Advances and Challenges of CAR T Cells in Clinical Trials

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Abbreviations

CAR	Chimeric antigen receptor
CRS	Cytokine release syndrome
CTLA-4	Cytotoxic T lymphocyte-associated antigen-4
EGFR	Epithelial growth factor receptor
GMP	Good manufacturing practice
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
MHC	Major histocompatibility complex
PD-1	Programmed cell death-1
scFv	Single-chain fragment of variable region
TCR	T cell receptor

1 Introduction

The concept of adoptive cell therapy with specifically redirected T cells is based on the observation that the immune system can control malignant diseases in the long term. In particular, tumor-infiltrating lymphocytes isolated from melanoma lesions,

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extensively amplified ex vivo, and re-administered to the patient are capable to induce tumor regression and even long-term remission in a substantial number of patients (Dudley et al. 2002). However, the antigen specificity of such isolated and amplified T cells is assumed to be predominantly tumor-specific, although frequently not known. To provide defined specificity in targeting cancer cells, patient's T cells are engineered with a transgenic chimeric antigen receptor (CAR), as discussed herein, or with T cell receptor (TCR) chains. The CAR is a recombinant composite transmembrane molecule which consists in the extracellular moiety of an antigen-binding domain and in the intracellular moiety of signaling domains capable to initiate T cell activation upon antigen engagement. The redirected activation of T cells and their therapeutic efficacy against cancer depend on multiple parameters including the CAR design, the CAR signaling, the binding affinity, the number of antigens on target cells, the spatial accessibility of the targeted antigen epitope, the maturation stage of T cells, and preconditioning of the patient's immune system. In the following, we summarize the major aspects and discuss developments in addressing the challenges of adoptive CAR T cell therapy in the clinical context.

2 The Evolution of the Prototype Chimeric Antigen Receptor

Adoptive cell therapy of cancer aims at redirecting T cells specifically toward the tumor lesion. Due to the limited number of available TCRs with known specificity for tumors and the frequent loss of major histocompatibility complex (MHC) presented antigen by cancer cells, a strategy was needed to overcome the limitations and to adapt the concept to a variety of targets. In this situation, Zelig Eshhar and colleagues (Weizmann Institute of Science) demonstrated that a composite receptor molecule with an antibody-derived binding domain in the extracellular domain and a TCR-derived signaling domain in the intracellular domain is capable of both recognizing a specific antigen on target cells and activating engineered T cells upon antigen engagement (Gross et al. 1989). Such modularly composed chimeric antigen receptor (CAR), at first named "T-body" or "immunoreceptor," allows targeting of a broad variety of antigens and signaling through various domains and combinations thereof initiating defined T cell functions. The prototype CAR is composed of a single-chain fragment of variable region (scFv) antibody for binding in the extracellular domain, a spacer of various lengths bridging to the transmembrane domain, and a signaling moiety mostly derived from the TCR CD3ζ intracellular chain with or without linked costimulatory domain. The scFv is engineered by joining the heavy and light chain variable (V) regions of an antibody by a linker, which provides some flexibility, in the order V_H-linker-V_L or V_L-linker-V_H. The primary activating domain is mostly the CD3ζ intracellular chain or a downstream kinase of the TCR; the Fc ε receptor-I (FcεRI) signaling chain is also used. The "first-generation" CARs contain only the primary signal (signal-1), while

the "second-generation" CARs in addition contain a costimulatory domain (signal-2), like CD28, 4-1BB, OX40, ICOS, or CD27. The CD28 and 4-1BB domain are usually at the membrane proximal position followed by CD3ζ in the distal position; OX40 is also active in the membrane distal position. The first-generation CAR T cells have limited activation potential, while both signal-1 and signal-2 are required for inducing full T cell activation (Alvarez-Vallina and Hawkins 1996; Finney et al. 1998; Hombach et al. 2001); the second-generation CAR T cells show durable in cytokine release, amplification, and anti-tumor activity and are currently in clinical exploration. The "third-generation" CARs contain a combination of costimulatory domains along with the primary signal and provide benefit for T cells progressed in terminal maturation (Hombach et al. 2013).

The different costimulatory domains impact T cell activity and persistence in a different fashion. In particular, CD28 costimulation increases glucose uptake and ATP generation, while 4-1BB increases catabolism and mitochondrial respiratory chain capacities (Kawalekar et al. 2016). The differences in metabolic addiction are due to different signaling pathways initiated by CD28 and 4-1BB costimulation. CD28 activates the PI3K/Akt/mTOR signaling pathway which stimulates aerobic glycolysis (Frauwirth et al. 2002), and 4-1BB stimulates the Wnt/β-catenin pathway which is linked to oxidative phosphorylation and fatty acid oxidation (Kawalekar et al. 2016). Canonical Wnt/β-catenin favors the formation of central memory cells and long-term survival of T cells, while CD28-induced PI3K/Akt signaling sustains the immediate response effector cell phenotype (van der Windt and Pearce 2012; van der Windt et al. 2012; Pearce et al. 2009; Sukumar et al. 2013; Gattinoni et al. 2009). Accordingly, Akt inhibition during ex vivo priming and expansion triggers a central memory T cell phenotype with high levels of fatty acid oxidation and finally improved anti-tumor activities (van der Waart et al. 2014). After repetitive stimulation, CD28 CAR T cells are converted to $CD45RO^{+}$ $CCR7^{-}$ effector memory cells, while 4-1BB CAR T cells predominantly show a $CD45RO^{+}$ $CCR7^{+}$ central memory phenotype (Kawalekar et al. 2016) with extended persistence in the blood (Hombach and Abken 2007; Zhang et al. 2015; Wang et al. 2016).

The modular composition of the prototype CAR has advantages for the use in adoptive cell therapy of various diseases.

(a) As a consequence of targeting by an antibody, the target recognition is independent of MHC presentation of antigen which is frequently deficient in cancer cells. Any antigen can basically be targeted including non-classical T cell antigens like carbohydrates, lipids, or structural variants of an antigen as far as a binding molecule is available.
(b) The CAR-recognized antigen needs to be on the surface of the target cell; intracellular antigens are usually not visible to CAR T cells. However, the CAR T cell can gain TCR-like specificity by binding to MHC-presented peptide through an antibody and thereby sense intracellular antigens, e.g., NY-ESO-1 peptide presented by HLA-A2 (Stewart-Jones et al. 2009; Ma et al. 2016).
(c) The use of a scFv single-chain antibody with linked heavy and light chain variable regions allows the design of a one-polypeptide-chain CAR. Since a

number of scFvs loose specificity and affinity compared with the native antibody, an alternative CAR is composed of two chains, i.e., the Ig heavy chain with the variable and constant region is linked to the transmembrane and signaling CAR moieties, while the Ig light chain is co-expressed and spontaneously associates with the heavy chain forming a fully functional antibody for CAR targeting (Faitschuk et al. 2016a).

(d) Naturally occurring binding domains or ligands are alternatively used for CAR targeting, including mutated IL-13 for targeting IL-13 receptor-α2 which is overexpressed by a broad variety of solid tumors but less by healthy tissues (Kahlon et al. 2004; Kong et al. 2012; Krebs et al. 2014). Alternatively, recombinant binding domains can be integrated into the CAR-like designed ankyrin repeat proteins (DARPins), which are composed of 33 amino acids ankyrin repeats and form a β-turn followed by two antiparallel α-helices and a loop reaching the β-turn of the next repeat (Hammill et al. 2015). Adnectin, derived from fibronectin, was used for CAR targeting epithelial growth factor receptor (EGFR) with high selectivity for high versus low expressing cells (Han et al. 2017).

(e) The spacer in the extracellular CAR moiety between the scFv and the transmembrane domain requires empiric optimization with respect to antigen binding and T cell activation. Assumed the optimal CAR T cell activation requires a distance of about 15 nm to the target cell as does the TCR (Grakoui et al. 1999), a longer spacer is capable to target an epitope near the target cell membrane, while a smaller spacer is optimal for a more distal epitope. The distance of the binding domain to the membrane can substantially be varied by using spacer of various lengths, e.g., IgG1 CH1–CH2–CH3 or CH2–CH3 or CH3 (Srivastava and Riddell 2015).

(f) CARs comprising the CD3ζ transmembrane domain engage signaling components of the TCR/CD3 complex and further downstream kinases which makes CAR T cell activation highly efficient (Bridgeman et al. 2010). However, the CAR is also functional in TCR knockout cells (Torikai et al. 2012) and in non-T cells like NK cells indicating that the signaling domain alone is sufficient to associate with kinases and to initiate a productive signaling cascade.

3 The Growing Family of CARs

(a) *TRUCK: a CAR T cell releasing a transgenic product*

CAR T cells can be used as "living factories" to release a transgenic polypeptide product "on demand" upon CAR signaling. The so-called TRUCKs (Chmielewski et al. 2014), the "fourth-generation" of CAR cells, are CAR T cells engineered with a constitutive or inducible expression cassette aiming at delivering the transgenic protein in therapeutic concentrations in the targeted tissue, while the concentrations in the periphery remain low. The strategy is of particular interest to combine the

redirected CAR T cell attack with the action of a locally deposited, biologically active protein while avoiding systemic toxicity. Technically, the induced protein expression is under control of the $NFAT_6$-IL-2 minimal promoter which is activated upon CAR signaling. So far, the release of transgenic cytokines by CAR T cells was reported, for instance, IL-12 or IL-18 (Chmielewski and Abken 2015, 2017; Pegram et al. 2014; Chmielewski et al. 2011; Pegram et al. 2012; Hu et al. 2017; Kunert et al. 2017); other cytokines or proteins are also feasible. CAR IL-12 T cells (IL-12 TRUCKs) recruited and activated an innate immune response in the targeted tumors (Chmielewski et al. 2011), resisted suppression by Treg cells (Pegram et al. 2012), and showed an increased cytokine release and expansion (Koneru et al. 2015a). CAR T cells targeting Muc16 and secreting IL-12 are currently tested in a clinical trial (NCT02498912) (Koneru et al. 2015b); other transgenic cytokines are also evaluated. For instance, IL-15 improved T cell amplification and anti-tumor activity (Xu et al. 2016), however, is potentially leukemogenic (Hsu et al. 2007) which demands a suicide gene to eliminate the CAR T cells in the case of uncontrolled amplification (Hoyos et al. 2010). Other applications can likewise be envisaged like protecting the attacking T cells from oxidative stress through the release of catalase (Ligtenberg et al. 2016) or sustaining tumor penetration by delivering the soluble HVEM ectodomain which targets the tumor vasculature (Boice et al. 2016).

