeiro, T. Holdich, S. Yanovich, N. Hardy, J. Yared, N. Kerr, S. Philip, S. Westphal, D.L. Siegel, B.L. Levine, B.K. Jakobsen, M. Kalos, C.H. June, NY-ESO-1-specific TCR-engineered T cells mediate sustained antigen-specific antitumor effects in myeloma, Nat. Med. 21 (2015) 914–921.
[29] C.J. Cohen, J.J. Gartner, M. Horovitz-Fried, K. Shamalov, K. Trebska-McGowan, V.V. Bliskovsky, M.R. Parkhurst, C. Ankri, T.D. Prickett, J.S. Crystal, Y.F. Li, M. El-Gamil,

S.A. Rosenberg, P.F. Robbins, Isolation of neoantigen-specific T cells from tumor and peripheral lymphocytes, J. Clin. Invest. 125 (2015) 3981–3991.
[30] M. Obenaus, C. Leitao, M. Leisegang, X. Chen, I. Gavvovidis, P. van der Bruggen, W. Uckert, D.J. Schendel, T. Blankenstein, Identification of human T-cell receptors with optimal affinity to cancer antigens using antigen-negative humanized mice, Nat. Biotechnol. 33 (2015) 402–407.
[31] R. Debets, E. Donnadieu, S. Chouaib, G. Coukos, TCR-engineered T cells to treat tumors: seeing but not touching? Semin. Immunol. 28 (2016) 10–21.
[32] T.M. Schmitt, D.H. Aggen, K. Ishida-Tsubota, S. Ochsenreither, D.M. Kranz, P.D. Greenberg, Generation of higher affinity T cell receptors by antigen-driven differentiation of progenitor T cells in vitro, Nat. Biotechnol. 35 (2017) 1188–1195.
[33] M.H. Gee, A. Han, S.M. Lofgren, J.F. Beausang, J.L. Mendoza, M.E. Birnbaum, M.T. Bethune, S. Fischer, X. Yang, R. Gomez-Eerland, D.B. Bingham, L.V. Sibener, R.A. Fernandes, A. Velasco, D. Baltimore, T.N. Schumacher, P. Khatri, S.R. Quake, M.M. Davis, K.C. Garcia, Antigen identification for orphan T cell receptors expressed on tumor-infiltrating lymphocytes, Cell 172 (2018) 549–563.
[34] T. Tsuji, A. Yoneda, J. Matsuzaki, A. Miliotto, C. Ryan, R.C. Koya, K. Odunsi, Rapid construction of antitumor T-cell receptor vectors from frozen tumors for engineered T-cell therapy, Cancer Immunol. Res. 6 (2018) 594–604.
[35] S. Walchli, G.A. Loset, S. Kumari, J.N. Johansen, W. Yang, I. Sandlie, J. Olweus, A practical approach to T-cell receptor cloning and expression, PLoS One 6 (2011), e27930. .
[36] G. Dossinger, M. Bunse, J. Bet, J. Albrecht, P.J. Paszkiewicz, B. Weissbrich, I. Schiedewitz, L. Henkel, M. Schiemann, M. Neuenhahn, W. Uckert, D.H. Busch, MHC multimer-guided and cell culture-independent isolation of functional T cell receptors from single cells facilitates TCR identification for immunotherapy, PLoS One 8 (2013), e61384. .
[37] Y.C. Lu, Z. Zheng, P.F. Robbins, E. Tran, T.D. Prickett, J.J. Gartner, Y.F. Li, S. Ray, Z. Franco, V. Bliskovsky, P.C. Fitzgerald, S.A. Rosenberg, An efficient single-Cell RNA-Seq approach to identify neoantigen-specific T cell receptors, Mol. Ther. 26 (2018) 379–389.
[38] C.J. Cohen, Y. Zhao, Z. Zheng, S.A. Rosenberg, R.A. Morgan, Enhanced antitumor activity of murine-human hybrid T-Cell receptor (TCR) in human lymphocytes is associated with improved pairing and TCR/CD3 stability, Cancer Res. 66 (2006) 8878–8886.
[39] G.M. Bendle, C. Linnemann, A.I. Hooijkaas, L. Bies, M.A. de Witte, A. Jorritsma, A.D. Kaiser, N. Pouw, R. Debets, E. Kieback, W. Uckert, J.Y. Song, J.B. Haanen, T.N. Schumacher, Lethal graft-versus-host disease in mouse models of T cell receptor gene therapy, Nat. Med. 16 (2010) 565–570.
[40] M.M. van Loenen, R. de Boer, A.L. Amir, R.S. Hagedoorn, G.L. Volbeda, R. Willemze, J.J. van Rood, J.H. Falkenburg, M.H. Heemskerk, Mixed T cell receptor dimers harbor potentially harmful neoreactivity, Proc. Natl. Acad. Sci. U. S. A 107 (2010) 10972–10977.
[41] I. Daniel-Meshulam, S. Ya'akobi, C. Ankri, C.J. Cohen, How (specific) would like your T-cells today? Generating T-cell therapeutic function through TCR-gene transfer, Front. Immunol. 3 (2012) 186.
[42] G. Bialer, M. Horovitz-Fried, S. Ya'acobi, R.A. Morgan, C.J. Cohen, Selected murine residues endow human TCR with enhanced tumor recognition, J. Immunol. 184 (2010) 6232–6241.
[43] D. Sommermeyer, W. Uckert, Minimal amino acid exchange in human TCR constant regions fosters improved function of TCR gene-modified T cells, J. Immunol. 184 (2010) 6223–6231.
[44] S.L. Goff, L.A. Johnson, M.A. Black, H. Xu, Z. Zheng, C.J. Cohen, R.A. Morgan, S.A. Rosenberg, S.A. Feldman, Enhanced receptor expression and in vitro effector function of a murine-human hybrid MART-1-reactive T cell receptor following a rapid expansion, Cancer Immunol. Immunother. 59 (2010) 1551–1560.
[45] S. Turcotte, A. Gros, E. Tran, C.C. Lee, J.R. Wunderlich, P.F. Robbins, S.A. Rosenberg, Tumor-reactive CD8+ T cells in metastatic gastrointestinal cancer refractory to chemotherapy, Clin. Cancer Res. 20 (2014) 331–343.
[46] T. Matsuda, M. Leisegang, J.H. Park, L. Ren, T. Kato, Y. Ikeda, M. Harada, K. Kiyotani, E. Lengyel, G.F. Fleming, Y. Nakamura, Induction of neoantigen-specific cytotoxic T cells and construction of t-cell receptor-engineered T cells for ovarian cancer, Clin. Cancer Res. 24 (21) (2018 Nov 1) 5357–5367, https://doi.org/10.1158/1078-0432.CCR-18-0142.
[47] C.J. Cohen, Y.F. Li, M. El Gamil, P.F. Robbins, S.A. Rosenberg, R.A. Morgan, Enhanced antitumor activity of T cells engineered to express T-cell receptors with a second disulfide bond, Cancer Res. 67 (2007) 3898–3903.
[48] J. Kuball, M.L. Dossett, M. Wolfl, W.Y. Ho, R.H. Voss, C. Fowler, P.D. Greenberg, Facilitating matched pairing and expression of TCR chains introduced into human T cells, Blood 109 (2007) 2331–2338.
[49] R.H. Voss, R.A. Willemsen, J. Kuball, M. Grabowski, R. Engel, R.S. Intan, P. Guillaume, P. Romero, C. Huber, M. Theobald, Molecular design of the Calphabeta interface favors specific pairing of introduced TCRalphabeta in human T cells, J. Immunol. 180 (2008) 391–401.
[50] M.T. Bethune, M.H. Gee, M. Bunse, M.S. Lee, E.H. Gschweng, M.S. Pagadala, J. Zhou, D. Cheng, J.R. Heath, D.B. Kohn, M.S. Kuhns, W. Uckert, D. Baltimore, Domain-swapped T cell receptors improve the safety of TCR gene therapy, Elife 5 (2016).
[51] Z. Sebestyen, E. Schooten, T. Sals, I. Zaldivar, E. San Jose, B. Alarcon, S. Bobisse, A. Rosato, J. Szollosi, J.W. Gratama, R.A. Willemsen, R. Debets, Human TCR that incorporate CD3zeta induce highly preferred pairing between TCRalpha and beta chains following gene transfer, J. Immunol. 180 (2008) 7736–7746.
[52] D.H. Aggen, A.S. Chervin, T.M. Schmitt, B. Engels, J.D. Stone, S.A. Richman, K.H. Piepenbrink, B.M. Baker, P.D. Greenberg, H. Schreiber, D.M. Kranz, Single-chain ValphaVbeta T-cell receptors function without mispairing with endogenous TCR chains, Gene Ther. 19 (2012) 365–374.
[53] S. Okamoto, J. Mineno, H. Ikeda, H. Fujiwara, M. Yasukawa, H. Shiku, I. Kato, Improved expression and reactivity of transduced tumor-specific TCRs in human lymphocytes by specific silencing of endogenous TCR, Cancer Res. 69 (2009) 9003–9011.
[54] M. Bunse, G.M. Bendle, C. Linnemann, L. Bies, S. Schulz, T.N. Schumacher, W. Uckert, RNAi-mediated TCR knockdown prevents autoimmunity in mice caused by mixed TCR dimers following TCR gene transfer, Mol. Ther. 22 (2014) 1983–1991.
[55] E. Provasi, P. Genovese, A. Lombardo, Z. Magnani, P.Q. Liu, A. Reik, V. Chu, D.E. Paschon, L. Zhang, J. Kuball, B. Camisa, A. Bondanza, G. Casorati, M. Ponzoni, F. Ciceri, C. Bordignon, P.D. Greenberg, M.C. Holmes, P.D. Gregory, L. Naldini, C. Bonini, Editing T cell specificity towards leukemia by zinc finger nucleases and lentiviral gene transfer, Nat. Med. 18 (5) (2012 May) 807–815, https://doi.org/10.1038/nm.2700.
[56] B. Berdien, U. Mock, D. Atanackovic, B. Fehse, TALEN-mediated editing of endogenous T-cell receptors facilitates efficient reprogramming of T lymphocytes by lentiviral gene transfer, Gene Ther. 21 (2014) 539–548.
[57] F. Knipping, M.J. Osborn, K. Petri, J. Tolar, H. Glimm, K.C. Von, M. Schmidt, R. Gabriel, Genome-wide specificity of highly efficient TALENs and CRISPR/Cas9 for T Cell receptor modification, Mol. Ther. Methods Clin. Dev. 4 (2017) 213–224.
[58] M. Legut, G. Dolton, A.A. Mian, O.G. Ottmann, A.K. Sewell, CRISPR-mediated TCR replacement generates superior anticancer transgenic T cells, Blood 131 (2018) 311–322.
[59] S. Thomas, H.J. Stauss, E.C. Morris, Molecular immunology lessons from therapeutic T-cell receptor gene transfer, Immunology 129 (2010) 170–177.
[60] E. Merhavi-Shoham, A. Haga-Friedman, C.J. Cohen, Genetically modulating T-cell function to target cancer, Semin. Cancer Biol. 22 (2012) 14–22.
[61] K.B. Scholten, D. Kramer, E.W. Kueter, M. Graf, T. Schoedl, C.J. Meijer, M.W. Schreurs, E. Hooijberg, Codon modification of T cell receptors allows enhanced functional expression in transgenic human T cells, Clin. Immunol. 119 (2006) 135–145.
[62] A. Jorritsma, R. Gomez-Eerland, M. Dokter, K.W. Van De, Y.M. Zoet, I.I. Doxiadis, N. Rufer, P. Romero, R.A. Morgan, T.N. Schumacher, J.B. Haanen, Selecting highly affine and well-expressed TCRs for gene therapy of melanoma, Blood 110 (2007) 3564–3572.
[63] M.H. Heemskerk, R.S. Hagedoorn, M.A. van der Hoorn, L.T. van der Veken, M. Hoogeboom, M.G. Kester, R. Willemze, J.H. Falkenburg, Efficiency of T-cell receptor expression in dual-specific T cells is controlled by the intrinsic qualities of the TCR chains within the TCR-CD3 complex, Blood 109 (2007) 235–243.
[64] M. Ahmadi, J.W. King, S.A. Xue, C. Voisine, A. Holler, G.P. Wright, J. Waxman, E. Morris, H.J. Stauss, CD3 limits the efficacy of TCR gene therapy in vivo, Blood 118 (2011) 3528–3537.
[65] J. Kuball, B. Hauptrock, V. Malina, E. Antunes, R.H. Voss, M. Wolfl, R. Strong, M. Theobald, P.D. Greenberg, Increasing functional avidity of TCR-redirected T cells by removing defined N-glycosylation sites in the TCR constant domain, J. Exp. Med. 206 (2009) 463–475.
[66] A. Haga-Friedman, M. Horovitz-Fried, C.J. Cohen, Incorporation of Transmembrane Hydrophobic Mutations in the TCR Enhance its Surface Expression and T Cell Functional Avidity, J. Immunol. 188 (2012) 5538–5546.
[67] A. Gros, M.R. Parkhurst, E. Tran, A. Pasetto, P.F. Robbins, S. Ilyas, T.D. Prickett, J.J. Gartner, J.S. Crystal, I.M. Roberts, K. Trebska-McGowan, J.R. Wunderlich, J.C. Yang, S.A. Rosenberg, Prospective identification of neoantigen-specific lymphocytes in the peripheral blood of melanoma patients, Nat. Med. 22 (2016) 433–438.
[68] R. Meir, K. Shamalov, O. Betzer, M. Motiei, M. Horovitz-Fried, R. Yehuda, A. Popovtzer, R. Popovtzer, C.J. Cohen, Nanomedicine for cancer immunotherapy: tracking cancer-specific T-cells in vivo with gold nanoparticles and CT imaging, ACS Nano 9 (2015) 6363–6372.
[69] D.C. Deniger, A. Pasetto, E. Tran, M.R. Parkhurst, C.J. Cohen, P.F. Robbins, L.J. Cooper, S.A. Rosenberg, Stable, nonviral expression of mutated tumor neoantigen-specific T-cell receptors using the sleeping beauty transposon/transposase system, Mol. Ther. 24 (2016) 1078–1089.
[70] B.Y. Jin, T.E. Campbell, L.M. Draper, S. Stevanovic, B. Weissbrich, Z. Yu, N.P. Restifo, S.A. Rosenberg, C.L. Trimble, C.S. Hinrichs, Engineered T cells targeting E7 mediate regression of human papillomavirus cancers in a murine model, JCI. Insight. 3 (2018).
[71] Y. Kuwana, Y. Asakura, N. Utsunomiya, M. Nakanishi, Y. Arata, S. Itoh, F. Nagase, Y. Kurosawa, Expression of chimeric receptor composed of immunoglobulin-derived V regions and T-cell receptor-derived C regions, Biochem. Biophys. Res. Commun. 149 (1987) 960–968.
[72] G. Gross, T. Waks, Z. Eshhar, Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity, Proc. Natl. Acad. Sci. U. S. A 86 (1989) 10024–10028.
[73] C. Romeo, B. Seed, Cellular immunity to HIV activated by CD4 fused to T cell or Fc receptor polypeptides, Cell 64 (1991) 1037–1046.
[74] B.A. Irving, A. Weiss, The cytoplasmic domain of the T cell receptor zeta chain is sufficient to couple to receptor-associated signal transduction pathways, Cell 64 (1991) 891–901.
[75] F. Letourneur, R.D. Klausner, T-cell and basophil activation through the cytoplasmic tail of T-cell-receptor zeta family proteins, Proc. Natl. Acad. Sci. U. S. A 88 (1991) 8905–8909.
[76] Z. Eshhar, T. Waks, G. Gross, D.G. Schindler, 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. U. S. A 90 (1993) 720–724.

[77] K. Shamalov, Y. Tal, C. Ankri, C.J. Cohen, Adoptive T-cell immunotherapy: perfecting self-defenses, in: M. Klink (Ed.), Interaction of Immune and Cancer Cells, Springer, Vienna 2014, pp. 207–232.
[78] P.P. Zheng, J.M. Kros, J. Li, Approved CAR T cell therapies: ice bucket challenges on glaring safety risks and long-term impacts, Drug Discov. Today 23 (2018) 1175–1182.
[79] S. Srivastava, S.R. Riddell, Engineering CAR-T cells: design concepts, Trends Immunol. 36 (2015) 494–502.
[80] M. Sadelain, R. Brentjens, I. Riviere, The basic principles of chimeric antigen receptor design, Cancer Discov. 3 (2013) 388–398.
[81] D. Abate-Daga, M.L. Davila, CAR models: next-generation CAR modifications for enhanced T-cell function, Mol. Ther. Oncolytics. 3 (2016) 16014.
[82] C.H. June, R.S. O'Connor, O.U. Kawalekar, S. Ghassemi, M.C. Milone, CAR T cell immunotherapy for human cancer, Science 359 (2018) 1361–1365.
[83] J.P. da Cunha, P.A. Galante, J.E. de Souza, R.F. de Souza, P.M. Carvalho, D.T. Ohara, R.P. Moura, S.M. Oba-Shinja, S.K. Marie, W.A. Silva Jr., R.O. Perez, B. Stransky, M. Pieprzyk, J. Moore, O. Caballero, J. Gama-Rodrigues, A. Habr-Gama, W.P. Kuo, A.J. Simpson, A.A. Camargo, L.J. Old, S.J. de Souza, Bioinformatics construction of the human cell surfaceome, Proc. Natl. Acad. Sci. U. S. A 106 (2009) 16752–16757.
[84] C.J. Cohen, G. Denkberg, A. Lev, M. Epel, Y. Reiter, Recombinant antibodies with MHC-restricted, peptide-specific, T-cell receptor-like specificity: new tools to study antigen presentation and TCR-peptide-MHC interactions, J. Mol. Recognit. 16 (2003) 324–332.
[85] C.J. Cohen, G. Denkberg, D. Segal, Y. Reiter, Generation of recombinant immunotoxins for specific targeting of tumor-related peptides presented by MHC molecules, Methods Mol. Biol. 207 (2003) 269–282.
[86] E. Walseng, H. Koksal, I.M. Sektioglu, A. Fane, G. Skorstad, G. Kvalheim, G. Gaudernack, E.M. Inderberg, S. Walchli, A TCR-based Chimeric Antigen Receptor, Sci. Rep. 7 (2017) 10713.
[87] C.J. Cohen, N. Hoffmann, M. Farago, H.R. Hoogenboom, L. Eisenbach, Y. Reiter, Direct detection and quantitation of a distinct T-cell epitope derived from tumor-specific epithelial cell-associated mucin using human recombinant antibodies endowed with the antigen-specific, major histocompatibility complex-restricted specificity of T cells, Cancer Res. 62 (2002) 5835–5844.
[88] G. Denkberg, C.J. Cohen, A. Lev, P. Chames, H.R. Hoogenboom, Y. Reiter, Direct visualization of distinct T cell epitopes derived from a melanoma tumor-associated antigen by using human recombinant antibodies with MHC- restricted T cell receptor-like specificity, Proc. Natl. Acad. Sci. U. S. A 99 (2002) 9421–9426.
[89] A. Lev, G. Denkberg, C.J. Cohen, M. Tzukerman, K.L. Skorecki, P. Chames, H.R. Hoogenboom, Y. Reiter, Isolation and characterization of human recombinant antibodies endowed with the antigen-specific, major histocompatibility complex-restricted specificity of T cells directed toward the widely expressed tumor T-cell epitopes of the telomerase catalytic subunit, Cancer Res. 62 (2002) 3184–3194.
[90] W.E. Biddison, R.V. Turner, S.J. Gagnon, A. Lev, C.J. Cohen, Y. Reiter, Tax and M1 peptide/HLA-A2-specific Fabs and T cell receptors recognize nonidentical structural features on peptide/HLA-A2 complexes, J. Immunol. 171 (2003) 3064–3074.
[91] C.J. Cohen, G. Denkberg, Y.S. Schiffenbauer, D. Segal, E. Trubniykov, G. Berke, Y. Reiter, Simultaneous monitoring of binding to and activation of tumor-specific T lymphocytes by peptide-MHC, J. Immunol. Methods 277 (2003) 39–52.
[92] C.J. Cohen, O. Sarig, Y. Yamano, U. Tomaru, S. Jacobson, Y. Reiter, Direct phenotypic analysis of human MHC class I antigen presentation: visualization, quantitation, and in situ detection of human viral epitopes using peptide-specific, MHC-restricted human recombinant antibodies, J. Immunol. 170 (2003) 4349–4361.
[93] P. Chames, R.A. Willemsen, G. Rojas, D. Dieckmann, L. Rem, G. Schuler, R.L. Bolhuis, H.R. Hoogenboom, TCR-like human antibodies expressed on human CTLs mediate antibody affinity-dependent cytolytic activity, J. Immunol. 169 (2002) 1110–1118.
[94] R. Oren, M. Hod-Marco, M. Haus-Cohen, S. Thomas, D. Blat, N. Duvshani, G. Denkberg, Y. Elbaz, F. Benchetrit, Z. Eshhar, H. Stauss, Y. Reiter, Functional comparison of engineered T cells carrying a native TCR versus TCR-like antibody-based chimeric antigen receptors indicates affinity/avidity thresholds, J. Immunol. 193 (2014) 5733–5743.
[95] M.V. Maus, J. Plotkin, G. Jakka, G. Stewart-Jones, I. Riviere, T. Merghoub, J. Wolchok, C. Renner, M. Sadelain, An MHC-restricted antibody-based chimeric antigen receptor requires TCR-like affinity to maintain antigen specificity, Mol. Ther. Oncolytics. 3 (2016) 1–9.
[96] M. Sadelain, I. Riviere, S. Riddell, Therapeutic T cell engineering, Nature 545 (2017) 423–431.
[97] Z. Grada, M. Hegde, T. Byrd, D.R. Shaffer, A. Ghazi, V.S. Brawley, A. Corder, K. Schonfeld, J. Koch, G. Dotti, H.E. Heslop, S. Gottschalk, W.S. Wels, M.L. Baker, N. Ahmed, TanCAR: a novel bispecific chimeric antigen receptor for cancer immunotherapy, Mol. Ther. Nucleic Acids 2 (2013), e105. .
[98] A. Martyniszyn, A.C. Krahl, M.C. Andre, A.A. Hombach, H. Abken, CD20-CD19 bispecific CAR T cells for the treatment of B-cell malignancies, Hum. Gene Ther. 28 (2017) 1147–1157.
[99] R.A. Morgan, J.C. Yang, M. Kitano, M.E. Dudley, C.M. Laurencot, S.A. Rosenberg, Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2, Mol. Ther. 18 (2010) 843–851.
[100] R. Brentjens, R. Yeh, Y. Bernal, I. Riviere, M. Sadelain, 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. 18 (2010) 666–668.
[101] X. Liu, S. Jiang, C. Fang, S. Yang, D. Olalere, E.C. Pequignot, A.P. Cogdill, N. Li, M. Ramones, B. Granda, L. Zhou, A. Loew, R.M. Young, C.H. June, Y. Zhao, Affinity-tuned ErbB2 or EGFR chimeric antigen receptor T cells exhibit an increased therapeutic index against tumors in mice, Cancer Res. 75 (2015) 3596–3607.
[102] H.G. Caruso, L.V. Hurton, A. Najjar, D. Rushworth, S. Ang, S. Olivares, T. Mi, K. Switzer, H. Singh, H. Huls, D.A. Lee, A.B. Heimberger, R.E. Champlin, L.J. Cooper, Tuning sensitivity of CAR to EGFR density limits recognition of normal tissue while maintaining potent antitumor activity, Cancer Res. 75 (2015) 3505–3518.
[103] E. Drent, M. Themeli, R. Poels, R. de Jong-Korlaar, H. Yuan, B.J. De, A.C.M. Martens, S. Zweegman, N.W.C.J. van de Donk, R.W.J. Groen, H.M. Lokhorst, T. Mutis, A rational strategy for reducing on-target off-tumor effects of CD38-chimeric antigen receptors by affinity optimization, Mol. Ther. 25 (2017) 1946–1958.
[104] Y. Zhao, Q.J. Wang, S. Yang, J.N. Kochenderfer, Z. Zheng, X. Zhong, M. Sadelain, Z. Eshhar, S.A. Rosenberg, R.A. Morgan, A herceptin-based chimeric antigen receptor with modified signaling domains leads to enhanced survival of transduced T lymphocytes and antitumor activity, J. Immunol. 183 (2009) 5563–5574.
[105] V. Eisenberg, K. Shamalov, S. Meir, S. Hoogi, R. Sarkar, S. Pinker, G. Markel, A. Porgador, C.J. Cohen, Targeting multiple tumors using T-cells engineered to express a natural cytotoxicity receptor 2-based chimeric receptor, Front. Immunol. 8 (2017) 1212.
[106] G. Dotti, S. Gottschalk, B. Savoldo, M.K. Brenner, Design and development of therapies using chimeric antigen receptor-expressing T cells, Immunol. Rev. 257 (2014) 107–126.
[107] R.D. Guest, R.E. Hawkins, N. Kirillova, E.J. Cheadle, J. Arnold, A. O'Neill, J. Irlam, K.A. Chester, J.T. Kemshead, D.M. Shaw, M.J. Embleton, P.L. Stern, D.E. Gilham, The role of extracellular spacer regions in the optimal design of chimeric immune receptors: evaluation of four different scFvs and antigens, J. Immunother. 28 (2005) 203–211.
[108] S.E. James, P.D. Greenberg, M.C. Jensen, Y. Lin, J. Wang, B.G. Till, A.A. Raubitschek, S.J. Forman, O.W. Press, Antigen sensitivity of CD22-specific chimeric TCR is modulated by target epitope distance from the cell membrane, J. Immunol. 180 (2008) 7028–7038.
[109] M. Hudecek, D. Sommermeyer, P.L. Kosasih, A. Silva-Benedict, L. Liu, C. Rader, M.C. Jensen, S.R. Riddell, The nonsignaling extracellular spacer domain of chimeric antigen receptors is decisive for in vivo antitumor activity, Cancer Immunol. Res. 3 (2015) 125–135.
[110] S. Wilkie, G. Picco, J. Foster, D.M. Davies, S. Julien, L. Cooper, S. Arif, S.J. Mather, J. Taylor-Papadimitriou, J.M. Burchell, J. Maher, Retargeting of human T cells to tumor-associated MUC1: the evolution of a chimeric antigen receptor, J. Immunol. 180 (2008) 4901–4909.
[111] N. Watanabe, P. Bajgain, S. Sukumaran, S. Ansari, H.E. Heslop, C.M. Rooney, M.K. Brenner, A.M. Leen, J.F. Vera, Fine-tuning the CAR spacer improves T-cell potency, Oncoimmunology. 5 (2016), e1253656. .
[112] A. Hombach, A.A. Hombach, H. Abken, Adoptive immunotherapy with genetically engineered T cells: modification of the IgG1 Fc 'spacer' domain in the extracellular moiety of chimeric antigen receptors avoids 'off-target' activation and unintended initiation of an innate immune response, Gene Ther. 17 (2010) 1206–1213.
[113] L. Liu, D. Sommermeyer, A. Cabanov, P. Kosasih, T. Hill, S.R. Riddell, Inclusion of Strep-tag II in design of antigen receptors for T-cell immunotherapy, Nat. Biotechnol. 34 (2016) 430–434.
[114] T. Zhang, A. Barber, C.L. Sentman, Generation of antitumor responses by genetic modification of primary human T cells with a chimeric NKG2D receptor, Cancer Res. 66 (2006) 5927–5933.
[115] C. Ankri, K. Shamalov, M. Horovitz-Fried, S. Mauer, C.J. Cohen, Human T cells engineered to express a programmed death 1/28 costimulatory retargeting molecule display enhanced antitumor activity, J. Immunol. 191 (2013) 4121–4129.
[116] A.T. Gacerez, B. Arellano, C.L. Sentman, How chimeric antigen receptor design affects adoptive T cell therapy, J. Cell. Physiol. 231 (2016) 2590–2598.
[117] N.M. Haynes, M.B. Snook, J.A. Trapani, L. Cerruti, S.M. Jane, M.J. Smyth, P.K. Darcy, Redirecting mouse CTL against colon carcinoma: superior signaling efficacy of single-chain variable domain chimeras containing TCR-zeta vs Fc epsilon RI-gamma, J. Immunol. 166 (2001) 182–187.
[118] C. Heuser, A. Hombach, C. Losch, K. Manista, H. Abken, T-cell activation by recombinant immunoreceptors: impact of the intracellular signalling domain on the stability of receptor expression and antigen-specific activation of grafted T cells, Gene Ther. 10 (2003) 1408–1419.
[119] P. Hwu, G.E. Shafer, J. Treisman, D.G. Schindler, G. Gross, R. Cowherd, S.A. Rosenberg, Z. Eshhar, Lysis of ovarian cancer cells by human lymphocytes redirected with a chimeric gene composed of an antibody variable region and the Fc receptor gamma chain, J. Exp. Med. 178 (1993) 361–366.
[120] P. Hwu, J.C. Yang, R. Cowherd, J. Treisman, G.E. Shafer, Z. Eshhar, S.A. Rosenberg, In vivo antitumor activity of T cells redirected with chimeric antibody/T-cell receptor genes, Cancer Res. 55 (1995) 3369–3373.
[121] M.A. Pule, B. Savoldo, G.D. Myers, C. Rossig, H.V. Russell, G. Dotti, M.H. Huls, E. Liu, A.P. Gee, Z. Mei, E. Yvon, H.L. Weiss, H. Liu, C.M. Rooney, H.E. Heslop, M.K. Brenner, Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma, Nat. Med. 14 (2008) 1264–1270.