(b) *CAR T cells with multiple specificities*

CD19 CAR T cell treatment of B cell leukemia/lymphoma is associated with a substantial risk of relapse by tumor cells lacking the targeted CD19 epitope or the entire CD19 protein. The situation is addressed by targeting two antigens which basically can be achieved by a mixture of CAR T cells with different specificities, by T cells with two co-expressed CARs or T cells with one CAR with two specificities. The latter is a bispecific or tandem CAR ("TanCAR") with two scFvs linked by a short linker; binding to either antigen is sufficient to induce CAR T cell activation (Grada et al. 2013). A TanCAR with anti-CD19 and anti-CD20 scFv is aimed at targeting even those leukemic cells which lost CD19 upon a primary CAR T cell attack (Zah et al. 2016). Pediatric acute lymphocytic leukemia with known high heterogeneity in CD19 and CD20 expression can be controlled by bispecific CD20-CD19 CAR T cells, while monospecific CD20 CAR T cells failed in a transplanted mouse model (Martyniszyn et al. 2017). Dual targeting CD19 and CD123 is aiming at eliminating CD123-positive blasts in the treatment of B-ALL (Ruella et al. 2016a); other antigens are also co-targeted like CD22 (Haso et al. 2013), ROR1 (Hudecek et al. 2010), and immunoglobulin kappa light chain (Igκ) (Vera et al. 2006). TanCAR T cells have an additional advantage in that they exhibit improved avidity to target cells with both antigens which helps to stabilize the CAR synapse.

T cells can also be equipped with two specificities by co-expressing two CARs, each recognizing a different antigen and each capable to initiate full T cell activation. In contrast, co-expressed CARs which provide complementary signals, e.g., through CD3ζ and CD28, require simultaneous recognition of the cognate antigens

to initiate full T cell activation; engagement of only one antigen is insufficient. Such a combination of CARs integrates antigen recognition in a Boolean "AND" logic computation and aims at reducing off-tumor toxicities toward healthy tissues. Examples of combinatorial antigen recognition are CARs targeting ErbB2 by the CD3ζ CAR and Muc1 by the CD28 CAR (Wilkie et al. 2012), or CD3ζ CAR targeting mesothelin and CD28 CAR targeting folate receptor-α (Lanitis et al. 2013). In contrast, a bispecific CAR with both primary and costimulatory signaling initiates full T cell activating also upon engagement of one target antigen providing a Boolean "OR" computation of antigen recognition.

An alternative "AND" gate recognition is based on Notch which upon activation mediates the proteolysis of the internal domain and the release of a transcription regulator which finally controls the transcription of a CAR (synNotch CAR) for cancer cell recognition and T cell activation (Roybal et al. 2016a, b; Morsut et al. 2016).

(c) *CARs with exchangeable antigen recognition*

The prototype CAR has a defined specificity for the targeted antigen; targeting a new antigen requires engineering and expressing a new CAR with novel specificity. In order to make the strategy more flexible, a high-affinity CD16 variant CAR was used to capture a tumor-specific antibody through binding the Ig Fc region, while the variable region of the captured antibody recognizes the tumor-associated antigen (Kudo et al. 2014). CD16 CAR T cells in the presence of the Herceptin antibody can target $Her2^+$ cancer cells; the specificity can be changed by using different antibodies for targeting. T cells with such "universal" CARs can be equipped with various specificities by adding a labeled targeting antibody which is recognized by the CAR. Toxicity can be controlled by titrating the amount of targeting antibody. In alternative developments, the CAR has specificity for epitopes linked to the targeting antibody, like fluorescein isothiocyanate (FITC) (Tamada et al. 2012), avidin (Urbanska et al. 2012), or a protein epitope (Cartellieri et al. 2016; Kim et al. 2015). Adding antibodies of different specificities allows redirecting CAR T cells toward a plethora of antigens without the need of de novo CAR T cell engineering which becomes relevant when targeting tumor lesions with a heterogeneous pattern of antigens.

(d) *Conditional CARs*

In the case of CAR-related toxicity, a "switch-on/switch-off" mechanism will help to fine-tune the CAR T cell response. The aim is achieved by a titrated dimerization of two co-expressed CAR chains, one of which is the "first-generation" CAR and the second is a rudimentary chain with a costimulatory moiety and without extracellular domains. Both chains dimerize and co-signal upon adding a small dimerizer molecule ("switch-on"), while without dimerizer the CAR remains "switched-off" (Kim et al. 2015; Rodgers et al. 2016; Wu et al. 2015). Increasing concentrations of the dimerizer improves CAR signaling upon antigen engagement which allows a fine-tuned titration of T cell response.

(e) *Switch CARs: converting a suppressor into an activator*

Since many solid tumors express inhibitory ligands at high levels, an activating CAR targeting the inhibitory ligand will convert the inhibitory into an activating signal. A CAR recognizing PD-L1 through its extracellular PD-1 domain and providing CD28 costimulation converts the inhibitory into an activating signal (Kobold et al. 2015; Liu et al. 2016; Prosser et al. 2012). Such PD-1:CD28 switch CAR competes with available PD-L1 and overruns the inhibitory PD-1 signal through CD28 signaling. Other inhibitory ligands may likewise be targeted by a switch CAR.

(f) *CARs providing inhibitory signals: iCARs*

Most currently used CARs provide an activating signal; CARs with inhibitory signals are also useful in certain situations. Such an inhibitory CAR (iCAR) blocks T cell activation, for instance, when engaging antigens on healthy cells in order to suppress off-tumor toxicities (Fedorov et al. 2013).

(g) *Armored CAR T cells with cytokine receptors*

In order to increase T cell amplification in response to cytokines, CAR T cells were equipped with the transgenic IL-7 receptor-α chain to restore responsiveness to IL-7 and to promote a Th1 response without stimulating Treg cells (Vera et al. 2009; Perna et al. 2014). Similarly, in prostate cancer with increased IL-4 levels, co-expression of the IL-4 binding/IL-7 signaling receptor improved anti-tumor activity of T cells with anti-PSCA CAR (Mohammed et al. 2017). On the other hand, a dominant negative receptor on CAR T cells can compete with an inhibitory cytokine, for instance, co-expression of the dominant negative TGF-β DNRII improved T cell anti-tumor activity in the presence of TGF-β in a melanoma model (Zhang et al. 2013).

4 Exploring Allogeneic Effector Cells: "Universal" T Cells and NK Cells

"Universal" T cells

In most adoptive cell therapy trials, patients were treated with autologous CAR T cells. Such individualized treatment is labor- and cost-intensive and hampers in the current fashion the widespread delivery of CAR T cells. T cells without HLA barriers are potential "universal" T cells that can be manufactured in advance and applied "off-the-shelf" to a number of patients. In this line, cells were derived from a non-HLA matched donor, disrupted in the TCR α chain locus using transcription activator-like effector nucleases (TALENs), thereby producing TCR-negative T cells which were finally engineered with an anti-CD19 CAR for the treatment of

pediatric B cell acute lymphoblastic leukemia (B-ALL) (Poirot et al. 2015; Qasim et al. 2017). Subsequent depleting of remaining TCRαβ T cells reduces the risk of graft versus host disease (GvHD) through contaminating allogeneic TCR+ cells (Poirot et al. 2015; Bertaina et al. 2014). In a first clinical application, TALEN-edited CAR T cells were administered to a pediatric patient with B-ALL for whom autologous T cells could not be produced in sufficient numbers; no substantial GvHD was induced (Qasim et al. 2017). However, genetic editing by TALENs produces translocations also between other target sites, although at low frequencies (Qasim et al. 2017), which basically also applies to other gene-editing procedures like virus-transmitted zinc-finger nucleases (Provasi et al. 2012) or non-virally transmitted megaTALs (Osborn et al. 2016). While CRISPR guide RNA and Cas9 were encoded by the viral vector for constitutive expression (Shalem et al. 2014), current research is aiming at providing both the CAR expression cassette and the gene-editing tools with one transducing vector. In the further development of gene editing, the endogenous TCR and β2-microglobulin locus were targeted by CRISPR RNA electroporation in order to disrupt TCR and MHC class I by transiently available tools in CAR T cells in order to minimize off-target editing (Ren et al. 2017a, b).

NK cells

Human NK cells can also be used to initiate a potent anti-tumor response in model systems and to secrete a panel of cytokines, like GM-CSF, IFN-γ and IL-3, required for a productive anti-tumor response (Kruschinski et al. 2008; Klingemann 2014; Huenecke et al. 2010). While the prototype CAR for T cells is also active in NK cells, a CAR with the NK cell signaling proteins 2DS and DAP12 produced higher levels of NK cell activation and anti-tumor activity (Wang et al. 2015a). However, NK cells have a limited life span and rapidly disappear from circulation. Instead of primary NK cells, cells of the established NK92 line were engineered with an anti-Her2 CAR which showed potent anti-tumor activity upon local installation in a glioblastoma xenograft (Zhang et al. 2016) and an orthotopic breast cancer model (Schönfeld et al. 2015). The advantage is the "off-the-shelf" manufacturing of the cell product for immediate use; however, the CAR NK92 cells need to be irradiated prior to infusion which results in short-term NK cell survival and requires repetitive administration.

5 CAR T Cell Production: Challenges in Translating Individualized CAR T Cell Therapy to the Clinic

Adoptive therapy with CAR-modified T cells requires the manufacturing of cell products in accordance with the good manufacturing practice (GMP) rules; the procedure includes collecting the cells by leukapheresis in most cases, genetic engineering by viral gene transfer or electroporation, T cell amplification, and quality control of the final cell product. T cells are stimulated ex vivo by incubation

with beads coated with agonistic anti-CD3 and anti-CD28 antibodies. In the majority of trials, T cells are ex vivo modified by γ-retroviral or lentiviral gene transfer; some trials use RNA-modified T cells obtained by electroporation. Viral transduction is performed at moderate-to-low virus titers, aiming to obtain less than 5 integrates per cell. Transposon-based vectors like Sleeping Beauty and PiggyBac were recently applied for clinical applications as well (Singh et al. 2013, 2015; Manuri et al. 2010). With the currently used transfer systems and the use of mature T cells, the risk of insertional mutagenesis and subsequent oncogenic transformation seems to be low; no oncogenic event due to transformed T cells was reported so far. Unintended engineering of a single leukemic B cell with the anti-CD19 CAR during the manufacturing process resulted in relapse of leukemia and resistance to CD19 CAR therapy mainly due to masking of the CD19 epitope (Ruella et al. 2018).