[122] M.H. Kershaw, J.A. Westwood, L.L. Parker, G. Wang, Z. Eshhar, S.A. Mavroukakis, D.E. White, J.R. Wunderlich, S. Canevari, L. Rogers-Freezer, C.C. Chen, J.C. Yang, S.A. Rosenberg, P. Hwu, A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer, Clin. Cancer Res. 12 (2006) 6106–6115.
[123] J. Scholler, T.L. Brady, G. Binder-Scholl, W.T. Hwang, G. Plesa, K.M. Hege, A.N. Vogel, M. Kalos, J.L. Riley, S.G. Deeks, R.T. Mitsuyasu, W.B. Bernstein, N.E. Aronson, B.L. Levine, F.D. Bushman, C.H. June, Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells, Sci. Transl. Med. 4 (2012) 132ra53.
[124] J. Maher, R.J. Brentjens, G. Gunset, I. Riviere, M. Sadelain, Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta/CD28 receptor, Nat. Biotechnol. 20 (2002) 70–75.

[125] C. Imai, K. Mihara, M. Andreansky, I.C. Nicholson, C.H. Pui, T.L. Geiger, D. Campana, Chimeric receptors with 4-1BB signaling capacity provoke potent cytotoxicity against acute lymphoblastic leukemia, Leukemia 18 (2004) 676–684.
[126] C.M. Kowolik, M.S. Topp, S. Gonzalez, T. Pfeiffer, S. Olivares, N. Gonzalez, D.D. Smith, S.J. Forman, M.C. Jensen, L.J. Cooper, CD28 costimulation provided through a CD19-specific chimeric antigen receptor enhances in vivo persistence and antitumor efficacy of adoptively transferred T cells, Cancer Res. 66 (2006) 10995–11004.
[127] B. Savoldo, C.A. Ramos, E. Liu, M.P. Mims, M.J. Keating, G. Carrum, R.T. Kamble, C.M. Bollard, A.P. Gee, Z. Mei, H. Liu, B. Grilley, C.M. Rooney, H.E. Heslop, M.K. Brenner, G. Dotti, CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients, J. Clin. Invest. 121 (2011) 1822–1826.
[128] Z. Zhao, M. Condomines, S.J.C. van der Stegen, F. Perna, C.C. Kloss, G. Gunset, J. Plotkin, M. Sadelain, Structural design of engineered costimulation determines tumor rejection kinetics and persistence of CAR T cells, Cancer Cell 28 (2015) 415–428.
[129] O.U. Kawalekar, R.S. O'Connor, J.A. Fraietta, L. Guo, S.E. McGettigan, A.D. Posey Jr., P.R. Patel, S. Guedan, J. Scholler, B. Keith, N.W. Snyder, I.A. Blair, M.C. Milone, C.H. June, Distinct signaling of coreceptors regulates specific metabolism pathways and impacts memory development in CAR T cells, Immunity 44 (2016) 380–390.
[130] S. Li, J. Zhang, M. Wang, G. Fu, Y. Li, L. Pei, Z. Xiong, D. Qin, R. Zhang, X. Tian, Z. Wei, R. Chen, X. Chen, J. Wan, J. Chen, X. Wei, Y. Xu, P. Zhang, P. Wang, X. Peng, S. Yang, J. Shen, Z. Yang, J. Chen, C. Qian, Treatment of acute lymphoblastic leukaemia with the second generation of CD19 CAR-T containing either CD28 or 4-1BB, Br. J. Haematol. 181 (2018) 360–371.
[131] D.L. Porter, W.T. Hwang, N.V. Frey, S.F. Lacey, P.A. Shaw, A.W. Loren, A. Bagg, K.T. Marcucci, A. Shen, V. Gonzalez, D. Ambrose, S.A. Grupp, A. Chew, Z. Zheng, M.C. Milone, B.L. Levine, J.J. Melenhorst, C.H. June, Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia, Sci. Transl. Med. 7 (2015) 303ra139.
[132] A.H. Long, W.M. Haso, J.F. Shern, K.M. Wanhainen, M. Murgai, M. Ingaramo, J.P. Smith, A.J. Walker, M.E. Kohler, V.R. Venkateshwara, R.N. Kaplan, G.H. Patterson, T.J. Fry, R.J. Orentas, C.L. Mackall, 4-1BB costimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors, Nat. Med. 21 (2015) 581–590.
[133] D. Gomes-Silva, M. Mukherjee, M. Srinivasan, G. Krenciute, O. Dakhova, Y. Zheng, J.M.S. Cabral, C.M. Rooney, J.S. Orange, M.K. Brenner, M. Mamonkin, Tonic 4-1BB costimulation in chimeric antigen receptors impedes T cell survival and is vector-dependent, Cell Rep. 21 (2017) 17–26.
[134] M. Mamonkin, M. Mukherjee, M. Srinivasan, S. Sharma, D. Gomes-Silva, F. Mo, G. Krenciute, J.S. Orange, M.K. Brenner, Reversible transgene expression reduces fratricide and permits 4-1BB costimulation of CAR T cells directed to T-cell malignancies, Cancer Immunol. Res. 6 (2018) 47–58.
[135] J. Wang, M. Jensen, Y. Lin, X. Sui, E. Chen, C.G. Lindgren, B. Till, A. Raubitschek, S.J. Forman, X. Qian, S. James, P. Greenberg, S. Riddell, O.W. Press, Optimizing adoptive polyclonal T cell immunotherapy of lymphomas, using a chimeric T cell receptor possessing CD28 and CD137 costimulatory domains, Hum. Gene Ther. 18 (2007) 712–725.
[136] C. Carpenito, M.C. Milone, R. Hassan, J.C. Simonet, M. Lakhal, M.M. Suhoski, A. Varela-Rohena, K.M. Haines, D.F. Heitjan, S.M. Albelda, R.G. Carroll, J.L. Riley, I. Pastan, C.H. June, Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains, Proc. Natl. Acad. Sci. U. S. A 106 (2009) 3360–3365.
[137] G. Enblad, H. Karlsson, J. Wenthe, K.I. Wikstrom, M. Essand, B. Savoldo, G. Dotti, M. Hoglund, H. Hallbook, M.K. Brenner, H. Hagberg, A. Loskog, 748. A clinical trial using third generation CD19 targeting CAR T cells for relapsed lymphoma and leukemia, Mol. Ther. 24 (2016) S295–S296.
[138] C.A. Ramos, R. Rouce, C.S. Robertson, A. Reyna, N. Narala, G. Vyas, B. Mehta, H. Zhang, O. Dakhova, G. Carrum, R.T. Kamble, A.P. Gee, Z. Mei, M.F. Wu, H. Liu, B. Grilley, C.M. Rooney, H.E. Heslop, M.K. Brenner, B. Savoldo, G. Dotti, In vivo fate and activity of second- versus third-generation CD19-specific CAR-T cells in B cell non-hodgkin's lymphomas, Mol. Ther. 26 (12) (2018 Dec 5) 2727–2737, https://doi.org/10.1016/j.ymthe.2018.09.009.
[139] S. Guedan, X. Chen, A. Madar, C. Carpenito, S.E. McGettigan, M.J. Frigault, J. Lee, A.D. Posey Jr., J. Scholler, N. Scholler, R. Bonneau, C.H. June, ICOS-based chimeric antigen receptors program bipolar TH17/TH1 cells, Blood 124 (2014) 1070–1080.
[140] S. Guedan, A.D. Posey Jr., C. Shaw, A. Wing, T. Da, P.R. Patel, S.E. McGettigan, V. Casado-Medrano, O.U. Kawalekar, M. Uribe-Herranz, D. Song, J.J. Melenhorst, S.F. Lacey, J. Scholler, B. Keith, R.M. Young, C.H. June, Enhancing CAR T cell persistence through ICOS and 4-1BB costimulation, JCI. Insight. 3 (2018).
[141] A.A. Hombach, J. Heiders, M. Foppe, M. Chmielewski, H. Abken, OX40 costimulation by a chimeric antigen receptor abrogates CD28 and IL-2 induced IL-10 secretion by redirected CD4(+) T cells, Oncoimmunology. 1 (2012) 458–466.
[142] K.V. Prasad, Z. Ao, Y. Yoon, M.X. Wu, M. Rizk, S. Jacquot, S.F. Schlossman, CD27, a member of the tumor necrosis factor receptor family, induces apoptosis and binds to Siva, a proapoptotic protein, Proc. Natl. Acad. Sci. U. S. A 94 (1997) 6346–6351.
[143] D.G. Song, Q. Ye, M. Poussin, G.M. Harms, M. Figini, D.J. Powell Jr., CD27 costimulation augments the survival and antitumor activity of redirected human T cells in vivo, Blood 119 (2012) 696–706.
[144] L. Mikkilineni, J.N. Kochenderfer, Chimeric antigen receptor T-cell therapies for multiple myeloma, Blood 130 (2017) 2594–2602.
[145] N.S. Raje, J.G. Berdeja, Y. Lin, N.C. Munshi, D.S. DiCapua Siegel, M. Liedtke, S. Jagannath, D. Madduri, J. Rosenblatt, M.V. Maus, A. Turka, L.P. Lam, R.A. Morgan, T. Quigley, M. Massaro, K. Hege, F. Petrocca, J.N. Kochenderfer, bb2121 anti-BCMA CAR T-cell therapy in patients with relapsed/refractory multiple myeloma: Updated results from a multicenter phase I study, J.Clin.Oncol. 36 (2018) 8007 15_suppl.
[146] F. Fan, W. Zhao, J. Liu, A. He, Y. Chen, X. Cao, N. Yang, B. Wang, P. Zhang, Y. Zhang, F. Wang, B. Lei, L. Gu, X. Wang, Q. Zhuang, W. Zhang, Durable remissions with BCMA-specific chimeric antigen receptor (CAR)-modified T cells in patients with refractory/relapsed multiple myeloma, JCO 35 (2017), LBA3001. .
[147] S. Li, Z. Tao, Y. Xu, J. Liu, N. An, Y. Wang, H. Xing, Z. Tian, K. Tang, X. Liao, Q. Rao, M. Wang, J. Wang, CD33-specific chimeric antigen receptor T cells with different co-stimulators showed potent anti-leukemia efficacy and different phenotype, Hum. Gene Ther. 29 (2018) 626–639.
[148] J.C. Petrov, M. Wada, K.G. Pinz, L.E. Yan, K.H. Chen, X. Shuai, H. Liu, X. Chen, L.H. Leung, H. Salman, N. Hagag, F. Liu, X. Jiang, Y. Ma, Compound CAR T-cells as a double-pronged approach for treating acute myeloid leukemia, Leukemia 32 (2018) 1317–1326.
[149] M. Ruella, D.M. Barrett, S.S. Kenderian, O. Shestova, T.J. Hofmann, J. Perazzelli, M. Klichinsky, V. Aikawa, F. Nazimuddin, M. Kozlowski, J. Scholler, S.F. Lacey, J.J. Melenhorst, J.J. Morrissette, D.A. Christian, C.A. Hunter, M. Kalos, D.L. Porter, C.H. June, S.A. Grupp, S. Gill, Dual CD19 and CD123 targeting prevents antigen-loss relapses after CD19-directed immunotherapies, J. Clin. Invest. 126 (2016) 3814–3826.
[150] D. Gomes-Silva, E. Atilla, P.A. Atilla, F. Mo, H. Tashiro, M. Srinivasan, P. Lulla, R.H. Rouce, J.M.S. Cabral, C.A. Ramos, M.K. Brenner, M. Mamonkin, CD7 CAR T cells for the therapy of acute myeloid leukemia, Mol. Ther. 27 (1) (2019 Jan 2) 272–280, https://doi.org/10.1016/j.ymthe.2018.10.001.
[151] P.M. Maciocia, P.A. Wawrzyniecka, B. Philip, I. Ricciardelli, A.U. Akarca, S.C. Onuoha, M. Legut, D.K. Cole, A.K. Sewell, G. Gritti, J. Somja, M.A. Piris, K.S. Peggs, D.C. Linch, T. Marafioti, M.A. Pule, Targeting the T cell receptor beta-chain constant region for immunotherapy of T cell malignancies, Nat. Med. 23 (2017) 1416–1423.
[152] L. Labanieh, R.G. Majzner, C.L. Mackall, Programming CAR-T cells to kill cancer, Nature Biomedical Engineering 2 (2018) 377–391.
[153] M.M. D'Aloia, I.G. Zizzari, B. Sacchetti, L. Pierelli, M. Alimandi, CAR-T cells: the long and winding road to solid tumors, Cell Death Dis. 9 (2018) 282.
[154] H. Abken, Adoptive therapy with CAR redirected T cells: the challenges in targeting solid tumors, Immunotherapy 7 (2015) 535–544.
[155] C.H. Lamers, S. Sleijfer, A.G. Vulto, W.H. Kruit, M. Kliffen, R. Debets, J.W. Gratama, G. Stoter, E. Oosterwijk, Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience, J. Clin. Oncol. 24 (2006) e20–e22.
[156] J.R. Park, D.L. DiGiusto, M. Slovak, C. Wright, A. Naranjo, J. Wagner, H.B. Meechoovet, C. Bautista, W.C. Chang, J.R. Ostberg, M.C. Jensen, Adoptive transfer of chimeric antigen receptor re-directed cytolytic T lymphocyte clones in patients with neuroblastoma, Mol. Ther. 15 (2007) 825–833.
[157] G.L. Beatty, A.R. Haas, M.V. Maus, D.A. Torigian, M.C. Soulen, G. Plesa, A. Chew, Y. Zhao, B.L. Levine, S.M. Albelda, M. Kalos, C.H. June, Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells induce anti-tumor activity in solid malignancies, Cancer Immunol. Res. 2 (2014) 112–120.
[158] N. Ahmed, V.S. Brawley, M. Hegde, C. Robertson, A. Ghazi, C. Gerken, E. Liu, O. Dakhova, A. Ashoori, A. Corder, T. Gray, M.F. Wu, H. Liu, J. Hicks, N. Rainusso, G. Dotti, Z. Mei, B. Grilley, A. Gee, C.M. Rooney, M.K. Brenner, H.E. Heslop, W.S. Wels, L.L. Wang, P. Anderson, S. Gottschalk, Human epidermal growth factor receptor 2 (HER2) -specific chimeric antigen receptor-modified T cells for the immunotherapy of HER2-positive sarcoma, J. Clin. Oncol. 33 (2015) 1688–1696.
[159] K. Feng, Y. Liu, Y. Guo, J. Qiu, Z. Wu, H. Dai, Q. Yang, Y. Wang, W. Han, Phase I study of chimeric antigen receptor modified T cells in treating HER2-positive advanced biliary tract cancers and pancreatic cancers, Protein Cell 9 (10) (2018 Oct) 838–847, https://doi.org/10.1007/s13238-017-0440-4.
[160] S.C. Katz, R.A. Burga, E. McCormack, L.J. Wang, W. Mooring, G.R. Point, P.D. Khare, M. Thorn, Q. Ma, B.F. Stainken, E.O. Assanah, R. Davies, N.J. Espat, R.P. Junghans, Phase I hepatic immunotherapy for metastases study of intra-arterial chimeric antigen receptor-modified T-Cell Therapy for CEA+ liver metastases, Clin. Cancer Res. 21 (2015) 3149–3159.
[161] D.M. O'Rourke, M.P. Nasrallah, A. Desai, J.J. Melenhorst, K. Mansfield, J.J.D. Morrissette, M. Martinez-Lage, S. Brem, E. Maloney, A. Shen, R. Isaacs, S. Mohan, G. Plesa, S.F. Lacey, J.M. Navenot, Z. Zheng, B.L. Levine, H. Okada, C.H. June, J.L. Brogdon, M.V. Maus, 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. 9 (2017).
[162] R.P. Junghans, Q. Ma, R. Rathore, E.M. Gomes, A.J. Bais, A.S. Lo, M. Abedi, R.A. Davies, H.J. Cabral, A.S. Al-Homsi, S.I. Cohen, Phase I trial of anti-PSMA designer CAR-T cells in prostate cancer: possible role for interacting interleukin 2-T cell pharmacodynamics as a determinant of clinical response, Prostate 76 (2016) 1257–1270.
[163] M. Suzuki, N.K. Cheung, Disialoganglioside GD2 as a therapeutic target for human diseases, Expert Opin. Ther. Targets 19 (2015) 349–362.
[164] I.C. Nicholson, K.A. Lenton, D.J. Little, T. Decorso, F.T. Lee, A.M. Scott, H. Zola, A.W. Hohmann, Construction and characterisation of a functional CD19 specific single chain Fv fragment for immunotherapy of B lineage leukaemia and lymphoma, Mol. Immunol. 34 (1997) 1157–1165.
[165] C.H. Lamers, R. Willemsen, E.P. Van, S. Van Steenbergen-Langeveld, M. Broertjes, J. Oosterwijk-Wakka, E. Oosterwijk, S. Sleijfer, R. Debets, J.W. Gratama, et al., Blood 117 (2011) 72–82.
[166] D. Sommermeyer, T. Hill, S.M. Shamah, A.I. Salter, Y. Chen, K.M. Mohler, S.R. Riddell, Fully human CD19-specific chimeric antigen receptors for T-cell therapy, Leukemia 31 (2017) 2191–2199.
[167] C. Berger, M.E. Flowers, E.H. Warren, S.R. Riddell, Analysis of transgene-specific immune responses that limit the in vivo persistence of adoptively transferred HSV-

TK-modified donor T cells after allogeneic hematopoietic cell transplantation, Blood 107 (2006) 2294–2302.
[168] M.C. Jensen, L. Popplewell, L.J. Cooper, D. DiGiusto, M. Kalos, J.R. Ostberg, S.J. Forman, Antitransgene rejection responses contribute to attenuated persistence of adoptively transferred CD20/CD19-specific chimeric antigen receptor redirected T cells in humans, Biol. Blood Marrow Transplant. 16 (2010) 1245–1256.
[169] C.J. Turtle, L.A. Hanafi, C. Berger, T.A. Gooley, S. Cherian, M. Hudecek, D. Sommermeyer, K. Melville, B. Pender, T.M. Budiarto, E. Robinson, N.N. Steevens, C. Chaney, L. Soma, X. Chen, C. Yeung, B. Wood, D. Li, J. Cao, S. Heimfeld, M.C. Jensen, S.R. Riddell, D.G. Maloney, CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients, J. Clin. Invest. 126 (2016) 2123–2138.
[170] M.V. Maus, A.R. Haas, G.L. Beatty, S.M. Albelda, B.L. Levine, X. Liu, Y. Zhao, M. Kalos, C.H. June, T cells expressing chimeric antigen receptors can cause anaphylaxis in humans, Cancer Immunol. Res 1 (2013) 26–31.
[171] J. Cao, G. Wang, H. Cheng, C. Wei, K. Qi, W. Sang, L. Zhenyu, M. Shi, H. Li, J. Qiao, B. Pan, J. Zhao, Q. Wu, L. Zeng, M. Niu, G. Jing, J. Zheng, K. Xu, Potent anti-leukemia activities of humanized CD19-targeted Chimeric antigen receptor T (CAR-T) cells in patients with relapsed/refractory acute lymphoblastic leukemia, Am. J. Hematol. 93 (2018) 851–858.
[172] L.A. Johnson, J. Scholler, T. Ohkuri, A. Kosaka, P.R. Patel, S.E. McGettigan, A.K. Nace, T. Dentchev, P. Thekkat, A. Loew, A.C. Boesteanu, A.P. Cogdill, T. Chen, J.A. Fraietta, C.C. Kloss, A.D. Posey Jr., B. Engels, R. Singh, T. Ezell, N. Idamakanti, M.H. Ramones, N. Li, L. Zhou, G. Plesa, J.T. Seykora, H. Okada, C.H. June, J.L. Brogdon, M.V. Maus, Rational development and characterization of humanized anti-EGFR variant III chimeric antigen receptor T cells for glioblastoma, Sci. Transl. Med. 7 (2015) 275ra22.
[173] M.S. De, J. Ingels, G. Goetgeluk, S. Bonte, M. Pille, K. Weening, T. Kerre, H. Abken, B. Vandekerckhove, Nanobody based dual specific CARs, Int. J. Mol. Sci. 19 (2018).
[174] F.R. Jamnani, F. Rahbarizadeh, M.A. Shokrgozar, F. Mahboudi, D. Ahmadvand, Z. Sharifzadeh, L. Parhamifar, S.M. Moghimi, T cells expressing VHH-directed oligoclonal chimeric HER2 antigen receptors: towards tumor-directed oligoclonal T cell therapy, Biochim. Biophys. Acta 1840 (2014) 378–386.
[175] T. Thayaparan, R.M. Petrovic, D.Y. Achkova, T. Zabinski, D.M. Davies, A. Klampatsa, A.C. Parente-Pereira, L.M. Whilding, S.J. van der Stegen, N. Woodman, M. Sheaff, J.R. Cochran, J.F. Spicer, J. Maher, CAR T-cell immunotherapy of MET-expressing malignant mesothelioma, Oncoimmunology. 6 (2017), e1363137. .
[176] J.A. Hammill, H. VanSeggelen, C.W. Helsen, G.F. Denisova, C. Evelegh, D.G. Tantalo, J.D. Bassett, J.L. Bramson, Designed ankyrin repeat proteins are effective targeting elements for chimeric antigen receptors, J. Immunother. Cancer 3 (2015) 55.
[177] E. Siegler, S. Li, Y.J. Kim, P. Wang, Designed ankyrin repeat proteins as Her2 targeting domains in chimeric antigen receptor-engineered T cells, Hum. Gene Ther. 28 (2017) 726–736.
[178] X. Han, G.E. Cinay, Y. Zhao, Y. Guo, X. Zhang, P. Wang, Adnectin-based design of chimeric antigen receptor for T cell engineering, Mol. Ther. 25 (2017) 2466–2476.
[179] W. Debinski, D.M. Gibo, S.W. Hulet, J.R. Connor, G.Y. Gillespie, Receptor for interleukin 13 is a marker and therapeutic target for human high-grade gliomas, Clin. Cancer Res. 5 (1999) 985–990.
[180] C.E. Brown, D. Alizadeh, R. Starr, L. Weng, J.R. Wagner, A. Naranjo, J.R. Ostberg, M.S. Blanchard, J. Kilpatrick, J. Simpson, A. Kurien, S.J. Priceman, X. Wang, T.L. Harshbarger, M. D'Apuzzo, J.A. Ressler, M.C. Jensen, M.E. Barish, M. Chen, J. Portnow, S.J. Forman, B. Badie, Regression of Glioblastoma after Chimeric Antigen Receptor T-Cell Therapy, N. Engl. J. Med. 375 (2016) 2561–2569.
[181] M. Hegde, M. Mukherjee, Z. Grada, A. Pignata, D. Landi, S.A. Navai, A. Wakefield, K. Fousek, K. Bielamowicz, K.K.H. Chow, V.S. Brawley, T.T. Byrd, S. Krebs, S. Gottschalk, W.S. Wels, M.L. Baker, G. Dotti, M. Mamonkin, M.K. Brenner, J.S. Orange, N. Ahmed, Tandem CAR T cells targeting HER2 and IL13R+¦2 mitigate tumor antigen escape, J. Clin. Invest. 126 (2016) 3036–3052.
[182] K. Bielamowicz, K. Fousek, T.T. Byrd, H. Samaha, M. Mukherjee, N. Aware, M.F. Wu, J.S. Orange, P. Sumazin, T.K. Man, S.K. Joseph, M. Hegde, N. Ahmed, Trivalent CAR T cells overcome interpatient antigenic variability in glioblastoma, Neuro-Oncology 20 (2018) 506–518.
[183] A.V. Stepanov, O.V. Markov, I.V. Chernikov, D.V. Gladkikh, H. Zhang, T. Jones, A.V. Sen'kova, E.L. Chernolovskaya, M.A. Zenkova, R.S. Kalinin, M.P. Rubtsova, A.N. Meleshko, D.D. Genkin, A.A. Belogurov Jr., J. Xie, A.G. Gabibov, R.A. Lerner, Autocrine-based selection of ligands for personalized CAR-T therapy of lymphoma, Sci. Adv. 4 (2018) eaau4580.
[184] E. Vivier, E. Tomasello, M. Baratin, T. Walzer, S. Ugolini, Functions of natural killer cells, Nat. Immunol. 9 (2008) 503–510.
[185] A.K. Purdy, K.S. Campbell, Natural killer cells and cancer: regulation by the killer cell Ig-like receptors (KIR), Cancer Biol. Ther. 8 (2009) 2211–2220.
[186] J. Pahl, A. Cerwenka, Tricking the balance: NK cells in anti-cancer immunity, Immunobiology 222 (2017) 11–20.
[187] .J. Koch, A. Steinle, C. Watzl, O. Mandelboim, Activating natural cytotoxicity receptors of natural killer cells in cancer and infection, Trends Immunol. 34 (2013) 182–191.
[188] P. Spear, M.R. Wu, M.L. Sentman, C.L. Sentman, NKG2D ligands as therapeutic targets, Cancer Immun. 13 (2013) 8.
[189] T. Zhang, B.A. Lemoi, C.L. Sentman, Chimeric NK-receptor-bearing T cells mediate antitumor immunotherapy, Blood 106 (2005) 1544–1551.
[190] A. Barber, K.R. Meehan, C.L. Sentman, Treatment of multiple myeloma with adoptively transferred chimeric NKG2D receptor-expressing T cells, Gene Ther. 18 (2011) 509–516.
[191] A. Barber, T. Zhang, L.R. DeMars, J. Conejo-Garcia, K.F. Roby, C.L. Sentman, Chimeric NKG2D receptor-bearing T cells as immunotherapy for ovarian cancer, Cancer Res. 67 (2007) 5003–5008.
[192] T. Weiss, M. Weller, M. Guckenberger, C.L. Sentman, P. Roth, NKG2D-based CAR T cells and radiotherapy exert synergistic efficacy in glioblastoma, Cancer Res. 78 (2018) 1031–1043.
[193] C. Lonez, A. Hendlisz, L. Shaza, P. Aftimos, M. Vouche, V. Donckier, J.H. Machiels, M. Van Den Eynde, J.L. Canon, J. Carrasco, K. Odunsi, S. Sahebjam, S. Rottey, N. Braun, B. Verma, D.E. Gilham, F.F. Lehmann, Celyad's novel CAR T-cell therapy for solid malignancies, Curr. Res. Transl. Med. 66 (2018) 53–56.
[194] O. Mandelboim, N. Lieberman, M. Lev, L. Paul, T.I. Arnon, Y. Bushkin, D.M. Davis, J.L. Strominger, J.W. Yewdell, A. Porgador, Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells, Nature 409 (2001) 1055–1060.
[195] B. Rosental, M. Brusilovsky, U. Hadad, D. Oz, M.Y. Appel, F. Afergan, R. Yossef, L.A. Rosenberg, A. Aharoni, A. Cerwenka, K.S. Campbell, A. Braiman, A. Porgador, Proliferating cell nuclear antigen is a novel inhibitory ligand for the natural cytotoxicity receptor NKp44, J. Immunol. 187 (2011) 5693–5702.
[196] B. Rosental, U. Hadad, M. Brusilovsky, K.S. Campbell, A. Porgador, A novel mechanism for cancer cells to evade immune attack by NK cells: the interaction between NKp44 and proliferating cell nuclear antigen, Oncoimmunology. 1 (2012) 572–574.
[197] M. Brusilovsky, O. Radinsky, L. Cohen, R. Yossef, A. Shemesh, A. Braiman, O. Mandelboim, K.S. Campbell, A. Porgador, Regulation of natural cytotoxicity receptors by heparan sulfate proteoglycans in -cis: a lesson from NKp44, Eur. J. Immunol. 45 (2015) 1180–1191.
[198] B. Martin-Antonio, G. Sune, L. Perez-Amill, M. Castella, A. Urbano-Ispizua, Natural killer cells: angels and devils for immunotherapy, Int. J. Mol. Sci. 18 (2017).
[199] Y. Tal, S. Yaakobi, M. Horovitz-Fried, E. Safyon, B. Rosental, A. Porgador, C.J. Cohen, An NCR1-based chimeric receptor endows T-cells with multiple anti-tumor specificities, Oncotarget 5 (2014) 10949–10958.
[200] T. Zhang, M.R. Wu, C.L. Sentman, An NKp30-based chimeric antigen receptor promotes T cell effector functions and antitumor efficacy in vivo, J. Immunol. 189 (2012) 2290–2299.
[201] D.E. Speiser, P.C. Ho, G. Verdeil, Regulatory circuits of T cell function in cancer, Nat. Rev. Immunol. 16 (2016) 599–611.
[202] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (2011) 646–674.
[203] C. Zhang, Z. Li, L. Xu, X. Che, T. Wen, Y. Fan, C. Li, S. Wang, Y. Cheng, X. Wang, X. Qu, Y. Liu, CXCL9/10/11, a regulator of PD-L1 expression in gastric cancer, BMC Cancer 18 (2018) 462.
[204] K. Abiko, N. Matsumura, J. Hamanishi, N. Horikawa, R. Murakami, K. Yamaguchi, Y. Yoshioka, T. Baba, I. Konishi, M. Mandai, IFN-gamma from lymphocytes induces PD-L1 expression and promotes progression of ovarian cancer, Br. J. Cancer 112 (2015) 1501–1509.
[205] G.M. Davoodzadeh, G.A. Kardar, Y. Saeedi, S. Heydari, J. Garssen, R. Falak, Exhaustion of T lymphocytes in the tumor microenvironment: significance and effective mechanisms, Cell. Immunol. 322 (2017) 1–14.