Modified cells are furthermore amplified in the presence of cytokines to high cell numbers using shaking reactors or bags; gas-permeable rapid expansion cultureware is currently preferred. Stimulation in the presence of IL-2 triggers effector T cell differentiation (Pipkin et al. 2010), while T cells amplified in the presence of IL-7 or IL-15 display a central memory phenotype with robust cytokine release, clonotypic persistence, and clinical anti-tumor activity (Kaneko et al. 2009; Butler et al. 2007). IL-21 is alternatively used to amplify cells with a less differentiated phenotype (Li et al. 2005; Hinrichs et al. 2008). Used for ex vivo amplification of CAR T cells, γ-cytokines also impact the metabolism in a specific fashion. IL-15 improves the oxidative metabolism as well as carnitine palmitoyl transferase expression which is involved in the rate-limiting step in fatty acid oxidation (van der Windt et al. 2012). IL-7 increases Glut1 by STAT5 and Akt activation (Wofford et al. 2008) and induces glycerol transport and triglyceride synthesis (Cui et al. 2015), all improving T cell persistence and survival.

While most CAR T cell products are currently manufactured in a manual process, great efforts are made to translate the process into a fully automated and supervised system. The aim is to allow manufacturing with high reproducibility and quality and to produce cells from multiple patients in the same production facility in parallel. The latter is of practical relevance to deliver sufficient numbers of cell products when the CAR T cell strategy becomes standard of treatment for a number of cancer patients.

The maturation stage of amplified T cells substantially impacts the redirected anti-tumor activity and CAR T cell persistence; the most suitable T cell population for CAR therapy is thought to be a naïve or young central memory cell with an acute inflammatory signature. The rationale is based on the observation that non-responding patients in trials accumulated T cells with an early memory and exhaustion signature, while responder patients did not (O'Rourke et al. 2017). $CD45RO^+$ $CD62L^+$ memory CAR T cells provide a more durable anti-tumor response than effector T cells in more advanced stages of differentiation (Klebanoff et al. 2012; Gattinoni et al. 2011; Singh et al. 2016). Therefore, $CD62L^+$-enriched CAR T cells are currently explored in trials. However, it is still unresolved how to keep CAR T cells in the early stage of maturation, in particular after repetitive CAR activation.

6 The Second-Generation CAR T Cells Produced Lasting Remissions in Leukemia and Lymphoma

While adoptive therapy with the "first-generation" CAR T cells failed to show therapeutic efficacy, the "second-generation" CAR T cells achieved spectacular remissions in so far refractory leukemia and lymphoma, changing the overall therapeutic landscape in the long term. The standard treatment procedure is a sequence of events starting with leukapheresis of the patient for T cell donation, non-myeloablative lymphodepletion, and administration of the CAR T cells to the patient in one or more doses by i.v. infusion with or without IL-2 support. The vast majority of trials are designed for the treatment of hematologic malignancies (Holzinger et al. 2016); still a minority of trials is aiming at treating solid cancer (Abken 2017). Since CAR T cell persistence is crucial for clinical efficacy (Porter et al. 2015) and T cell persistence depends on appropriate costimulation, CARs with one or two costimulatory endodomains are used in trials, mostly providing CD28 or 4-1BB costimulation. CARs with alternative costimulatory domains are also clinically explored including CARs with OX40 (Hombach and Abken 2011), ICOS (Shen et al. 2013; Guedan et al. 2014), CD27 (Song et al. 2012), CD40-MyD88 (Foster et al. 2017), CD2 (Cheadle et al. 2012), and CD244 (Altvater et al. 2009).

One of the first successfully treated patients received anti-CD19 CAR T cells for the treatment of chronic lymphocytic leukemia (CLL) resulting in complete and maintained remission (Porter et al. 2011); other groups also successfully treated patients with CLL at the same time with CD19 CAR T cells (Porter et al. 2015; Grupp et al. 2013; Kochenderfer et al. 2013, 2015; Cruz et al. 2013; Brentjens et al. 2013; Maude et al. 2014a; Davila et al. 2014; Lee et al. 2015). CAR T cells with 4-1BB costimulation appear superior to CD28 CAR T cells (Porter et al. 2015) with prolonged persistence of 4-1BB CAR T cells for more than 4 years compared with 30 days of CD28 CAR T cells (Brentjens et al. 2011). All patients experienced lasting depletion of healthy B cells, at least as long as CD19 CAR T cells persisted. For the treatment of chronic lymphocytic leukemia (CLL), the Fcμ receptor is potentially a more tumor-selective target sparing healthy B cells from elimination by CAR T cells (Faitschuk et al. 2016b). Pediatric and adult patients with B cell acute lymphocytic leukemia (B-ALL) and follicular lymphoma were also successfully treated, even with higher frequencies of remissions. Remarkably, patients with multiple myeloma were also experienced remissions after CD19 CAR T cell therapy (Garfall et al. 2015) although multiple myeloma consists entirely of CD19-negative plasma cells. The observation led to the speculation that CD19 CAR T cells eliminated a $CD19^+$ cancer stem cell population responsible for tumor repopulation; alternatively, a suppressor B cell population may have been eliminated by CAR T cells. Apart from CD19, alternative antigens are also targeted, i.e., CD20, CD22, the Igκ light chain, ROR-1 for B-NHL and B-ALL, and CD30 for Hodgkin's lymphoma.

Currently, nearly 400 early-phase trials using the "second-generation" CAR T cells are in clinical exploration, mostly performed by academic centers or major pharmaceutical companies like Novartis, Juno Therapeutics, and Kite Pharma (now

Gilead). The anti-CD19 CAR for the treatment of pediatric B-ALL (Kymriah™, tisagenlecleucel, Novartis) and adult large B cell lymphoma (Yescarta™, axicabtagene ciloleucel, Gilead) have recently obtained FDA approval in 2017 and subsequently EMA approval in 2018. The CAR provides specificity by a murine anti-CD19 scFv and mediates T cell activation through CD28-CD3ζ signaling; fully humanized CARs are currently developed to avoid an anti-CAR immune response which potentially may deplete CAR T cells by the patient's immune system in the long term.

The success of CAR T cell therapy in various trials is difficult to compare due to a number of differences in the trial design, CAR composition, targeted antigen, preconditioning, and others. Apart thereof, CAR T cell dose and lymphodepletion were recently identified as key factors which impact CAR T cell amplification and persistence and finally therapeutic efficacy (Zhang et al. 2015). It is therefore reasonable that much effort is currently put into optimizing the "preconditioning" regimen in order to optimize the engraftment and initial amplification of CAR T cells. Only a small number of trials do not perform preconditioning.

During complete remission, most patients treated with 4-1BB CAR T cells did not receive further cancer-specific treatment; patients with CD28 CAR therapy frequently underwent allogeneic stem cell transplantation. Further exploration needs to identify a more successful strategy. However, the clinical observation that CD28 CAR T cells less persist than 4-1BB CAR T cells, i.e., few months compared with some years, underlines a potential benefit of transplantation after CD28 CAR T cell therapy.

Persistence of CAR T cells in the periphery is crucial for a lasting remission; repetitive re-stimulation of CAR T cells may improve persistence and finally anti-tumor activity. Therefore, virus-specific T cells, which are re-stimulated upon contact with viral antigens, are being used for a CAR-redirected anti-tumor response. In particular, T cells specific for Epstein–Barr virus (EBV) were engineered with a CAR with cancer specificity; EBV viral antigens are recognized by the endogenous TCR of the engineered T cells triggering their repetitive activation and amplification (Savoldo et al. 2007). EBV-specific CAR T cells persisted substantially longer after infusion to the patient than CAR T cells without virus specificity (Louis et al. 2011).

7 CAR T Cell Therapy of Solid Cancer Is Still Challenging

In the treatment of solid cancer lesions, some specific properties of T cells provide advantages over standard drug treatment regimens. Basically, CAR T cells have the capability to migrate through nearly all tissues, to amplify upon activation, and to execute their cytolytic and pro-inflammatory activity in a repetitive fashion. These properties make CAR T cells ideal for targeting widespread solid tumor lesions and metastases; however, the therapy of solid cancer is still challenging (Fig. 1, Table 1).

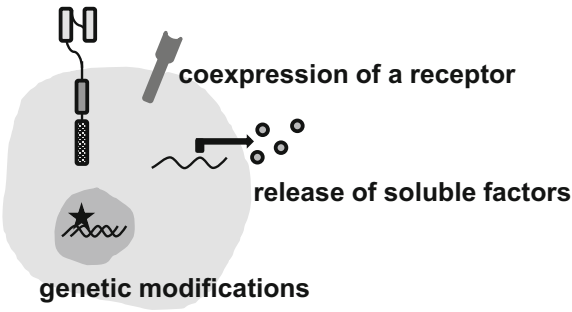

Fig. 1 Challenges and modifications of CAR T cells to overcome the barriers in solid tumors

Trafficking of T cells to specific targets depends on sensing chemokines; however, the process is impaired since most tumors exhibit an altered chemokine milieu (Franciszkiewicz et al. 2012) and some adhesion factors are lost on tumor endothelia (Bouzin et al. 2007), making T cell penetration and migration less efficient. Locally deposited TNF-α increased vascular adhesion molecules, such as vascular cell adhesion protein-1 and intracellular adhesion molecule-2 on endothelial cells, resulting in enhanced T cell extravasation and tumor accumulation (Calcinotto et al. 2012). Endothelial cell adhesion and/or transmigration of T cells is improved by targeting vascular endothelial growth factor (VEGF) receptor-2 (Chinnasamy et al. 2010) or blocking migration inhibitory factors like the endothelin B receptor (Kandalaft et al. 2009). T cells can also accumulate in privileged tissues like testes and eyes (Brudno and Kochenderfer 2016), penetrate the blood–brain barrier, and infiltrate the brain (Pule et al. 2008), which is thought to be the cause of neurotoxicity (Mackall and Miklos 2017). On the other hand, several chemokine receptors are downregulated on the T cell surface upon extensive ex vivo propagation, making amplified T cell products less sensitive to chemokine-driven trafficking. Transgenic re-expression of chemokine receptors, like CXCR2 (CXCL1 receptor) for targeting melanoma (Kershaw et al. 2002) or CCR2b for targeting neuroblastoma (Craddock et al. 2010), is aiming at improving specific trafficking of CAR T cells toward the tumor lesion.

Infiltration into the tumor tissue is a major hurdle for CAR-modified T cells (Joyce and Fearon 2015). Local T cell installation circumvents this limitation and may improve therapeutic efficacy (Adusumilli et al. 2014). For instance, CAR T cells were applied intrapleurally and intraperitoneally for the treatment of mesothelioma and ovarian cancer, respectively (Koneru et al. 2015b). Anti-CEA CAR T cells were applied by endoscopy into hepatic metastases (Katz et al. 2015); anti-c-Met CAR T cells were applied by intratumoral injections into breast cancer metastases inducing necrosis of injected tumor lesions (Tchou et al. 2017) (NCT01837602). On the other hand, T cell penetration can be improved by transgenic expression of heparanase which degrades heparan sulfate proteoglycans in the stroma; moreover, endogenous heparanase expression is frequently downregulated during the manufacturing process (Caruana et al. 2015).

Table 1 Challenges and modifications of CAR T cells to overcome the barriers in solid tumors

Challenge	Barriers in the tumor environment	CAR T cell engineering	References
Co-expression of a receptor			
T cell activation	Lack of costimulation, e.g., 4-1BB-L, OX40-L, CD80/86	Expression of costimulatory receptors, e.g., CD28, 4-1BB, OX40	
		Expression of stimulatory ligands, e.g., CD40L	Curran et al. (2015)
T cell homing	Reduced release of chemokines by tumors	Expression of chemokine receptors, e.g., CCR4, CXCR2	Craddock et al. (2010), Di Stasi et al. (2009), Peng et al. (2010)
T cell suppression	Treg cells	Modified CD28 signaling deficient in IL-2 induction	Kofler et al. (2011)
Nutrient resources	Low glucose, glutamine, arginine, tryptophan	Expression of transporters, e.g., for glucose or amino acids	Cretenet et al. (2016)
Inhibitory pathways	Inhibitory ligands displayed by cancer cells, e.g., PD-L1/2	Expression of a switch receptor, e.g., PD-1:CD28, expression of dnPD-1	Kobold et al. (2015), Liu et al. (2016), Prosser et al. (2012)
	Inhibitory cytokines, e.g., TGF-β	Expression of dnTGF-β receptor; expression of synthetic receptor	Zhang et al. (2013); Golumba-Nagy et al. (2018)
Extravasation	Tumor-associated vasculature	Expression of anti-VEGF-R2 CAR	Chinnasamy et al. (2010)
Penetration	Stromal tissue	Expression of anti-FAP CAR	Kakarla et al. (2013)
Release of soluble factors			
Extravasation	Tumor-associated vasculature	Secretion of endothelin B receptor blocking Ab	Kandalaft et al. (2009)
Penetration	Stromal tissue	Release of degrading enzymes, e.g., heparanase	Caruana et al. (2015)
Immune suppression	Inhibitory ligands on cancer cells, e.g., PD-L1/L2, VISTA	Checkpoint blockade, e.g., expression of anti-PD-1 Ab, anti-PD-L1 Ab, anti-CTLA-4 Ab	
	Suppressor cells, e.g., Tregs, MDSCs	Release of IL-18	Chmielewski and Abken (2017)
	Deficient innate immune response, M2 macrophages	Release of IL-12	Chmielewski et al. (2011)

(continued)

Table 1 (continued)

Challenge	Barriers in the tumor environment	CAR T cell engineering	References
Metabolic situation	Metabolic exhaustion	Overexpression of intracellular proteins, e.g., transcription co-activator PGC1-α	
	Oxidative stress	Overexpression of catalase	Ligtenberg et al. (2016)
Genetic modifications			
Immune suppression	Inhibitory ligands, e.g., PD-L1	PD-1 suppression by siRNA, shRNA, Gene knockout by CRISPR/Cas9, e.g., PD-1, Cbl-b	Ren et al. (2017a)
	Low γ-cytokine levels	Enhanced sensitivity to γ-cytokines by miR-155 overexpression	Ji et al. (2015)

CAR T cells are facing a hostile environment after successful penetration into the tumor tissue. CAR T cells need to break the stroma and extracellular matrix barrier to get in near vicinity to the cancer cells; IFN-γ is required to eliminate the stromal cells (Textor et al. 2014). As a consequence, targeting tumor stroma by CAR T cells in addition to targeting the cancer cells likely improves the overall efficacy in eliminating solid tumor lesions. Fibroblast activation protein (FAP), a serine protease involved in extracellular matrix remodeling and expressed by stromal cells of a majority of epithelial cancers, is a candidate protein for targeting the stroma. Consequently, targeting FAP in addition to cancer cell targeting improved the overall anti-tumor activity (Kakarla et al. 2013).

Myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages deprive CAR T cells in the tumor tissue of essential amino acids through decreasing tryptophan levels (Ninomiya et al. 2015). Regulatory T (Treg) cells, MDSCs, and tumor-associated M2 macrophages release suppressive cytokines, like IL-4, IL-10, leukemia inhibitory factor, and TGF-β; MDSCs and Tregs can be suppressed by sunitinib, a multi-kinase inhibitor, which may be used in combination with CAR T cell treatment. The stromal cells are releasing IDO and deprive the tissue of glucose and other nutrients; profound acidosis moreover counteracts the anti-tumor activity of CAR T cells. IDO inhibits CAR T cells through accumulating kynurenine which blocks expansion, cytotoxicity, and cytokine secretion by CAR T cells (Ninomiya et al. 2015). On the other hand, fludarabine and cyclophosphamide, used for pre-conditioning in patients, decrease IDO levels through depletion from Treg cells. Low levels of arginine in the tumor tissue result in CD3ζ repression and inhibition of T cell amplification and cytokine release (Rodriguez et al. 2007). MDSCs moreover suppress T cell function in a direct fashion through arginase-mediated TCR CD3ζ chain repression (Rodriguez et al. 2002). Protein kinase A (PKA) is the effector molecule in the downstream cascade of prostaglandin E2 and adenosine, both produced in the tumor tissue and both inhibiting T cell function. Consequently, disruption of the PKA membrane anchoring increases CAR T cell infiltration, chemotaxis, persistence, and anti-tumor activity (Newick et al. 2016).

Inhibitory ligands suppress CAR T cell activity by binding to programmed cell death-1 (PD-1), cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), or Fas, among others. Much effort is currently undertaken to make CAR T cells resistant to this type of suppression, e.g., by suppressing PD-1 expression (Cherkassky et al. 2016) or a PD-1 switch receptor which binds to PD-L1 and conveys the suppressing into an activating signal (Liu et al. 2016; Prosser et al. 2012). Alternatively, checkpoint inhibitors to block the PD-1/PD-L1, e.g., nivolumab, or CTLA-4 axis, e.g., ipilimumab, are currently explored as adjuvant in CAR T cell trials. Along this line, CAR T cell therapy combined with PD-1 blockade increased the anti-tumor efficacy (John et al. 2013). In a case report, a patient showed tumor reduction and increase in circulating CAR T cells upon PD-1 blockade by pembrolizumab (Chong et al. 2017); a trial is exploring PD-1 blockade in CD19 CAR T cell-resistant or relapsing leukemia patients (NCT02650999). A PD-L1 mini-body improved the anti-tumor activity of CAR T cells in a preclinical model (Tanoue et al. 2017).

Taken together, blocking inhibitory checkpoints can enhance the efficacy of CAR T cell therapy against tumors.

On the other hand, CAR T cell therapy is combined with agonistic activation of 4-1BB (Mardiana et al. 2017) or vaccination with viral antigen recognized by anti-tumor CAR and antivirus TCR-engineered T cells (Slaney et al. 2017). Other strategies including the use of EBV-specific T cells are in line with specifically re-stimulating CAR T cells by non-tumor antigens.

8 CAR T Cell Therapy-Associated Toxicities

CAR T cell therapy so far showed efficacy in the treatment of B cell leukemia, however, provokes side effects which need clinical intervention (Table 2). An updated review on grading and management of CRS was recently published by Riegler et al. (2019).

Table 2 Clinical management of toxicities associated with CAR T cell therapy

Toxicity	Potential prevention or treatment
Cytokine release syndrome (CRS)	Blocking the IL-6R/IL-6 axis by tocilizumab or siltuximab or sarilumab Depleting from CAR T cells Reducing or fractionating CAR T cell dose
Vascular leakage syndrome (VLS)	Plasma expansion Plasmapheresis to deplete serum factors
Tumor lysis syndrome (TLS)	Plasmapheresis Reducting tumor mass prior cell therapy Reducing or fractionating CAR T cell dose
Macrophage activation syndrome (MAS)	Blocking the IL-6R/IL-6 axis by tocilizumab or siltuximab
Neurotoxicity	Corticosteroids
“On-target off-tumor” toxicities	Targeting of tumor-selective antigens, e.g., neo-antigens Blocking the target antigen on healthy cells Co-expression of iCARs to protect healthy cells Combinatorial antigen recognition Transient CAR expression after RNA transfer Conditional CAR activation by a dimerizer Local CAR T cell application CAR T cell elimination by suicide gene activation, e.g., iCasp9, or by depleting antibodies
GvHD after allogeneic T cell therapy	TCR-negative CAR T cells
Tumor relapse by antigen escape of cancer cells	Targeting of co-expressed antigens
Poor in vivo expansion	Intensifying lymphodepletion Increasing cytokine substitution
B cell aplasia after CD19 CAR T cell therapy	Replacement of immunoglobulins Antibiotic and antifungal prophylaxis

(a) Most CAR-targeted antigens are not exclusively expressed by cancer cells but also by healthy cells. The lack of tumor selectivity becomes obvious, for instance, when targeting CD19 to treat B cell leukemia; also, healthy B cells are eliminated resulting in a lasting B cell depletion which requires immunoglobulin substitution and antibiotic and antifungal protection. Such "on-target off-tumor" toxicity in the treatment of leukemia is clinically manageable and, however, is more severe when the targeted antigen is expressed by vital tissues. For instance, targeting ErB2 by the third-generation CAR T cells resulted in a fatal cardiopulmonary failure likely due to the attack against healthy lung tissues (Morgan et al. 2010). The toxicity depends also on the particular binding domain and on CAR signaling since CAR T cells with another anti-Her2 binding domain and with one costimulatory domain produced no dose-limiting toxicity (Ahmed et al. 2015; Feng et al. 2017).