[206] E.J. Wherry, M. Kurachi, Molecular and cellular insights into T cell exhaustion, Nat. Rev. Immunol. 15 (2015) 486–499.
[207] B. Seliger, F.M. Marincola, S. Ferrone, H. Abken, The complex role of B7 molecules in tumor immunology, Trends Mol. Med. 14 (2008) 550–559.
[208] G. Driessens, J. Kline, T.F. Gajewski, Costimulatory and coinhibitory receptors in anti-tumor immunity, Immunol. Rev. 229 (2009) 126–144.
[209] D.S. Vinay, E.P. Ryan, G. Pawelec, W.H. Talib, J. Stagg, E. Elkord, T. Lichtor, W.K. Decker, R.L. Whelan, H.M.C.S. Kumara, E. Signori, K. Honoki, A.G. Georgakilas, A. Amin, W.G. Helferich, C.S. Boosani, G. Guha, M.R. Ciriolo, S. Chen, S.I. Mohammed, A.S. Azmi, W.N. Keith, A. Bilsland, D. Bhakta, D. Halicka, H. Fujii, K. Aquilano, S.S. Ashraf, S. Nowsheen, X. Yang, B.K. Choi, B.S. Kwon, Immune evasion in cancer: Mechanistic basis and therapeutic strategies, Semin. Cancer Biol. 35 (Suppl) (2015) S185–S198.
[210] M. Fusek, J. Vetvickova, V. Vetvicka, Secretion of cytokines in breast cancer cells: the molecular mechanism of procathepsin D proliferative effects, J. Interf. Cytokine Res. 27 (2007) 191–199.
[211] E.I. Buchbinder, A. Desai, CTLA-4 and PD-1 Pathways: Similarities, differences, and Implications of their Inhibition, Am. J. Clin. Oncol. 39 (2016) 98–106.
[212] A.H. Sharpe, K.E. Pauken, The diverse functions of the PD1 inhibitory pathway, Nat. Rev. Immunol. 18 (2018) 153–167.
[213] S.L. Topalian, Targeting Immune Checkpoints in Cancer Therapy, JAMA 318 (2017) 1647–1648.
[214] R. Meir, K. Shamalov, T. Sadan, M. Motiei, G. Yaari, C.J. Cohen, R. Popovtzer, Fast image-guided stratification using anti-programmed death ligand 1 gold nanoparticles for cancer immunotherapy, ACS Nano 11 (2017) 11127–11134.
[215] H.M. Knochelmann, A.S. Smith, C.J. Dwyer, M.M. Wyatt, S. Mehrotra, C.M. Paulos, CAR T cells in solid tumors: blueprints for building effective therapies, Front. Immunol. 9 (2018) 1740.
[216] M.S. Topp, S.R. Riddell, Y. Akatsuka, M.C. Jensen, J.N. Blattman, P.D. Greenberg, Restoration of CD28 expression in CD28- CD8+ memory effector T cells reconstitutes antigen-induced IL-2 production, J. Exp. Med. 198 (2003) 947–955.
[217] M.T. Stephan, V. Ponomarev, R.J. Brentjens, A.H. Chang, K.V. Dobrenkov, G. Heller, M. Sadelain, T cell-encoded CD80 and 4-1BBL induce auto- and transcostimulation, resulting in potent tumor rejection, Nat. Med. 13 (2007) 1440–1449.
[218] I. Daniel-Meshulam, M. Horovitz-Fried, C.J. Cohen, Enhanced antitumor activity mediated by human 4-1BB-engineered T cells, Int. J. Cancer 133 (2013) 2903–2913.
[219] R. Engelstein, S. Merims, G. Eisenberg, J. Cohen, S. Frank, T. Hamburger, S. Frankenburg, I. Ron, R. Isacson, T. Grenader, H. Steinberg, C.J. Cohen, T. Peretz, M. Lotem, Immune monitoring of patients Treated with a Whole-Cell Melanoma Vaccine Engineered to Express 4-1BBL, J. Immunother. 39 (2016) 321–328.
[220] J.C. Tay, S. Zha, S. Wang, Chimeric switch receptor: switching for improved adoptive T-cell therapy against cancers, Immunotherapy 9 (2017) 1339–1349.

[221] X. Liu, R. Ranganathan, S. Jiang, C. Fang, J. Sun, S. Kim, K. Newick, A. Lo, C.H. June, Y. Zhao, E.K. Moon, A chimeric switch-receptor targeting PD1 Augments the efficacy of second-generation CAR T cells in advanced solid tumors, Cancer Res. 76 (2016) 1578–1590.
[222] M.E. Prosser, C.E. Brown, A.F. Shami, S.J. Forman, M.C. Jensen, Tumor PD-L1 co-stimulates primary human CD8(+) cytotoxic T cells modified to express a PD1: CD28 chimeric receptor, Mol. Immunol. 51 (2012) 263–272.
[223] C. Ankri, C.J. Cohen, Out of the bitter came forth sweet: activating CD28-dependent co-stimulation via PD-1 ligands, Oncoimmunology 3 (2014), e27399. .
[224] S. Kobold, S. Grassmann, M. Chaloupka, C. Lampert, S. Wenk, F. Kraus, M. Rapp, P. Duwell, Y. Zeng, J.C. Schmollinger, M. Schnurr, S. Endres, S. Rothenfusser, Impact of a new fusion receptor on PD-1-mediated immunosuppression in adoptive T cell therapy, J. Natl. Cancer Inst. 107 (2015).
[225] F. Rataj, F.B.T. Kraus, M. Chaloupka, S. Grassmann, C. Heise, B.L. Cadilha, P. Duewell, S. Endres, S. Kobold, PD1-CD28 fusion protein enables CD4+ T cell help for adoptive T cell therapy in models of pancreatic cancer and non-hodgkin lymphoma, Front. Immunol. 9 (2018) 1955.
[226] R. Schlenker, L.F. Olguin-Contreras, M. Leisegang, J. Schnappinger, A. Disovic, S. Ruhland, P.J. Nelson, H. Leonhardt, H. Harz, S. Wilde, D.J. Schendel, W. Uckert, G. Willimsky, E. Noessner, Chimeric PD-1:28 receptor upgrades low-avidity T cells and restores effector function of tumor-infiltrating lymphocytes for adoptive cell therapy, Cancer Res. 77 (2017) 3577–3590.
[227] X. Tang, Q. Li, Y. Zhu, D. Zheng, J. Dai, W. Ni, J. Wei, Y. Xue, K. Chen, W. Hou, C. Zhang, X. Feng, Y. Liang, The advantages of PD1 activating chimeric receptor (PD1-ACR) engineered lymphocytes for PDL1(+) cancer therapy, Am. J. Transl. Res. 7 (2015) 460–473.
[228] J.H. Shin, H.B. Park, Y.M. Oh, D.P. Lim, J.E. Lee, H.H. Seo, S.J. Lee, H.S. Eom, I.H. Kim, S.H. Lee, K. Choi, Positive conversion of negative signaling of CTLA4 potentiates antitumor efficacy of adoptive T-cell therapy in murine tumor models, Blood 119 (2012) 5678–5687.
[229] H.B. Park, J.E. Lee, Y.M. Oh, S.J. Lee, H.S. Eom, K. Choi, CTLA4-CD28 chimera gene modification of T cells enhances the therapeutic efficacy of donor lymphocyte infusion for hematological malignancy, Exp. Mol. Med. 49 (2017), e360. .
[230] H.Y. Yoo, P. Kim, W.S. Kim, S.H. Lee, S. Kim, S.Y. Kang, H.Y. Jang, J.E. Lee, J. Kim, S.J. Kim, Y.H. Ko, S. Lee, Frequent CTLA4-CD28 gene fusion in diverse types of T-cell lymphoma, Haematologica 101 (2016) 757–763.
[231] S.K. Oda, A.W. Daman, N.M. Garcia, F. Wagener, T.M. Schmitt, X. Tan, A.G. Chapuis, P.D. Greenberg, A CD200R-CD28 fusion protein appropriates an inhibitory signal to enhance T-cell function and therapy of murine leukemia, Blood 130 (2017) 2410–2419.
[232] A. Krause, H.F. Guo, J.B. Latouche, C. Tan, N.K. Cheung, M. Sadelain, Antigen-dependent CD28 signaling selectively enhances survival and proliferation in genetically modified activated human primary T lymphocytes, J. Exp. Med. 188 (1998) 619–626.
[233] C.C. Kloss, M. Condomines, M. Cartellieri, M. Bachmann, M. Sadelain, Combinatorial antigen recognition with balanced signaling promotes selective tumor eradication by engineered T cells, Nat. Biotechnol. 31 (2013) 71–75.
[234] K. Miyao, S. Terakura, S. Okuno, J. Julamanee, K. Watanabe, H. Hamana, H. Kishi, R. Sakemura, D. Koyama, T. Goto, T. Nishida, M. Murata, H. Kiyoi, Introduction of genetically modified CD3zeta improves proliferation and persistence of antigen-specific CTLs, Cancer Immunol. Res. 6 (2018) 733–744.
[235] B. Heemskerk, K. Liu, M.E. Dudley, L.A. Johnson, A. Kaiser, S. Downey, Z. Zheng, T.E. Shelton, K. Matsuda, P.F. Robbins, R.A. Morgan, S.A. Rosenberg, Adoptive cell therapy for patients with melanoma, using tumor-infiltrating lymphocytes genetically engineered to secrete interleukin-2, Hum. Gene Ther. 19 (2008) 496–510.
[236] L. Zhang, S.P. Kerkar, Z. Yu, Z. Zheng, S. Yang, N.P. Restifo, S.A. Rosenberg, R.A. Morgan, Improving adoptive T cell therapy by targeting and controlling IL-12 expression to the tumor environment, Mol. Ther. 19 (2011) 751–759.
[237] C. Hsu, S.A. Jones, C.J. Cohen, Z. Zheng, K. Kerstann, J. Zhou, P.F. Robbins, P.D. Peng, X. Shen, T.J. Gomes, C.E. Dunbar, D.J. Munroe, C. Stewart, K. Cornetta, D. Wangsa, T. Ried, S.A. Rosenberg, R.A. Morgan, Cytokine independent growth and clonal expansion of a primary human CD8+ T cell clone following retroviral transduction with the IL-15 gene, Blood 109 (12) (2007 Jun 15) 5168–5177.
[238] J.F. Vera, V. Hoyos, B. Savoldo, C. Quintarelli, G.M. Giordano Attianese, A.M. Leen, H. Liu, A.E. Foster, H.E. Heslop, C.M. Rooney, M.K. Brenner, G. Dotti, Genetic manipulation of tumor-specific cytotoxic T lymphocytes to restore responsiveness to IL-7, Mol. Ther. 17 (2009) 880–888.
[239] Y. Kagoya, S. Tanaka, T. Guo, M. Anczurowski, C.H. Wang, K. Saso, M.O. Butler, M.D. Minden, N. Hirano, A novel chimeric antigen receptor containing a JAK-STAT signaling domain mediates superior antitumor effects, Nat. Med. 24 (2018) 352–359.
[240] H.J. Pegram, J.C. Lee, E.G. Hayman, G.H. Imperato, T.F. Tedder, M. Sadelain, R.J. Brentjens, Tumor-targeted T cells modified to secrete IL-12 eradicate systemic tumors without need for prior conditioning, Blood 119 (2012) 4133–4141.
[241] G. Kueberuwa, M. Kalaitsidou, E. Cheadle, R.E. Hawkins, D.E. Gilham, CD19 CAR T cells expressing IL-12 eradicate lymphoma in fully lymphoreplete mice through induction of host immunity, Mol. Ther. Oncolytics. 8 (2018) 41–51.
[242] D. Chinnasamy, Z. Yu, S.P. Kerkar, L. Zhang, R.A. Morgan, N.P. Restifo, S.A. Rosenberg, Local delivery of interleukin-12 using T cells targeting VEGF receptor-2 eradicates multiple vascularized tumors in mice, Clin. Cancer Res. 18 (2012) 1672–1683.
[243] E.K. Moon, L.C. Wang, D.V. Dolfi, C.B. Wilson, R. Ranganathan, J. Sun, V. Kapoor, J. Scholler, E. Pure, M.C. Milone, C.H. June, J.L. Riley, E.J. Wherry, S.M. Albelda, Multifactorial T-cell hypofunction that is reversible can limit the efficacy of chimeric antigen receptor-transduced human T cells in solid tumors, Clin. Cancer Res. 20 (2014) 4262–4273.
[244] M. Koneru, R. O'Cearbhaill, S. Pendharkar, D.R. Spriggs, R.J. Brentjens, 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. 13 (2015) 102.
[245] J.E. Zuniga, J.C. Groppe, Y. Cui, C.S. Hinck, V. Contreras-Shannon, O.N. Pakhomova, J. Yang, Y. Tang, V. Mendoza, F. Lopez-Casillas, L. Sun, A.P. Hinck, Assembly of TbetaRI:TbetaRII:TGFbeta ternary complex in vitro with receptor extracellular domains is cooperative and isoform-dependent, J. Mol. Biol. 354 (2005) 1052–1068.
[246] L. Gorelik, R.A. Flavell, Abrogation of TGFbeta signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease, Immunity 12 (2000) 171–181.
[247] L. Zhang, Z. Yu, P. Muranski, D.C. Palmer, N.P. Restifo, S.A. Rosenberg, R.A. Morgan, Inhibition of TGF-beta signaling in genetically engineered tumor antigen-reactive T cells significantly enhances tumor treatment efficacy, Gene Ther. 20 (5) (2013 May) 575–580, https://doi.org/10.1038/gt.2012.75.
[248] G.M. Bendle, C. Linnemann, L. Bies, J.Y. Song, T.N. Schumacher, Blockade of TGF-beta signaling greatly enhances the efficacy of TCR gene therapy of cancer, J. Immunol. 191 (2013) 3232–3239.
[249] Z. Wang, L. Guo, Y. Song, Y. Zhang, D. Lin, B. Hu, Y. Mei, D. Sandikin, H. Liu, Augmented anti-tumor activity of NK-92 cells expressing chimeric receptors of TGF-betaR II and NKG2D, Cancer Immunol. Immunother. 66 (2017) 537–548.
[250] P. Muranski, N.P. Restifo, Adoptive immunotherapy of cancer using CD4(+) T cells, Curr. Opin. Immunol. 21 (2009) 200–208.
[251] A.M. Leen, S. Sukumaran, N. Watanabe, S. Mohammed, J. Keirnan, R. Yanagisawa, U. Anurathapan, D. Rendon, H.E. Heslop, C.M. Rooney, M.K. Brenner, J.F. Vera, Reversal of tumor immune inhibition using a chimeric cytokine receptor, Mol. Ther. 22 (2014) 1211–1220.