(b) Rapid destruction of a large tumor mass may induce a tumor lysis syndrome which is initiated by the release of tumor cell components and accompanied by electrolyte and metabolic disturbances with the risk of multi-organ failure.

(c) The CAR itself can induce "off-target off-tumor" toxicity through the IgG1 Fc spacer which binds to the Fc γ receptor (FcγR) (CD64) and can thereby activate innate cells like NK cells and macrophages. Deleting the IgG1 CH2 domain or mutating the Asn297 side (Hudecek et al. 2015; Hombach et al. 2010) reduces the risk; IgG4 or extracellular CD8 is used as an alternative spacer.

(d) The cytokine release syndrome (CRS) is an acute immune activation resulting in elevated serum levels of pro-inflammatory cytokines including IFN-γ and TNF-α, IL-10 and in particular IL-6 (Maude et al. 2014a, b; Davila et al. 2014; Lee et al. 2015). CRS is clinically characterized by high fever, malaise, fatigue, myalgia, nausea, anorexia, tachycardia, hypotension, capillary leak, cardiac dysfunction, renal impairment, hepatic failure, and disseminated intravascular coagulation (Lee et al. 2014). The severity of CRS may, but must not, correlate with tumor burden (Maude et al. 2014a; Teachey et al. 2016), often occurs together with the vascular leakage syndrome (VLS), and is closely associated with the systemic macrophage activation syndrome, clinically resembling hemophagocytic lymphohistiocytosis, which makes clinical diagnosis and management difficult. A score to identify CRS/VLS in early stages and clinical guidelines in management were recently proposed (Davila et al. 2014; Maude et al. 2014b; Teachey et al. 2016). Three markers were identified to predict CRS, i.e., in adults, soluble gp130 (sgp130), IFN-γ, and IL1Rα and in pediatric patients, IFN-γ, IL-13, and MIP1α. C-reactive protein, which is released by hepatocytes in response to IL-6, currently serves as a laboratory marker of CRS onset and severity (Davila et al. 2014).

Systemic corticosteroid treatment rapidly reversed CRS without compromising the initial anti-tumor response as long as steroids are applied short term, i.e., below 14 days (Davila et al. 2014; Lee et al. 2015). Current CRS therapy is based on blocking the IL-6/IL-6 receptor signaling axis by tocilizumab application which neutralizes the IL-6 receptor and does not interfere with CAR T cell efficacy (Grupp et al. 2013; Teachey et al. 2016; Chen et al. 2016);

the IL-6 blocking antibody siltuximab has also been used. The long-term impact of blocking IL-6 on the anti-tumor efficacy needs to be explored in detail.

(e) Neurotoxicity with aphasia, hallucinations, confusion, delirium, expressive aphasia, obtundation, myoclonus, and delirium occurs in about 40% of patients during CAR T cell therapy, is reversible, and is often observed after CD19 CAR T cell therapy (Maude et al. 2014a; Davila et al. 2014; Lee et al. 2015; Teachey et al. 2016). The mechanism is less understood; a diffuse encephalopathy caused by infiltrating CAR T cells is thought to be the cause.

(f) Anaphylaxis with elevated IgE levels was reported for one patient after repeated doses of CAR T cells; the patient developed antibodies against murine domains of the CAR (Maus et al. 2013).

9 Strategies to Improve Safety of CAR T Cell Therapy

(a) *CAR T cells recognizing more than one antigen*

The strategy is based on the rationale that a pattern of antigens is more indicative for cancer cells versus healthy cells than one antigen only; this is particularly the case since a truly cancer-specific antigen is rare. To drive T cell activation upon recognizing two antigens, two CARs are co-expressed, one CAR providing the primary activating signal and the other CAR, the costimulatory signal, thereby complementing the signals for full T cell activation only in the presence of both antigens (Wilkie et al. 2012; Lanitis et al. 2013; Kloss et al. 2012).

(b) *Inhibitory CARs*

The inhibitory CAR (iCAR) is co-expressed by T cells together with an activating CAR and aimed at providing an inhibitory signal when engaging an antigen on healthy cells which is absent on cancer cells. The iCAR signaling domain is derived from PD-1 or CTLA-4 which is dominant over the activating signals through CD3ζ and costimulation (Fedorov et al. 2013). The T cell is blocked by the inhibitory signal as long as the iCAR engages healthy cells; without iCAR signaling, the T cell can be activated through the co-expressed tumor-specific CAR.

(c) *Transient CAR expression*

In the case of potential toxicity, transient CAR expression by the T cell may limit the side effects. The CAR is transiently expressed upon RNA transfer due to the short RNA half-life and RNA dilution upon T cell division which is even more rapid after T cell activation. However, the CAR is present on the T cell surface in the order of several days and mediates efficient T cell activation upon target cell engagement (Birkholz et al. 2009). Such RNA-modified T cells were applied in trials with some, although transient efficacy (Maus et al. 2013; Beatty et al. 2014).

(d) *CAR T cell elimination*

In the case of uncontrolled toxicity, CAR T cells need to be rapidly and efficiently eliminated. High-dose steroid treatment was applied to stop autoimmunity after treatment with carboanhydrase IX-specific CAR T cells (Lamers et al. 2006). More selective elimination of CAR T cells is achieved by antibody targeting a specific domain in the extracellular CAR moiety (Philip et al. 2014) or by targeting a co-expressed marker, for instance, the truncated EGFR which can be targeted by cetuximab (Wang et al. 2011). The CAR binding domain can also be targeted by an anti-idiotypic antibody (Jena et al. 2013). Alternatively, a suicide gene is co-expressed with the CAR, for instance, the truncated caspase-9 and a mutated FK506 binding protein; the apoptotic cascade is initiated upon applying a synthetic drug for dimerizing caspase-9 (Straathof et al. 2005).

(e) Routes of T cell administration

Usually, CAR T cells are applied by i.v. injection to approach the target side through blood circulation. Local administration by endoscopy or by intrapleural or intraperitoneal application may avoid off-tumor T cell activation to some extent while providing high CAR T cell doses at the tumor side (Parente-Pereira et al. 2011; Katz et al. 2016). However, in most tumor patients, puncture of tumor lesions is technically not feasible and is not applicable in a disseminated tumor disease.

10 Future Developments in CAR T Cell Therapy: Challenges Remain

Current clinical trials in phase I and II are promising to establish CAR T cell therapy in the front-line treatment of leukemia and lymphoma within the next years. However, major hurdles remain, in particular in the CAR T cell therapy of solid cancer.

(a) *Which antigen serves best in targeting solid tumors while avoiding off-tumor toxicities?*

Extensive research is aiming at identifying new and more selective antigens suitable for safe targeting tumor lesions while sparing healthy tissues. Truly tumor-selective antigens are rare; however, more selective antigens are tumor-specific mutations of surface proteins or glycosylation variants like Muc1 or Muc16 which can be targeted by CAR T cells (Posey et al. 2016). Apart from tumor-specific antigens, CAR T cell treatment of solid tumors proved safe by targeting carcinoembryonic antigen (CEA) as an auto-antigen which is strictly luminal expressed by healthy epithelial cells while depolarized on cancer cells. Two trials provided some clinical efficacy in the treatment of gastrointestinal adenocarcinoma by systemic application of CEA-specific CAR T cells (NCT01212887, NCT02349724) (Thistlethwaite et al.

2017; Zhang et al. 2017); local administration of anti-CEA CAR T cells by hepatic artery infusion also declined tumor progression (NCT01373047) without the induction of treatment-related colitis (Katz et al. 2015).

(b) *How to prevent tumor relapse after CAR T cell therapy?*

CD19 CAR T cell therapy induces complete remissions in pediatric B-ALL patients with high frequencies; however, leukemia relapses in about 40% of patients despite persisting CAR T cells (Grupp et al. 2013; Maude et al. 2014a; Lee et al. 2015). A frequent cause of relapse is the expression of a functionally active CD19 isoform which is not recognized by the CAR due to the lack of exon-2 (Sotillo et al. 2015). Targeting a CD19 epitope which is not lost by splicing or co-targeting a second antigen, e.g., CD20 by a bispecific CAR, likely increases the therapeutic pressure on leukemic cells. Switching to a CD19-negative myeloid lineage was observed in the relapse of two cases of B-ALL after CD19 CAR treatment (Gardner et al. 2016), again pointing to the need to target leukemic cells by two independent antigens. Profound heterogeneity in the expression of the targeted antigen may also be the cause of early tumor relapse after initial tumor regression. A CAR T cell-initiated antigen-independent anti-tumor response through innate immune cells in the tumor lesion may improve the overall therapeutic efficacy. Designed for these purposes, IL-12 or IL-18 TRUCK cells, i.e., CAR T cells with the inducible release of transgenic cytokines, are capable to induce an innate response against antigen-negative cancer cells in an experimental model (Chmielewski et al. 2011; Chmielewski and Abken 2017).

(c) *What is the optimal CAR design?*

Research during the last two decades established the prototype design of a CAR; however, each CAR needs to be optimized with respect to the potential target antigen and the T cell subset. In particular, the binding affinity, the targeted antigen epitope, the extracellular spacer length, the transmembrane domain, and finally the primary and costimulatory signaling domains need to be individually evaluated with respect to the specific tumor situation. Early preclinical research established that CAR T cell activation depends on the affinity of antigen binding and the epitope of the targeted antigen (Chmielewski et al. 2004; Hombach et al. 2007). Recently confirmed by others (Liu et al. 2015; Caruso et al. 2015), there is an affinity window in which CAR T cells target tumor cells with high antigen load while sparing healthy cells with low antigen levels. Consequently, a trial targeting Her2 caused no toxicity (Ahmed et al. 2015), while a high-affinity CAR targeting a different epitope caused fatal adverse events (Morgan et al. 2010).

(d) *Which T cell subset performs best in the long term against solid tumors?*

The most suitable stage in T cell maturation for adoptive cell therapy seems to be a naïve or early central memory cell with an enhanced capacity for amplification and

long-lived persistence. In some trials, T cells with a $CD62L^+$ phenotype are selected prior engineering with a CAR (Sabatino et al. 2016). Reducing T cell amplification during manufacturing improves the anti- tumor activity of CAR T cells (Ghassemi et al. 2018). On the other hand, the T cell maturation can be directed by costimulation and/or cytokine signals; 4-1BB costimulation initiates a central memory T cell response in young T cells, while CD28 mediates a more short-lived effector cell response (Kawalekar et al. 2016). In more matured stages of T cell development, other costimuli or combinations thereof are needed; for instance, $CCR7^-$ T cells require combined CD28-OX40 costimulation for lasting persistence, while young T cells respond upon CD28 costimulation (Hombach et al. 2013). The T cell phenotype and functional capacities can also be modulated by co-treatment with kinase inhibitors. Ibrutinib, a Bruton tyrosine kinase (BTK) inhibitor used for CLL treatment, reduces PD-1 and exhaustion of CAR T cells and thereby increases persistence and anti-tumor activity in the long term (Ruella et al. 2016b).

(e) *How can a high-quality T cell product be manufactured for an increasing number of patients in a standardized process?*

Currently, most CAR T cells are produced by a manual process in specialized GMP units, frozen, and shipped to the patient's hospital (Köhl et al. 2018). Manufacturing a growing number of cell products in this fashion will come to its limits, in particular, when thousands of patients require their own cell products in due time. A decentralized, in-hospital manufacturing by an automated, fully controlled and entirely closed system needs to be established. This will also require a high degree of standardization in the manufacturing process, will be less cost-intensive, and would avoid sophisticated logistics in transportation of blood and cell products.

(f) *Will "universal" CAR T cells outsmart patient's "individualized" CAR T cells?*

So far, patient's T cells are genetically engineered with the CAR for the individual patient and the individual tumor. A number of efforts are aiming at generating "universal" T cells which can be applied to a number of patients independently of their MHC which requires making the CAR T cell invisible to the patient's immune system. Moreover, the allogeneic CAR T cell needs to be deficient in alloreactivity against the patient's healthy tissues which is achieved by targeted disruption of the TCR α-chain locus (Qasim et al. 2017). In this line, CAR-modified virus-specific T cells and T cells with silenced endogenous TCR are explored toward a "universal" cell product (Poirot et al. 2015; Cruz et al. 2013; Wang et al. 2015b). While additional manipulations need to be performed to avoid immune destruction of such "universal" CAR T cells, a cell product "off-the-shelf" or a "third-party" cell bank would provide much more flexibility in the clinical application and would help to establish adoptive cell therapy for a higher number of patients.

(g) *Will major CAR T cell therapy-associated adverse events be controlled?*

CAR T cell treatment can cause severe side effects which need intensive care hospitalization; the cytokine release syndrome (CRS) is a frequently occurring; first,

steps to standardize grading and treatment regimens are made (Davila et al. 2014; Maude et al. 2014b; Riegler et al. 2019; Teachey et al. 2016). However, as long as the CAR T cell protocols and treatment procedures differ and the various parameters were not clinically evaluated in a comparative clinical setting, more general conclusions cannot be drawn from individual trials and further optimization in mono- and combo-immune therapies is difficult to perform in a timely fashion. In the case of uncontrolled toxicity, CAR T cells need to be selectively and rapidly eliminated; co-expressed suicide genes or domains targeted by depleting antibodies may be mandatory. An example is the induced apoptosis by dimerization of the inducible caspase-9 (iCasp9) upon addition of the dimerizing agent AP1903 resulting in the elimination of >90% of T cells within 30 min (Thomis et al. 2001; Tey et al. 2007; Di Stasi et al. 2011; Zhou et al. 2014). However, spontaneous dimerization occurs in a substantial basal frequency producing a constant level of apoptotic cells. Alternatively, CAR T cells can be cleared by antibody-dependent cellular cytotoxicity (ADCC) using antibodies targeting a CAR domain, for instance, rituximab for a co-expressed CD20 epitope or cetuximab for EGFR targeting (Philip et al. 2014; Wang et al. 2011; Serafini et al. 2004). The caveat is that cancer patients with a dysfunctional immune system may have limited capacities to remove the CAR cells by ADCC, especially in the case of toxicity.

(h) *Will there be a specific preconditioning for each type of cancer?*

In order to sustain CAR T cell engraftment and amplification, patients are subjected to a non-myeloablative lymphodepletion prior to adoptive T cell transfer and IL-2 substitution in the following weeks. The pretreatment with fludarabine and cyclophosphamide also impacts the tumor tissue by depleting suppressor cells and mild cell destruction releasing tumor-associated antigens to the immune system. Although basically effective, the currently used preconditioning regimen still needs further optimization. A cancer-specific protocol may be required to meet the particular situation of solid or disseminated tumors. For instance, the non-myeloablative conditioning regimen used in the treatment of $Her2^+$ tumors (Morgan et al. 2010) was modified to nab-paclitaxel and cyclophosphamide pretreatment of biliary tract and pancreatic cancers in order to deplete from desmoplastic stroma and to increase T cell infiltration (Von Hoff et al. 2011). Depleting tumor stroma by nab-paclitaxel may promote HER2 antigen presentation; cyclophosphamide can deplete inhibitory cells like Tregs and MDSCs among others. These and other preconditioning regimens may create a more appropriate environment for CAR T cell activities. On the other hand, preconditioning can be highly toxic in the context of CAR T cell therapy. Cerebral edema and CAR T cells in cerebral spinal fluid are commonly observed in CD19 CAR T cell trials (Maude et al. 2014a; Davila et al. 2014; Hu et al. 2016). Following intensified lymphodepletion with fludarabine, neurologic toxicities caused fatal complications in a recent trial, reducing lymphodepletion still induced uncontrolled toxicities and deaths (NCT02535364). Further research is needed to elucidate the mechanism of toxicity and to establish more effective pretreatment regimens.

(i) *Can the immune network be manipulated in order to induce a broad inflammatory response?*

The host immune system is substantially involved in tumor rejection initiated by CAR T cell transfer. Evidences raised in experimental tumor models in which anti-EGFRvIII CAR T cells conferred resistance to EGFRvIII-negative tumors (Sampson et al. 2014). A secondary innate cell response can be induced by treatment with IL-12-releasing CAR T cells (IL-12 TRUCKs) which attract and activate M1 macrophages in the tumor tissue to eliminate those cancer cells which are invisible to CAR T cells (Chmielewski et al. 2011). IL-18 CAR T cells shape the immune cell environment of targeted tumors in a specific fashion by increasing the numbers of tumor-associated $CD206^{-}$ M1 macrophages and $NKG2D^{+}$ NK cells and reducing Treg cells, suppressive $CD103^{+}$ dendritic cells, and M2 macrophages (Chmielewski and Abken 2017). Other immune response modifiers deposited in the tumor tissue by CAR T cells will be explored in the near future in order to shape a broader anti-tumor immune response. Checkpoint blockade is the first step in this direction; targeting PD-1 in the context of CAR T cell therapy is currently explored in a trial (NCT02650999); other checkpoints or combinations thereof need likewise clinical exploration, in particular, since checkpoints are part of a regulatory network and specific checkpoints like TIM-3 are upregulated upon PD-1 blockade (Koyama et al. 2016).

11 CAR T Cell Therapy Beyond Cancer

Redirected T cell activation by a CAR is not limited to targets on cancer cells; moreover, it can be used to target other diseased tissues including infected cells. CARs were engineered to target viral antigens on the surface of cells infected by hepatitis B virus (Krebs et al. 2013), hepatitis C virus (Sautto et al. 2016), cytomegalovirus (Full et al. 2010), and HIV (Romeo and Seed 1991; Deeks et al. 2002). Carbohydrate epitopes on aspergillus can be targeted by using dectin-1, a pattern-recognition receptor from the innate immune system, as binder to disrupt germination of the fungus (Kumaresan et al. 2014). B cells can also be targeted by CAR T cells which are used to eliminate memory B cells expressing an anti-Dsg3 antibody, responsible for the pathology of pemphigus vulgaris (Ellebrecht et al. 2016). Auto-reactive T cells were targeted by CAR T cells recognizing MHC-presented auto-antigen (Jyothi et al. 2002; Margalit et al. 2003). Of broader clinical interest is the development of CAR Treg cells for use in the long-term control of autoimmune diseases like colitis (Elinav et al. 2008), allergic asthma (Skuljec et al. 2017), and graft versus host disease by targeting HLA (MacDonald et al. 2016; Boardman et al. 2017; Noyan et al. 2017). The experimental data sustain the concept that CAR Tregs can be used to promote immune tolerance in the therapy of autoimmune diseases.

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References

Abken H (2017) Driving CARs on the highway to solid cancer: some considerations on the adoptive therapy with CAR T cells. Hum Gene Ther 28(11):1047–1060

Adusumilli PS, Cherkassky L, Villena-Vargas J, Colovos C, Servais E, Plotkin J et al (2014) Regional delivery of mesothelin-targeted CAR T cell therapy generates potent and long-lasting CD4-dependent tumor immunity. Sci Transl Med 6(261):261ra151

Ahmed N, Brawley VS, Hegde M, Robertson C, Ghazi A, Gerken C et al (2015) Human epidermal growth factor receptor 2 (HER2)—specific chimeric antigen receptor-modified T cells for the immunotherapy of HER2-positive sarcoma. J Clin Oncol Off J Am Soc Clin Oncol 33 (15):1688–1696

Altvater B, Landmeier S, Pscherer S, Temme J, Juergens H, Pule M et al (2009) 2B4 (CD244) signaling via chimeric receptors costimulates tumor-antigen specific proliferation and in vitro expansion of human T cells. Cancer Immunol Immunother CII 58(12):1991–2001

Alvarez-Vallina L, Hawkins RE (1996) Antigen-specific targeting of CD28-mediated T cell co-stimulation using chimeric single-chain antibody variable fragment-CD28 receptors. Eur J Immunol 26(10):2304–2309

Beatty GL, Haas AR, Maus MV, Torigian DA, Soulen MC, Plesa G et al (2014) Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells induce anti-tumor activity in solid malignancies. Cancer Immunol Res 2(2):112–120

Bertaina A, Merli P, Rutella S, Pagliara D, Bernardo ME, Masetti R et al (2014) HLA-haploidentical stem cell transplantation after removal of αβ+ T and B cells in children with nonmalignant disorders. Blood 124(5):822–826

Birkholz K, Hombach A, Krug C, Reuter S, Kershaw M, Kämpgen E et al (2009) Transfer of mRNA encoding recombinant immunoreceptors reprograms CD4+ and CD8+ T cells for use in the adoptive immunotherapy of cancer. Gene Ther 16(5):596–604

Boardman DA, Philippeos C, Fruhwirth GO, Ibrahim MAA, Hannen RF, Cooper D et al (2017) Expression of a chimeric antigen receptor specific for donor HLA class I enhances the potency of human regulatory T cells in preventing human skin transplant rejection. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg 17(4):931–943