[252] M.H. Kershaw, G. Wang, J.A. Westwood, R.K. Pachynski, H.L. Tiffany, F.M. Marincola, E. Wang, H.A. Young, P.M. Murphy, P. Hwu, Redirecting migration of T cells to chemokine secreted from tumors by genetic modification with CXCR2, Hum. Gene Ther. 13 (2002) 1971–1980.
[253] A. Di Stasi, B. De Angelis, C.M. Rooney, L. Zhang, A. Mahendravada, A.E. Foster, H.E. Heslop, M.K. Brenner, G. Dotti, B. Savoldo, T lymphocytes coexpressing CCR4 and a chimeric antigen receptor targeting CD30 have improved homing and antitumor activity in a Hodgkin tumor model, Blood 113 (2009) 6392–6402.
[254] J.A. Craddock, A. Lu, A. Bear, M. Pule, M.K. Brenner, C.M. Rooney, A.E. Foster, Enhanced tumor trafficking of GD2 chimeric antigen receptor T cells by expression of the chemokine receptor CCR2b, J. Immunother. 33 (2010) 780–788.
[255] E.K. Moon, C. Carpenito, J. Sun, L.C. Wang, V. Kapoor, J. Predina, D.J. Powell Jr., J.L. Riley, C.H. June, S.M. Albelda, Expression of a functional CCR2 receptor enhances tumor localization and tumor eradication by retargeted human T cells expressing a mesothelin-specific chimeric antibody receptor, Clin. Cancer Res. 17 (2011) 4719–4730.
[256] S. Sapoznik, R. Ortenberg, G. Galore-Haskel, S. Kozlovski, D. Levy, C. Avivi, I. Barshack, C.J. Cohen, M.J. Besser, J. Schachter, G. Markel, CXCR1 as a novel target for directing reactive T cells toward melanoma: implications for adoptive cell transfer immunotherapy, Cancer Immunol. Immunother. 61 (2012) 1833–1847.
[257] M.A. Ligtenberg, D. Mougiakakos, M. Mukhopadhyay, K. Witt, A. Lladser, M. Chmielewski, T. Riet, H. Abken, R. Kiessling, Coexpressed catalase protects chimeric antigen receptor-redirected T cells as well as bystander cells from oxidative stress-induced loss of antitumor activity, J. Immunol. 196 (2016) 759–766.
[258] I. Caruana, B. Savoldo, V. Hoyos, G. Weber, H. Liu, E.S. Kim, M.M. Ittmann, D. Marchetti, G. Dotti, Heparanase promotes tumor infiltration and antitumor activity of CAR-redirected T lymphocytes, Nat. Med. 21 (2015) 524–529.
[259] E.R. Suarez, D.K. Chang, J. Sun, J. Sui, G.J. Freeman, S. Signoretti, Q. Zhu, W.A. Marasco, Chimeric antigen receptor T cells secreting anti-PD-L1 antibodies more effectively regress renal cell carcinoma in a humanized mouse model, Oncotarget 7 (2016) 34341–34355.
[260] S. Li, N. Siriwon, X. Zhang, S. Yang, T. Jin, F. He, Y.J. Kim, J. Mac, Z. Lu, S. Wang, X. Han, P. Wang, Enhanced cancer immunotherapy by chimeric antigen receptor-modified T cells engineered to secrete checkpoint inhibitors, Clin. Cancer Res. 23 (2017) 6982–6992.
[261] S. Rafiq, O.O. Yeku, H.J. Jackson, T.J. Purdon, D.G. van Leeuwen, D.J. Drakes, M. Song, M.M. Miele, Z. Li, P. Wang, S. Yan, J. Xiang, X. Ma, V.E. Seshan, R.C. Hendrickson, C. Liu, R.J. Brentjens, Targeted delivery of a PD-1-blocking scFv by CAR-T cells enhances anti-tumor efficacy in vivo, Nat. Biotechnol. 36 (2018) 847–856.
[262] M. Boice, D. Salloum, F. Mourcin, V. Sanghvi, R. Amin, E. Oricchio, M. Jiang, A. Mottok, N. Denis-Lagache, G. Ciriello, W. Tam, J. Teruya-Feldstein, S.E. De, W.C. Chan, S.N. Malek, D. Ennishi, R.J. Brentjens, R.D. Gascoyne, M. Cogne, K. Tarte, H.G. Wendel, Loss of the HVEM tumor suppressor in lymphoma and restoration by modified CAR-T cells, Cell 167 (2016) 405–418.
[263] E. Jacoby, Y. Yang, H. Qin, C.D. Chien, J.N. Kochenderfer, T.J. Fry, Murine allogeneic CD19 CAR T cells harbor potent antileukemic activity but have the potential to mediate lethal GVHD, Blood 127 (2016) 1361–1370.
[264] W. Uckert, T.N. Schumacher, TCR transgenes and transgene cassettes for TCR gene therapy: status in 2008, Cancer Immunol. Immunother. 58 (2009) 809–822.
[265] T. Tsuji, M. Yasukawa, J. Matsuzaki, T. Ohkuri, K. Chamoto, D. Wakita, T. Azuma, H. Niiya, H. Miyoshi, K. Kuzushima, Y. Oka, H. Sugiyama, H. Ikeda, T. Nishimura, Generation of tumor-specific, HLA class I-restricted human Th1 and Tc1 cells by cell engineering with tumor peptide-specific T-cell receptor genes, Blood 106 (2005) 470–476.
[266] S. Yang, C.J. Cohen, P.D. Peng, Y. Zhao, L. Cassard, Z. Yu, Z. Zheng, S. Jones, N.P. Restifo, S.A. Rosenberg, R.A. Morgan, Development of optimal bicistronic lentiviral vectors facilitates high-level TCR gene expression and robust tumor cell recognition, Gene Ther. 15 (2008) 1411–1423.
[267] S. Jones, P.D. Peng, S. Yang, C. Hsu, C.J. Cohen, Y. Zhao, J. Abad, Z. Zheng, S.A. Rosenberg, R.A. Morgan, Lentiviral vector design for optimal T cell receptor gene

expression in the transduction of peripheral blood lymphocytes and tumor-infiltrating lymphocytes, Hum. Gene Ther. 20 (2009) 630–640.
[268] P. Circosta, L. Granziero, A. Follenzi, E. Vigna, S. Stella, A. Vallario, A.R. Elia, L. Gammaitoni, K. Vitaggio, F. Orso, M. Geuna, D. Sangiolo, M. Todorovic, C. Giachino, A. Cignetti, T cell receptor (TCR) gene transfer with lentiviral vectors allows efficient redirection of tumor specificity in naive and memory T cells without prior stimulation of endogenous TCR, Hum. Gene Ther. 20 (2009) 1576–1588.
[269] C. Frecha, C. Levy, F.L. Cosset, E. Verhoeyen, Advances in the field of lentivector-based transduction of T and B lymphocytes for gene therapy, Mol. Ther. 18 (2010) 1748–1757.
[270] B.L. Levine, L.M. Humeau, J. Boyer, R.R. MacGregor, T. Rebello, X. Lu, G.K. Binder, V. Slepushkin, F. Lemiale, J.R. Mascola, F.D. Bushman, B. Dropulic, C.H. June, Gene transfer in humans using a conditionally replicating lentiviral vector, Proc. Natl. Acad. Sci. U. S. A 103 (2006) 17372–17377.
[271] H.E. Heslop, M.K. Brenner, Seek and you will not find: ending the hunt for replication-competent retroviruses during human gene therapy, Mol. Ther. 26 (2018) 1–2.
[272] A.S. Bear, R.A. Morgan, K. Cornetta, C.H. June, G. Binder-Scholl, M.E. Dudley, S.A. Feldman, S.A. Rosenberg, S.A. Shurtleff, C.M. Rooney, H.E. Heslop, G. Dotti, Replication-competent retroviruses in gene-modified T cells used in clinical trials: is it time to revise the testing requirements? Mol. Ther. 20 (2012) 246–249.
[273] K. Cornetta, L. Duffy, C.J. Turtle, M. Jensen, S. Forman, G. Binder-Scholl, T. Fry, A. Chew, D.G. Maloney, C.H. June, Absence of replication-competent lentivirus in the clinic: analysis of infused T cell products, Mol. Ther. 26 (2018) 280–288.
[274] P.D. Peng, C.J. Cohen, S. Yang, C. Hsu, S. Jones, Y. Zhao, Z. Zheng, S.A. Rosenberg, R.A. Morgan, Efficient nonviral Sleeping beauty transposon-based TCR gene transfer to peripheral blood lymphocytes confers antigen-specific antitumor reactivity, Gene Ther. 16 (2009) 1042–1049.
[275] P. Kebriaei, H. Singh, M.H. Huls, M.J. Figliola, R. Bassett, S. Olivares, B. Jena, M.J. Dawson, P.R. Kumaresan, S. Su, S. Maiti, J. Dai, B. Moriarity, M.A. Forget, V. Senyukov, A. Orozco, T. Liu, J. McCarty, R.N. Jackson, J.S. Moyes, G. Rondon, M. Qazilbash, S. Ciurea, A. Alousi, Y. Nieto, K. Rezvani, D. Marin, U. Popat, C. Hosing, E.J. Shpall, H. Kantarjian, M. Keating, W. Wierda, K.A. Do, D.A. Largaespada, D.A. Lee, P.B. Hackett, R.E. Champlin, L.J. Cooper, Phase I trials using sleeping beauty to generate CD19-specific CAR T cells, J Clin Invest 126 (2016) 3363–3376.
[276] T. L. Roth, C. Puig-Saus, R. Yu, E. Shifrut, J. Carnevale, P. J. Li, J. Hiatt, J. Saco, P. Krystofinski, H. Li, V. Tobin, D. N. Nguyen, M. R. Lee, A. L. Putnam, A. L. Ferris, J. W. Chen, J. N. Schickel, L. Pellerin, D. Carmody, G. Alkorta-Aranburu, G. D. Del, H. Matsumoto, M. Morell, Y. Mao, M. Cho, R. M. Quadros, C. B. Gurumurthy, B. Smith, M. Haugwitz, S. H. Hughes, J. S. Weissman, K. Schumann, J. H. Esensten, A. P. May, A. Ashworth, G. M. Kupfer, S. A. W. Greeley, R. Bacchetta, E. Meffre, M. G. Roncarolo, N. Romberg, K. C. Herold, A. Ribas, M. D. Leonetti, and A. Marson, Reprogramming human T cell function and specificity with non-viral genome targeting, Nature, (2018).
[277] Y. Zhao, Z. Zheng, C.J. Cohen, L. Gattinoni, D.C. Palmer, N.P. Restifo, S.A. Rosenberg, R.A. Morgan, High-efficiency transfection of primary human and mouse T lymphocytes using RNA electroporation, Mol. Ther. 13 (2006) 151–159.
[278] R.M. Gupta, K. Musunuru, Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9, J. Clin. Invest. 124 (2014) 4154–4161.
[279] J. Ren, Y. Zhao, Advancing chimeric antigen receptor T cell therapy with CRISPR/Cas9, Protein Cell 8 (2017) 634–643.
[280] J.D. Beane, G. Lee, Z. Zheng, M. Mendel, D. Abate-Daga, M. Bharathan, M. Black, N. Gandhi, Z. Yu, S. Chandran, M. Giedlin, D. Ando, J. Miller, D. Paschon, D. Guschin, E.J. Rebar, A. Reik, M.C. Holmes, P.D. Gregory, N.P. Restifo, S.A. Rosenberg, R.A. Morgan, S.A. Feldman, Clinical scale zinc finger nuclease-mediated gene editing of PD-1 in tumor infiltrating lymphocytes for the treatment of metastatic melanoma, Mol. Ther. 23 (2015) 1380–1390.
[281] S. Su, B. Hu, J. Shao, B. Shen, J. Du, Y. Du, J. Zhou, L. Yu, L. Zhang, F. Chen, H. Sha, L. Cheng, F. Meng, Z. Zou, X. Huang, B. Liu, CRISPR-Cas9 mediated efficient PD-1 disruption on human primary T cells from cancer patients, Sci. Rep. 6 (2016) 20070.
[282] J. Ren, X. Zhang, X. Liu, C. Fang, S. Jiang, C.H. June, Y. Zhao, A versatile system for rapid multiplex genome-edited CAR T cell generation, Oncotarget 8 (2017) 17002–17011.
[283] I.Y. Jung, Y.Y. Kim, H.S. Yu, M. Lee, S. Kim, J. Lee, CRISPR/Cas9-mediated knockout of DGK improves antitumor activities of human T cells, Cancer Res. 78 (2018) 4692–4703.
[284] A. Seki, S. Rutz, Optimized RNP transfection for highly efficient CRISPR/Cas9-mediated gene knockout in primary T cells, J. Exp. Med. 215 (2018) 985–997.
[285] J.A. Fraietta, C.L. Nobles, M.A. Sammons, S. Lundh, S.A. Carty, T.J. Reich, A.P. Cogdill, J.J.D. Morrissette, J.E. DeNizio, S. Reddy, Y. Hwang, M. Gohil, I. Kulikovskaya, F. Nazimuddin, M. Gupta, F. Chen, J.K. Everett, K.A. Alexander, E. Lin-Shiao, M.H. Gee, X. Liu, R.M. Young, D. Ambrose, Y. Wang, J. Xu, M.S. Jordan, K.T. Marcucci, B.L. Levine, K.C. Garcia, Y. Zhao, M. Kalos, D.L. Porter, R.M. Kohli, S.F. Lacey, S.L. Berger, F.D. Bushman, C.H. June, J.J. Melenhorst, Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells, Nature 558 (2018) 307–312.
[286] J. Eyquem, J. Mansilla-Soto, T. Giavridis, S.J. van der Stegen, M. Hamieh, K.M. Cunanan, A. Odak, M. Gonen, M. Sadelain, Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection, Nature 543 (2017) 113–117.
[287] C. Georgiadis, R. Preece, L. Nickolay, A. Etuk, A. Petrova, D. Ladon, A. Danyi, N. Humphryes-Kirilov, A. Ajetunmobi, D. Kim, J.S. Kim, W. Qasim, Long terminal repeat CRISPR-CAR-coupled "Universal" T cells mediate potent anti-leukemic effects, Mol. Ther. 26 (2018) 1215–1227.
[288] S. Mastaglio, P. Genovese, Z. Magnani, E. Ruggiero, E. Landoni, B. Camisa, G. Schiroli, E. Provasi, A. Lombardo, A. Reik, N. Cieri, M. Rocchi, G. Oliveira, G. Escobar, M. Casucci, B. Gentner, A. Spinelli, A. Mondino, A. Bondanza, L. Vago, M. Ponzoni, F. Ciceri, M.C. Holmes, L. Naldini, C. Bonini, NY-ESO-1 TCR single edited stem and central memory T cells to treat multiple myeloma without graft-versus-host disease, Blood 130 (2017) 606–618.