Boice M, Salloum D, Mourcin F, Sanghvi V, Amin R, Oricchio E et al (2016) Loss of the HVEM tumor suppressor in lymphoma and restoration by modified CAR-T cells. Cell 167(2):405–418. e13

Bouzin C, Brouet A, De Vriese J, Dewever J, Feron O (2007) Effects of vascular endothelial growth factor on the lymphocyte-endothelium interactions: identification of caveolin-1 and nitric oxide as control points of endothelial cell anergy. J Immunol Baltim Md 1950 178 (3):1505–1511

Brentjens RJ, Rivière I, Park JH, Davila ML, Wang X, Stefanski J et al (2011) Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. Blood 118(18):4817–4828

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

Bridgeman JS, Hawkins RE, Bagley S, Blaylock M, Holland M, Gilham DE (2010) The optimal antigen response of chimeric antigen receptors harboring the CD3 transmembrane domain is dependent upon incorporation of the receptor into the endogenous TCR/CD3 complex. J Immunol 184(12):6938–6949

Brudno JN, Kochenderfer JN (2016) Toxicities of chimeric antigen receptor T cells: recognition and management. Blood 127(26):3321–3330

Butler MO, Lee J-S, Ansén S, Neuberg D, Hodi FS, Murray AP et al (2007) Long-lived antitumor CD8+ lymphocytes for adoptive therapy generated using an artificial antigen-presenting cell. Clin Cancer Res Off J Am Assoc Cancer Res 13(6):1857–1867

Calcinotto A, Grioni M, Jachetti E, Curnis F, Mondino A, Parmiani G et al (2012) Targeting TNF-α to neoangiogenic vessels enhances lymphocyte infiltration in tumors and increases the therapeutic potential of immunotherapy. J Immunol Baltim Md 1950 188(6):2687–2694

Cartellieri M, Feldmann A, Koristka S, Arndt C, Loff S, Ehninger A et al (2016) Switching CAR T cells on and off: a novel modular platform for retargeting of T cells to AML blasts. Blood Cancer J 6(8):e458

Caruana I, Savoldo B, Hoyos V, Weber G, Liu H, Kim ES et al (2015) Heparanase promotes tumor infiltration and antitumor activity of CAR-redirected T lymphocytes. Nat Med 21(5):524–529

Caruso HG, Hurton LV, Najjar A, Rushworth D, Ang S, Olivares S et al (2015) Tuning sensitivity of CAR to EGFR density limits recognition of normal tissue while maintaining potent antitumor activity. Cancer Res 75(17):3505–3518

Cheadle EJ, Rothwell DG, Bridgeman JS, Sheard VE, Hawkins RE, Gilham DE (2012) Ligation of the CD2 co-stimulatory receptor enhances IL-2 production from first-generation chimeric antigen receptor T cells. Gene Ther 19(11):1114–1120

Chen F, Teachey DT, Pequignot E, Frey N, Porter D, Maude SL et al (2016) Measuring IL-6 and sIL-6R in serum from patients treated with tocilizumab and/or siltuximab following CAR T cell therapy. J Immunol Methods

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

Chinnasamy D, Yu Z, Theoret MR, Zhao Y, Shrimali RK, Morgan RA et al (2010) Gene therapy using genetically modified lymphocytes targeting VEGFR-2 inhibits the growth of vascularized syngenic tumors in mice. J Clin Invest 120(11):3953–3968

Chmielewski M, Abken H (2015) TRUCKs: the fourth generation of CARs. Expert Opin Biol Ther 15(8):1145–1154

Chmielewski M, Abken H (2017) CAR T cells releasing IL-18 convert to T-bet^{high} FoxO1^{low} effectors which exhibit augmented activity against advanced solid tumors. Cell Rep (in press)

Chmielewski M, Hombach A, Heuser C, Adams GP, Abken H (2004) T cell activation by antibody-like immunoreceptors: increase in affinity of the single-chain fragment domain above threshold does not increase T cell activation against antigen-positive target cells but decreases selectivity. J Immunol Baltim Md 1950 173(12):7647–7653

Chmielewski M, Kopecky C, Hombach AA, Abken H (2011) IL-12 release by engineered T cells expressing chimeric antigen receptors can effectively Muster an antigen-independent macrophage response on tumor cells that have shut down tumor antigen expression. Cancer Res 71(17):5697–5706

Chmielewski M, Hombach AA, Abken H (2014) Of CARs and TRUCKs: chimeric antigen receptor (CAR) T cells engineered with an inducible cytokine to modulate the tumor stroma. Immunol Rev 257(1):83–90

Chong EA, Melenhorst JJ, Lacey SF, Ambrose DE, Gonzalez V, Levine BL et al (2017) PD-1 blockade modulates chimeric antigen receptor (CAR)-modified T cells: refueling the CAR. Blood 129(8):1039–1041

Craddock JA, Lu A, Bear A, Pule M, Brenner MK, Rooney CM et al (2010) Enhanced tumor trafficking of GD2 chimeric antigen receptor T cells by expression of the chemokine receptor CCR2b. J Immunother Hagerstown Md 1997 33(8):780–788

Cretenet G, Clerc I, Matias M, Loisel S, Craveiro M, Oburoglu L et al (2016) Cell surface Glut1 levels distinguish human CD4 and CD8 T lymphocyte subsets with distinct effector functions. Sci Rep 12(6):24129

Cruz CRY, Micklethwaite KP, Savoldo B, Ramos CA, Lam S, Ku S et al (2013) 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(17):2965–2973

Cui G, Staron MM, Gray SM, Ho P-C, Amezquita RA, Wu J et al (2015) IL-7-induced glycerol transport and TAG synthesis promotes memory CD8+ T cell longevity. Cell 161(4):750–761

Curran KJ, Seinstra BA, Nikhamin Y, Yeh R, Usachenko Y, van Leeuwen DG et al (2015) Enhancing antitumor efficacy of chimeric antigen receptor T cells through constitutive CD40L expression. Mol Ther J Am Soc Gene Ther 23(4):769–778

Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K et al (2014) Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med 6(224):224ra25

Deeks SG, Wagner B, Anton PA, Mitsuyasu RT, Scadden DT, Huang C et al (2002) A phase II randomized study of HIV-specific T-cell gene therapy in subjects with undetectable plasma viremia on combination antiretroviral therapy. Mol Ther J Am Soc Gene Ther 5(6):788–797

Di Stasi A, De Angelis B, Rooney CM, Zhang L, Mahendravada A, Foster AE et al (2009) T lymphocytes coexpressing CCR207 and a chimeric antigen receptor targeting CD30 have improved homing and antitumor activity in a Hodgkin tumor model. Blood 113(25):6392–6402

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

Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ et al (2002) Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science 298(5594):850–854

Elinav E, Waks T, Eshhar Z (2008) Redirection of regulatory T cells with predetermined specificity for the treatment of experimental colitis in mice. Gastroenterology 134(7):2014–2024

Ellebrecht CT, Bhoj VG, Nace A, Choi EJ, Mao X, Cho MJ et al (2016) Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease. Science 353(6295):179–184

Faitschuk E, Nagy V, Hombach AA, Abken H (2016a) A dual chain chimeric antigen receptor (CAR) in the native antibody format for targeting immune cells towards cancer cells without the need of an scFv. Gene Ther

Faitschuk E, Hombach AA, Frenzel LP, Wendtner C-M, Abken H (2016b) Chimeric antigen receptor T cells targeting Fc μ receptor selectively eliminate CLL cells while sparing healthy B cells. Blood

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

Feng K, Liu Y, Guo Y, Qiu J, Wu Z, Dai H et al (2017) Phase I study of chimeric antigen receptor modified T cells in treating HER2-positive advanced biliary tract cancers and pancreatic cancers. Protein Cell

Finney HM, Lawson AD, Bebbington CR, Weir AN (1998) Chimeric receptors providing both primary and costimulatory signaling in T cells from a single gene product. J Immunol Baltim Md 1950 161(6):2791–2797

Foster AE, Mahendravada A, Shinners NP, Chang W-C, Crisostomo J, Lu A et al (2017) Regulated expansion and survival of chimeric antigen receptor-modified T cells using small molecule-dependent inducible MyD88/CD40. Mol Ther J Am Soc Gene Ther 25(9):2176–2188

Franciszkiewicz K, Boissonnas A, Boutet M, Combadière C, Mami-Chouaib F (2012) Role of chemokines and chemokine receptors in shaping the effector phase of the antitumor immune response. Cancer Res 72(24):6325–6332

Frauwirth KA, Riley JL, Harris MH, Parry RV, Rathmell JC, Plas DR et al (2002) The CD28 signaling pathway regulates glucose metabolism. Immunity 16(6):769–777

Full F, Lehner M, Thonn V, Goetz G, Scholz B, Kaufmann KB et al (2010) T cells engineered with a cytomegalovirus-specific chimeric immunoreceptor. J Virol 84(8):4083–4088

Gardner R, Wu D, Cherian S, Fang M, Hanafi L-A, Finney O et al (2016) Acquisition of a CD19-negative myeloid phenotype allows immune escape of MLL-rearranged B-ALL from CD19 CAR-T-cell therapy. Blood 127(20):2406–2410

Garfall AL, Maus MV, Hwang W-T, Lacey SF, Mahnke YD, Melenhorst JJ et al (2015) Chimeric antigen receptor T cells against CD19 for multiple myeloma. N Engl J Med 373(11):1040–1047

Gattinoni L, Zhong X-S, Palmer DC, Ji Y, Hinrichs CS, Yu Z et al (2009) Wnt signaling arrests effector T cell differentiation and generates CD8+ memory stem cells. Nat Med 15(7):808–813

Gattinoni L, Lugli E, Ji Y, Pos Z, Paulos CM, Quigley MF et al (2011) A human memory T cell subset with stem cell-like properties. Nat Med 17(10):1290–1297

Ghassemi S, Nunez-Cruz S, O'Connor RS, Fraietta JA, Patel PR, Scholler J, Barrett DM, Lundh SM, Davis MM, Bedoya F, C Zhang, Leferovich J, Lacey SF, Levine BL, Grupp SA, June CH, Melenhorst JJ, Milone MC (2018) Reducing culture improves the antileukemic activity of chimeric antigen receptor (CAR) T cells. Cancer Immunol Res 6(9):1100–1109

Golumba-Nagy V, Kuehle J, Hombach AA, Abken H (2018) CD28-ζ CAR T cells resist TGF-β repression through IL-2 signaling, which can be mimicked by an engineered IL-7 autocrine loop. Mol Ther 26(9):2218–2230