[289] X. Liu, Y. Zhang, C. Cheng, A.W. Cheng, X. Zhang, N. Li, C. Xia, X. Wei, X. Liu, H. Wang, CRISPR-Cas9-mediated multiplex gene editing in CAR-T cells, Cell Res. 27 (2017) 154–157.
[290] J. Rasaiyaah, C. Georgiadis, R. Preece, U. Mock, W. Qasim, TCRab/CD3 disruption enables CD3-specific antileukemic T cell immunotherapy, JCI Insight 3 (2018).
[291] M.Y. Kim, K.R. Yu, S.S. Kenderian, M. Ruella, S. Chen, T.H. Shin, A.A. Aljanahi, D. Schreeder, M. Klichinsky, O. Shestova, M.S. Kozlowski, K.D. Cummins, X. Shan, M. Shestov, A. Bagg, J.J.D. Morrissette, P. Sekhri, C.R. Lazzarotto, K.R. Calvo, D.B. Kuhns, R.E. Donahue, G.K. Behbehani, S.Q. Tsai, C.E. Dunbar, S. Gill, Genetic Inactivation of CD33 in hematopoietic stem cells to enable CAR T cell immunotherapy for acute myeloid leukemia, Cell 173 (2018) 1439–1453.
[292] F. Ciceri, C. Bonini, M.T. Stanghellini, A. Bondanza, C. Traversari, M. Salomoni, L. Turchetto, S. Colombi, M. Bernardi, J. Peccatori, A. Pescarollo, P. Servida, Z. Magnani, S.K. Perna, V. Valtolina, F. Crippa, L. Callegaro, E. Spoldi, R. Crocchiolo, K. Fleischhauer, M. Ponzoni, L. Vago, S. Rossini, A. Santoro, E. Todisco, J. Apperley, E. Olavarria, S. Slavin, E.M. Weissinger, A. Ganser, M. Stadler, E. Yannaki, A. Fassas, A. Anagnostopoulos, M. Bregni, C.G. Stampino, P. Bruzzi, C. Bordignon, 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 (2009) 489–500.
[293] V. Hoyos, B. Savoldo, C. Quintarelli, A. Mahendravada, M. Zhang, J. Vera, H.E. Heslop, C.M. Rooney, M.K. Brenner, G. Dotti, Engineering CD19-specific T lymphocytes with interleukin-15 and a suicide gene to enhance their anti-lymphoma/leukemia effects and safety, Leukemia 24 (2010) 1160–1170.
[294] I. Diaconu, B. Ballard, M. Zhang, Y. Chen, J. West, G. Dotti, B. Savoldo, Inducible Caspase-9 Selectively Modulates the Toxicities of CD19-specific Chimeric Antigen Receptor-Modified T Cells, Mol. Ther. 25 (2017) 580–592.
[295] E. Kieback, J. Charo, D. Sommermeyer, T. Blankenstein, W. Uckert, A safeguard eliminates T cell receptor gene-modified autoreactive T cells after adoptive transfer, Proc. Natl. Acad. Sci. U. S. A 105 (2008) 623–628.
[296] X. Wang, W.C. Chang, C.W. Wong, D. Colcher, M. Sherman, J.R. Ostberg, S.J. Forman, S.R. Riddell, M.C. Jensen, A transgene-encoded cell surface polypeptide for selection, in vivo tracking, and ablation of engineered cells, Blood 118 (2011) 1255–1263.
[297] R. Sakemura, S. Terakura, K. Watanabe, J. Julamanee, E. Takagi, K. Miyao, D. Koyama, T. Goto, R. Hanajiri, T. Nishida, M. Murata, H. Kiyoi, A Tet-on Inducible System for Controlling CD19-Chimeric Antigen Receptor Expression upon Drug Administration, Cancer Immunol. Res. 4 (2016) 658–668.
[298] C.Y. Wu, K.T. Roybal, E.M. Puchner, J. Onuffer, W.A. Lim, Remote control of therapeutic T cells through a small molecule-gated chimeric receptor, Science 350 (2015) aab4077.
[299] A. Juillerat, A. Marechal, J.M. Filhol, Y. Valogne, J. Valton, A. Duclert, P. Duchateau, L. Poirot, An oxygen sensitive self-decision making engineered CAR T-cell, Sci. Rep. 7 (2017) 39833.
[300] X. Han, P.D. Bryson, Y. Zhao, G.E. Cinay, S. Li, Y. Guo, N. Siriwon, P. Wang, Masked chimeric antigen receptor for tumor-specific activation, Mol. Ther. 25 (2017) 274–284.
[301] C.W. Helsen, J.A. Hammill, V.W.C. Lau, K.A. Mwawasi, A. Afsahi, K. Bezverbnaya, L. Newhook, D.L. Hayes, C. Aarts, B. Bojovic, G.F. Denisova, J.M. Kwiecien, I. Brain, H. Derocher, K. Milne, B.H. Nelson, J.L. Bramson, The chimeric TAC receptor co-opts the T cell receptor yielding robust anti-tumor activity without toxicity, Nat. Commun. 9 (2018) 3049.
[302] S. Wilkie, M.C. van Schalkwyk, S. Hobbs, D.M. Davies, S.J. van der Stegen, A.C. Pereira, S.E. Burbridge, C. Box, S.A. Eccles, J. Maher, Dual targeting of ErbB2 and MUC1 in breast cancer using chimeric antigen receptors engineered to provide complementary signaling, J. Clin. Immunol. 32 (2012) 1059–1070.
[303] E. Lanitis, M. Poussin, A.W. Klattenhoff, D. Song, R. Sandaltzopoulos, C.H. June, D.J. Powell Jr., Chimeric antigen receptor T Cells with dissociated signaling domains exhibit focused antitumor activity with reduced potential for toxicity in vivo, Cancer Immunol. Res. 1 (2013) 43–53.
[304] W.R. Gordon, B. Zimmerman, L. He, L.J. Miles, J. Huang, K. Tiyanont, D.G. McArthur, J.C. Aster, N. Perrimon, J.J. Loparo, S.C. Blacklow, Mechanical allostery: evidence for a force requirement in the proteolytic activation of notch, Dev. Cell 33 (2015) 729–736.
[305] K.T. Roybal, L.J. Rupp, L. Morsut, W.J. Walker, K.A. McNally, J.S. Park, W.A. Lim, Precision tumor recognition by T cells with combinatorial antigen-sensing circuits, Cell 164 (2016) 770–779.
[306] K.T. Roybal, J.Z. Williams, L. Morsut, L.J. Rupp, I. Kolinko, J.H. Choe, W.J. Walker, K.A. McNally, W.A. Lim, Engineering T cells with customized therapeutic response programs using synthetic notch receptors, Cell 167 (2016) 419–432.
[307] V.D. Fedorov, M. Themeli, M. Sadelain, PD-1- and CTLA-4-based inhibitory chimeric antigen receptors (iCARs) divert off-target immunotherapy responses, Sci. Transl. Med. 5 (2013) 215ra172.
[308] . K. Urbanska, E. Lanitis, M. Poussin, R.C. Lynn, B.P. Gavin, S. Kelderman, J. Yu, N. Scholler, D.J. Powell Jr., A universal strategy for adoptive immunotherapy of cancer through use of a novel T-cell antigen receptor, Cancer Res. 72 (2012) 1844–1852.
[309] J.J. Lohmueller, J.D. Ham, M. Kvorjak, O.J. Finn, mSA2 affinity-enhanced biotin-binding CAR T cells for universal tumor targeting, Oncoimmunology. 7 (2017), e1368604. .
[310] D.T. Rodgers, M. Mazagova, E.N. Hampton, Y. Cao, N.S. Ramadoss, I.R. Hardy, A. Schulman, J. Du, F. Wang, O. Singer, J. Ma, V. Nunez, J. Shen, A.K. Woods, T.M. Wright, P.G. Schultz, C.H. Kim, T.S. Young, Switch-mediated activation and

retargeting of CAR-T cells for B-cell malignancies, Proc. Natl. Acad. Sci. U. S. A 113 (2016) E459–E468.
[311] K. Tamada, D. Geng, Y. Sakoda, N. Bansal, R. Srivastava, Z. Li, E. Davila, Redirecting gene-modified T cells toward various cancer types using tagged antibodies, Clin. Cancer Res. 18 (2012) 6436–6445.
[312] M.S. Kim, J.S. Ma, H. Yun, Y. Cao, J.Y. Kim, V. Chi, D. Wang, A. Woods, L. Sherwood, D. Caballero, J. Gonzalez, P.G. Schultz, T.S. Young, C.H. Kim, Redirection of genetically engineered CAR-T cells using bifunctional small molecules, J. Am. Chem. Soc. 137 (2015) 2832–2835.
[313] K. Kudo, C. Imai, P. Lorenzini, T. Kamiya, K. Kono, A.M. Davidoff, W.J. Chng, D. Campana, T lymphocytes expressing a CD16 signaling receptor exert antibody-dependent cancer cell killing, Cancer Res. 74 (2014) 93–103.
[314] J.H. Cho, J.J. Collins, W.W. Wong, Universal Chimeric Antigen Receptors for Multiplexed and Logical Control of T Cell responses, Cell 173 (2018) 1426–1438.
[315] V. Golubovskaya, L. Wu, Different Subsets of T Cells, Memory, Effector Functions, and CAR-T Immunotherapy, Cancers. (Basel) 8 (2016).
[316] W.H. Fridman, F. Pages, C. Sautes-Fridman, J. Galon, The immune contexture in human tumours: impact on clinical outcome, Nat. Rev. Cancer 12 (2012) 298–306.
[317] L. Gattinoni, C.A. Klebanoff, N.P. Restifo, Paths to stemness: building the ultimate antitumour T cell, Nat. Rev. Cancer 12 (2012) 671–684.
[318] C.A. Klebanoff, C.D. Scott, A.J. Leonardi, T.N. Yamamoto, A.C. Cruz, C. Ouyang, M. Ramaswamy, R. Roychoudhuri, Y. Ji, R.L. Eil, M. Sukumar, J.G. Crompton, D.C. Palmer, Z.A. Borman, D. Clever, S.K. Thomas, S. Patel, Z. Yu, P. Muranski, H. Liu, E. Wang, F.M. Marincola, A. Gros, L. Gattinoni, S.A. Rosenberg, R.M. Siegel, N.P. Restifo, Memory T cell-driven differentiation of naive cells impairs adoptive immunotherapy, J. Clin. Invest. 126 (2016) 318–334.
[319] V.P. Zediak, J.B. Johnnidis, E.J. Wherry, S.L. Berger, Cutting edge: persistently open chromatin at effector gene loci in resting memory CD8+ T cells independent of transcriptional status, J. Immunol. 186 (2011) 2705–2709.
[320] E.L. Pearce, M.C. Poffenberger, C.H. Chang, R.G. Jones, Fueling immunity: insights into metabolism and lymphocyte function, Science 342 (2013) 1242454.
[321] C. Berger, M.C. Jensen, P.M. Lansdorp, M. Gough, C. Elliott, S.R. Riddell, Adoptive transfer of effector CD8+ T cells derived from central memory cells establishes persistent T cell memory in primates, J. Clin. Invest. 118 (2008) 294–305.
[322] X. Wang, C.W. Wong, R. Urak, E. Taus, B. Aguilar, W.C. Chang, A. Mardiros, L.E. Budde, C.E. Brown, C. Berger, S.J. Forman, M.C. Jensen, Comparison of naive and central memory derived CD8(+) effector cell engraftment fitness and function following adoptive transfer, Oncoimmunology. 5 (2016), e1072671. .
[323] X. Wang, L.L. Popplewell, J.R. Wagner, A. Naranjo, M.S. Blanchard, M.R. Mott, A.P. Norris, C.W. Wong, R.Z. Urak, W.C. Chang, S.K. Khaled, T. Siddiqi, L.E. Budde, J. Xu, B. Chang, N. Gidwaney, S.H. Thomas, L.J. Cooper, S.R. Riddell, C.E. Brown, M.C. Jensen, S.J. Forman, Phase 1 studies of central memory-derived CD19 CAR T-cell therapy following autologous HSCT in patients with B-cell NHL, Blood 127 (2016) 2980–2990.
[324] L.V. Hurton, H. Singh, A.M. Najjar, K.C. Switzer, T. Mi, S. Maiti, S. Olivares, B. Rabinovich, H. Huls, M.A. Forget, V. Datar, P. Kebriaei, D.A. Lee, R.E. Champlin, L.J. Cooper, Tethered IL-15 augments antitumor activity and promotes a stem-cell memory subset in tumor-specific T cells, Proc. Natl. Acad. Sci. U. S. A 113 (2016) E7788–E7797.
[325] Y. Zhao, M.R. Parkhurst, Z. Zheng, C.J. Cohen, J.P. Riley, L. Gattinoni, N.P. Restifo, S.A. Rosenberg, R.A. Morgan, Extrathymic generation of tumor-specific T cells from genetically engineered human hematopoietic stem cells via Notch signaling, Cancer Res. 67 (2007) 2425–2429.
[326] L. Yang, D. Baltimore, Long-term in vivo provision of antigen-specific T cell immunity by programming hematopoietic stem cells, Proc. Natl. Acad. Sci. U. S. A 102 (2005) 4518–4523.
[327] M. Themeli, C.C. Kloss, G. Ciriello, V.D. Fedorov, F. Perna, M. Gonen, M. Sadelain, Generation of tumor-targeted human T lymphocytes from induced pluripotent stem cells for cancer therapy, Nat. Biotechnol. 31 (2013) 928–933.
[328] E. Gschweng, O.S. De, D.B. Kohn, Hematopoietic stem cells for cancer immunotherapy, Immunol. Rev. 257 (2014) 237–249.
[329] D. Sommermeyer, M. Hudecek, P.L. Kosasih, T. Gogishvili, D.G. Maloney, C.J. Turtle, S.R. Riddell, Chimeric antigen receptor-modified T cells derived from defined CD8+ and CD4+ subsets confer superior antitumor reactivity in vivo, Leukemia 30 (2016) 492–500.
[330] F. Blaeschke, D. Stenger, T. Kaeuferle, S. Willier, R. Lotfi, A.D. Kaiser, M. Assenmacher, M. Doring, J. Feucht, T. Feuchtinger, Induction of a central memory and stem cell memory phenotype in functionally active CD4(+) and CD8(+) CAR T cells produced in an automated good manufacturing practice system for the treatment of CD19(+) acute lymphoblastic leukemia, Cancer Immunol. Immunother. 67 (2018) 1053–1066.
[331] C. Rossig, C.M. Bollard, J.G. Nuchtern, C.M. Rooney, M.K. Brenner, Epstein-Barr virus-specific human T lymphocytes expressing antitumor chimeric T-cell receptors: potential for improved immunotherapy, Blood 99 (2002) 2009–2016.
[332] C.R. Cruz, K.P. Micklethwaite, B. Savoldo, C.A. Ramos, S. Lam, S. Ku, O. Diouf, E. Liu, A.J. Barrett, S. Ito, E.J. Shpall, R.A. Krance, R.T. Kamble, G. Carrum, C.M. Hosing, A.P. Gee, Z. Mei, B.J. Grilley, H.E. Heslop, C.M. Rooney, M.K. Brenner, C.M. Bollard, G. Dotti, Infusion of donor-derived CD19-redirected virus-specific T cells for B-cell malignancies relapsed after allogeneic stem cell transplant: a phase 1 study, Blood 122 (2013) 2965–2973.
[333] Y. Xu, M. Zhang, C.A. Ramos, A. Durett, E. Liu, O. Dakhova, H. Liu, C.J. Creighton, A.P. Gee, H.E. Heslop, C.M. Rooney, B. Savoldo, G. Dotti, Closely related T-memory stem cells correlate with in vivo expansion of CAR.CD19-T cells and are preserved by IL-7 and IL-15, Blood 123 (2014) 3750–3759.
[334] C.H. Lamers, S. van Steenbergen-Langeveld, B.M. Van, C.M. Groot-van Ruijven, P.M. van Elzakker, K.B. Van, S. Sleijfer, R. Debets, T cell receptor-engineered T cells to treat solid tumors: T cell processing toward optimal T cell fitness, Hum. Gene Ther. Methods 25 (2014) 345–357.
[335] C.A. Klebanoff, S.E. Finkelstein, D.R. Surman, M.K. Lichtman, L. Gattinoni, M.R. Theoret, N. Grewal, P.J. Spiess, P.A. Antony, D.C. Palmer, Y. Tagaya, S.A. Rosenberg, T.A. Waldmann, N.P. Restifo, IL-15 enhances the in vivo antitumor activity of tumor-reactive CD8+ T cells, Proc. Natl. Acad. Sci. U. S. A 101 (2004) 1969–1974.
[336] R.S. Mehta, K. Rezvani, Chimeric antigen receptor expressing natural killer cells for the immunotherapy of cancer, Front. Immunol. 9 (2018) 283.
[337] C. Imai, S. Iwamoto, D. Campana, Genetic modification of primary natural killer cells overcomes inhibitory signals and induces specific killing of leukemic cells, Blood 106 (2005) 376–383.
[338] C. Zhang, P. Oberoi, S. Oelsner, A. Waldmann, A. Lindner, T. Tonn, W.S. Wels, Chimeric antigen receptor-engineered NK-92 Cells: An off-the-shelf cellular therapeutic for targeted elimination of cancer cells and induction of protective antitumor immunity, Front. Immunol. 8 (2017) 533.
[339] . A. Romanski, C. Uherek, G. Bug, E. Seifried, H. Klingemann, W.S. Wels, O.G. Ottmann, T. Tonn, CD19-CAR engineered NK-92 cells are sufficient to overcome NK cell resistance in B-cell malignancies, J. Cell. Mol. Med. 20 (2016) 1287–1294.
[340] E. Liu, Y. Tong, G. Dotti, H. Shaim, B. Savoldo, M. Mukherjee, J. Orange, X. Wan, X. Lu, A. Reynolds, M. Gagea, P. Banerjee, R. Cai, M.H. Bdaiwi, R. Basar, M. Muftuoglu, L. Li, D. Marin, W. Wierda, M. Keating, R. Champlin, E. Shpall, K. Rezvani, Cord blood NK cells engineered to express IL-15 and a CD19-targeted CAR show long-term persistence and potent antitumor activity, Leukemia 32 (2018) 520–531.
[341] N. Muller, S. Michen, S. Tietze, K. Topfer, A. Schulte, K. Lamszus, M. Schmitz, G. Schackert, I. Pastan, A. Temme, Engineering NK Cells modified with an EGFRvIII-specific chimeric antigen receptor to overexpress CXCR4 Improves immunotherapy of CXCL12/SDF-1alpha-secreting glioblastoma, J. Immunother. 38 (2015) 197–210.
[342] J.N. Kochenderfer, M.E. Dudley, R.O. Carpenter, S.H. Kassim, J.J. Rose, W.G. Telford, F.T. Hakim, D.C. Halverson, D.H. Fowler, N.M. Hardy, A.R. Mato, D.D. Hickstein, J.C. Gea-Banacloche, S.Z. Pavletic, C. Sportes, I. Maric, S.A. Feldman, B.G. Hansen, J.S. Wilder, B. Blacklock-Schuver, B. Jena, M.R. Bishop, R.E. Gress, S.A. Rosenberg, Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation, Blood 122 (2013) 4129–4139.
[343] J.N. Brudno, R.P. Somerville, V. Shi, J.J. Rose, D.C. Halverson, D.H. Fowler, J.C. Gea-Banacloche, S.Z. Pavletic, D.D. Hickstein, T.L. Lu, S.A. Feldman, A.T. Iwamoto, R. Kurlander, I. Maric, A. Goy, B.G. Hansen, J.S. Wilder, B. Blacklock-Schuver, F.T. Hakim, S.A. Rosenberg, R.E. Gress, J.N. Kochenderfer, 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. 34 (2016) 1112–1121.
[344] A. Ghosh, M. Smith, S.E. James, M.L. Davila, E. Velardi, K.V. Argyropoulos, G. Gunset, F. Perna, F.M. Kreines, E.R. Levy, S. Lieberman, H.V. Jay, A.Z. Tuckett, J.L. Zakrzewski, L. Tan, L.F. Young, K. Takvorian, J.A. Dudakov, R.R. Jenq, A.M. Hanash, A.C. Motta, G.F. Murphy, C. Liu, A. Schietinger, M. Sadelain, M.R. van den Brink, Donor CD19 CAR T cells exert potent graft-versus-lymphoma activity with diminished graft-versus-host activity, Nat. Med. 23 (2017) 242–249.
[345] H. Torikai, A. Reik, P.Q. Liu, Y. Zhou, L. Zhang, S. Maiti, H. Huls, J.C. Miller, P. Kebriaei, B. Rabinovitch, D.A. Lee, R.E. Champlin, C. Bonini, L. Naldini, E.J. Rebar, P.D. Gregory, M.C. Holmes, L.J. Cooper, 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 (2012) 5697–5705.
[346] W. Qasim, H. Zhan, S. Samarasinghe, S. Adams, P. Amrolia, S. Stafford, K. Butler, C. Rivat, G. Wright, K. Somana, S. Ghorashian, D. Pinner, G. Ahsan, K. Gilmour, G. Lucchini, S. Inglott, W. Mifsud, R. Chiesa, K.S. Peggs, L. Chan, F. Farzeneh, A.J. Thrasher, A. Vora, M. Pule, P. Veys, Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells, Sci. Transl. Med. 9 (2017).
[347] J. Ren, X. Liu, C. Fang, S. Jiang, C.H. June, Y. Zhao, Multiplex genome editing to generate universal CAR T cells resistant to PD1 inhibition, Clin. Cancer Res. 23 (2017) 2255–2266.
[348] G.G. Gornalusse, R.K. Hirata, S.E. Funk, L. Riolobos, V.S. Lopes, G. Manske, D. Prunkard, A.G. Colunga, L.A. Hanafi, D.O. Clegg, C. Turtle, D.W. Russell, HLA-E-expressing pluripotent stem cells escape allogeneic responses and lysis by NK cells, Nat. Biotechnol. 35 (2017) 765–772.
[349] L. Zhang, T. Sosinowski, A.R. Cox, J.R. Cepeda, N.S. Sekhar, S.M. Hartig, D. Miao, L. Yu, M. Pietropaolo, H.W. Davidson, Chimeric antigen receptor (CAR) T cells targeting a pathogenic MHC class II:peptide complex modulate the progression of autoimmune diabetes, J. Autoimmun. 96 (2019) 50–58.
[350] K. Fujio, A. Okamoto, Y. Araki, H. Shoda, H. Tahara, N.H. Tsuno, K. Takahashi, T. Kitamura, K. Yamamoto, Gene therapy of arthritis with TCR isolated from the inflamed paw, J. Immunol. 177 (2006) 8140–8147.
[351] G.P. Wright, C.A. Notley, S.A. Xue, G.M. Bendle, A. Holler, T.N. Schumacher, M.R. Ehrenstein, H.J. Stauss, Adoptive therapy with redirected primary regulatory T cells results in antigen-specific suppression of arthritis, Proc. Natl. Acad. Sci. U. S. A 106 (2009) 19078–19083.
[352] C.T. Ellebrecht, V.G. Bhoj, A. Nace, E.J. Choi, X. Mao, M.J. Cho, Z.G. Di, A. Lanzavecchia, J.T. Seykora, G. Cotsarelis, M.C. Milone, A.S. Payne, Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease, Science 353 (2016) 179–184.
[353] Y. Zhang, Y. Liu, K.M. Moxley, L. Golden-Mason, M.G. Hughes, T. Liu, M.H. Heemskerk, H.R. Rosen, M.I. Nishimura, Transduction of human T cells with a novel T-cell receptor confers anti-HCV reactivity, PLoS Pathog. 6 (2010), e1001018. .

[354] A. Schub, I.G. Schuster, W. Hammerschmidt, A. Moosmann, CMV-specific TCR-transgenic T cells for immunotherapy, J. Immunol. 183 (2009) 6819–6830.
[355] N. Schaft, B. Lankiewicz, J. Drexhage, C. Berrevoets, D.J. Moss, V. Levitsky, M. Bonneville, S.P. Lee, A.J. McMichael, J.W. Gratama, R.L. Bolhuis, R. Willemsen, R. Debets, T cell re-targeting to EBV antigens following TCR gene transfer: CD28-containing receptors mediate enhanced antigen-specific IFNgamma production, Int. Immunol. 18 (2006) 591–601.
[356] D.P. Hart, S.A. Xue, S. Thomas, M. Cesco-Gaspere, A. Tranter, B. Willcox, S.P. Lee, N. Steven, E.C. Morris, H.J. Stauss, Retroviral transfer of a dominant TCR prevents surface expression of a large proportion of the endogenous TCR repertoire in human T cells, Gene Ther. 15 (2008) 625–631.
[357] A.J. Gehring, S.A. Xue, Z.Z. Ho, D. Teoh, C. Ruedl, A. Chia, S. Koh, S.G. Lim, M.K. Maini, H. Stauss, A. Bertoletti, Engineering virus-specific T cells that target HBV infected hepatocytes and hepatocellular carcinoma cell lines, J. Hepatol. 55 (2011) 103–110.
[358] K.B. Scholten, M.W. Schreurs, J.J. Ruizendaal, E.W. Kueter, D. Kramer, S. Veenbergen, C.J. Meijer, E. Hooijberg, Preservation and redirection of HPV16E7-specific T cell receptors for immunotherapy of cervical cancer, Clin. Immunol. 114 (2005) 119–129.
[359] K.B. Scholten, A.W. Turksma, J.J. Ruizendaal, H.M. Van Den, S.H. van der Burg, M.H. Heemskerk, C.J. Meijer, E. Hooijberg, Generating HPV specific T helper cells for the treatment of HPV induced malignancies using TCR gene transfer, J. Transl. Med. 9 (2011) 147.
[360] P.R. Kumaresan, P.R. Manuri, N.D. Albert, S. Maiti, H. Singh, T. Mi, J. Roszik, B. Rabinovich, S. Olivares, J. Krishnamurthy, L. Zhang, A.M. Najjar, M.H. Huls, D.A. Lee, R.E. Champlin, D.P. Kontoyiannis, L.J. Cooper, Bioengineering T cells to target carbohydrate to treat opportunistic fungal infection, Proc. Natl. Acad. Sci. U. S. A 111 (2014) 10660–10665.
[361] C.J. Cohen, J.J. Gartner, M. Horovitz-Fried, K. Shamalov, K. Trebska-McGowan, V.V. Bliskovsky, M.R. Parkhurst, C. Ankri, T.D. Prickett, J.S. Crystal, Y.F. Li, M. El-Gamil, S.A. Rosenberg, P.F. Robbins, Isolation of neoantigen-specific T cells from tumor and peripheral lymphocytes, J. Clin. Invest. 125 (2015) 3981–3991.
[362] J.A. Fraietta, S.F. Lacey, E.J. Orlando, I. Pruteanu-Malinici, M. Gohil, S. Lundh, A.C. Boesteanu, Y. Wang, R.S. O'Connor, W.T. Hwang, E. Pequignot, D.E. Ambrose, C. Zhang, N. Wilcox, F. Bedoya, C. Dorfmeier, F. Chen, L. Tian, H. Parakandi, M. Gupta, R.M. Young, F.B. Johnson, I. Kulikovskaya, L. Liu, J. Xu, S.H. Kassim, M.M. Davis, B.L. Levine, N.V. Frey, D.L. Siegel, A.C. Huang, E.J. Wherry, H. Bitter, J.L. Brogdon, D.L. Porter, C.H. June, J.J. Melenhorst, Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia, Nat. Med. 24 (2018) 563–571.
[363] M. Ruella, J. Xu, D.M. Barrett, J.A. Fraietta, T.J. Reich, D.E. Ambrose, M. Klichinsky, O. Shestova, P.R. Patel, I. Kulikovskaya, F. Nazimuddin, V.G. Bhoj, E.J. Orlando, T.J. Fry, H. Bitter, S.L. Maude, B.L. Levine, C.L. Nobles, F.D. Bushman, R.M. Young, J. Scholler, S.I. Gill, C.H. June, S.A. Grupp, S.F. Lacey, J.J. Melenhorst, Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell, Nat. Med. 24 (2018) 1499–1503.
[364] M. Norelli, B. Camisa, G. Barbiera, L. Falcone, A. Purevdorj, M. Genua, F. Sanvito, M. Ponzoni, C. Doglioni, P. Cristofori, C. Traversari, C. Bordignon, F. Ciceri, R. Ostuni, C. Bonini, M. Casucci, A. Bondanza, Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells, Nat. Med. 24 (2018) 739–748.
[365] S.S. Yaghoubi, M.C. Jensen, N. Satyamurthy, S. Budhiraja, D. Paik, J. Czernin, S.S. Gambhir, Noninvasive detection of therapeutic cytolytic T cells with 18F-FHBG PET in a patient with glioma, Nat. Clin Pract. Oncol. 6 (2009) 53–58.
[366] A. Pfeiffer, F.B. Thalheimer, S. Hartmann, A.M. Frank, R.R. Bender, S. Danisch, C. Costa, W.S. Wels, U. Modlich, R. Stripecke, E. Verhoeyen, C.J. Buchholz, In vivo generation of human CD19-CAR T cells results in B-cell depletion and signs of cytokine release syndrome, EMBO Mol. Med. 10 (2018).
[367] T.T. Smith, S.B. Stephan, H.F. Moffett, L.E. McKnight, W. Ji, D. Reiman, E. Bonagofski, M.E. Wohlfahrt, S.P.S. Pillai, M.T. Stephan, In situ programming of leukaemia-specific T cells using synthetic DNA nanocarriers, Nat. Nanotechnol. 12 (2017) 813–820.