Grada Z, Hegde M, Byrd T, Shaffer DR, Ghazi A, Brawley VS et al (2013) TanCAR: a novel bispecific chimeric antigen receptor for cancer immunotherapy. Mol Ther Nucleic Acids 2: e105

Grakoui A, Bromley SK, Sumen C, Davis MM, Shaw AS, Allen PM et al (1999) The immunological synapse: a molecular machine controlling T cell activation. Science 285 (5425):221–227

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

Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR et al (2013) Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med 368(16):1509–1518

Guedan S, Chen X, Madar A, Carpenito C, McGettigan SE, Frigault MJ et al (2014) ICOS-based chimeric antigen receptors program bipolar TH17/TH1 cells. Blood 124(7):1070–1080

Hammill JA, VanSeggelen H, Helsen CW, Denisova GF, Evelegh C, Tantalo DGM et al (2015) Designed ankyrin repeat proteins are effective targeting elements for chimeric antigen receptors. J Immunother Cancer 3:55

Han X, Cinay GE, Zhao Y, Guo Y, Zhang X, Wang P (2017) Adnectin-based design of chimeric antigen receptor for T cell engineering. Mol Ther J Am Soc Gene Ther 25(11):2466–2476

Haso W, Lee DW, Shah NN, Stetler-Stevenson M, Yuan CM, Pastan IH et al (2013) Anti-CD22-chimeric antigen receptors targeting B-cell precursor acute lymphoblastic leukemia. Blood 121(7):1165–1174

Hinrichs CS, Spolski R, Paulos CM, Gattinoni L, Kerstann KW, Palmer DC et al (2008) IL-2 and IL-21 confer opposing differentiation programs to CD8+ T cells for adoptive immunotherapy. Blood 111(11):5326–5333

Holzinger A, Barden M, Abken H (2016) The growing world of CAR T cell trials: a systematic review. Cancer Immunol Immunother CII

Hombach A, Abken H (2007) Costimulation tunes tumor-specific activation of redirected T cells in adoptive immunotherapy. Cancer Immunol Immunother CII. 56(5):731–737

Hombach AA, Abken H (2011) Costimulation by chimeric antigen receptors revisited the T cell antitumor response benefits from combined CD28-OX40 signalling. Int J Cancer 129 (12):2935–2944

Hombach A, Sent D, Schneider C, Heuser C, Koch D, Pohl C et al (2001) T-cell activation by recombinant receptors CD28 costimulation is required for interleukin 2 secretion and receptor-mediated T-cell proliferation but does not affect receptor-mediated target cell lysis. Cancer Res 61(5):1976–1982

Hombach AA, Schildgen V, Heuser C, Finnern R, Gilham DE, Abken H (2007) T cell activation by antibody-like immunoreceptors: the position of the binding epitope within the target molecule determines the efficiency of activation of redirected T cells. J Immunol Baltim Md 1950 178(7):4650–4657

Hombach A, Hombach AA, Abken H (2010) 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(10):1206–1213

Hombach AA, Chmielewski M, Rappl G, Abken H (2013) Adoptive immunotherapy with redirected T cells produces CCR6− cells that are trapped in the periphery and benefit from combined CD28-OX40 costimulation. Hum Gene Ther 24(3):259–269

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

Hsu C, Jones SA, Cohen CJ, Zheng Z, Kerstann K, Zhou J et al (2007) 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):5168–5177

Hu Y, Sun J, Wu Z, Yu J, Cui Q, Pu C et al (2016) Predominant cerebral cytokine release syndrome in CD19-directed chimeric antigen receptor-modified T cell therapy. J Hematol Oncol 9(1):70

Hu B, Ren J, Luo Y, Keith B, Young RM, Scholler J et al (2017) Augmentation of antitumor immunity by human and mouse CAR T cells secreting IL-18. Cell Rep 20(13):3025–3033

Hudecek M, Schmitt TM, Baskar S, Lupo-Stanghellini MT, Nishida T, Yamamoto TN et al (2010) The B-cell tumor-associated antigen ROR1 can be targeted with T cells modified to express a ROR1-specific chimeric antigen receptor. Blood 116(22):4532–4541

Hudecek M, Sommermeyer D, Kosasih PL, Silva-Benedict A, Liu L, Rader C et al (2015) The nonsignaling extracellular spacer domain of chimeric antigen receptors is decisive for in vivo antitumor activity. Cancer Immunol Res 3(2):125–135

Huenecke S, Zimmermann SY, Kloess S, Esser R, Brinkmann A, Tramsen L et al (2010) IL-2-driven regulation of NK cell receptors with regard to the distribution of CD16+ and CD16 − subpopulations and in vivo influence after haploidentical NK cell infusion. J Immunother Hagerstown Md 1997 33(2):200–210

Jena B, Maiti S, Huls H, Singh H, Lee DA, Champlin RE et al (2013) Chimeric antigen receptor (CAR)-specific monoclonal antibody to detect CD19-specific T cells in clinical trials. PLoS ONE 8(3):e57838

Ji Y, Wrzesinski C, Yu Z, Hu J, Gautam S, Hawk NV et al (2015) miR-155 augments CD8+ T-cell antitumor activity in lymphoreplete hosts by enhancing responsiveness to homeostatic γc cytokines. Proc Natl Acad Sci U S A 112(2):476–481

John LB, Devaud C, Duong CPM, Yong CS, Beavis PA, Haynes NM et al (2013) Anti-PD-1 antibody therapy potently enhances the eradication of established tumors by gene-modified T cells. Clin Cancer Res Off J Am Assoc Cancer Res 19(20):5636–5646

Joyce JA, Fearon DT (2015) T cell exclusion, immune privilege, and the tumor microenvironment. Science 348(6230):74–80

Jyothi MD, Flavell RA, Geiger TL (2002) Targeting autoantigen-specific T cells and suppression of autoimmune encephalomyelitis with receptor-modified T lymphocytes. Nat Biotechnol 20 (12):1215–1220

Kahlon KS, Brown C, Cooper LJN, Raubitschek A, Forman SJ, Jensen MC (2004) Specific recognition and killing of glioblastoma multiforme by interleukin 13-zetakine redirected cytolytic T cells. Cancer Res 64(24):9160–9166

Kakarla S, Chow KKH, Mata M, Shaffer DR, Song X-T, Wu M-F et al (2013) Antitumor effects of chimeric receptor engineered human T cells directed to tumor stroma. Mol Ther J Am Soc Gene Ther 21(8):1611–1620

Kandalaft LE, Facciabene A, Buckanovich RJ, Coukos G (2009) Endothelin B receptor, a new target in cancer immune therapy. Clin Cancer Res Off J Am Assoc Cancer Res 15(14):4521–4528

Kaneko S, Mastaglio S, Bondanza A, Ponzoni M, Sanvito F, Aldrighetti L et al (2009) IL-7 and IL-15 allow the generation of suicide gene-modified alloreactive self-renewing central memory human T lymphocytes. Blood 113(5):1006–1015

Katz SC, Burga RA, McCormack E, Wang LJ, Mooring W, Point GR et al (2015) Phase I hepatic immunotherapy for metastases study of intra-arterial chimeric antigen receptor-modified T-cell therapy for CEA+ liver metastases. Clin Cancer Res Off J Am Assoc Cancer Res 21(14):3149–3159

Katz SC, Point GR, Cunetta M, Thorn M, Guha P, Espat NJ et al (2016) Regional CAR-T cell infusions for peritoneal carcinomatosis are superior to systemic delivery. Cancer Gene Ther

Kawalekar OU, O'Connor RS, Fraietta JA, Guo L, McGettigan SE, Posey AD et al (2016) Distinct signaling of coreceptors regulates specific metabolism pathways and impacts memory development in CAR T Cells. Immunity 44(2):380–390

Kershaw MH, Wang G, Westwood JA, Pachynski RK, Tiffany HL, Marincola FM et al (2002) Redirecting migration of T cells to chemokine secreted from tumors by genetic modification with CXCR2. Hum Gene Ther 13(16):1971–1980

Kim MS, Ma JSY, Yun H, Cao Y, Kim JY, Chi V et al (2015) Redirection of genetically engineered CAR-T cells using bifunctional small molecules. J Am Chem Soc 137(8):2832–2835

Klebanoff CA, Gattinoni L, Restifo NP (2012) Sorting through subsets: which T-cell populations mediate highly effective adoptive immunotherapy? J Immunother Hagerstown Md 1997 35 (9):651–660

Klingemann H (2014) Are natural killer cells superior CAR drivers? Oncoimmunology 3:e28147

Kloss CC, Condomines M, Cartellieri M, Bachmann M, Sadelain M (2012) Combinatorial antigen recognition with balanced signaling promotes selective tumor eradication by engineered T cells. Nat Biotechnol 31(1):71–75

Kobold S, Grassmann S, Chaloupka M, Lampert C, Wenk S, Kraus F et al (2015) Impact of a new fusion receptor on PD-1-mediated immunosuppression in adoptive T cell therapy. J Natl Cancer Inst 107(8)

Kochenderfer JN, Dudley ME, Carpenter RO, Kassim SH, Rose JJ, Telford WG et al (2013) Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation. Blood 122(25):4129–4139

Kochenderfer JN, Dudley ME, Kassim SH, Somerville RPT, Carpenter RO, Stetler-Stevenson M et al (2015) Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. J Clin Oncol 33(6):540–549

Kofler DM, Chmielewski M, Rappl G, Hombach A, Riet T, Schmidt A et al (2011) CD28 costimulation Impairs the efficacy of a redirected t-cell antitumor attack in the presence of regulatory t cells which can be overcome by preventing Lck activation. Mol Ther J Am Soc Gene Ther 19(4):760–767

Köhl U, Arsenieva S, Holzinger A, Abken H (2018) CAR T cells in Trials: recent achievements and challenges that remain in the production of modified T cells for clinical applications. Hum Gene Ther 29(5):559–568

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

Koneru M, O'Cearbhaill R, Pendharkar S, Spriggs DR, Brentjens RJ (2015b) 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 28(13):102

Kong S, Sengupta S, Tyler B, Bais AJ, Ma Q, Doucette S et al (2012) Suppression of human glioma xenografts with second-generation IL13R-specific chimeric antigen receptor-modified T cells. Clin Cancer Res Off J Am Assoc Cancer Res 18(21):5949–5960

Koyama S, Akbay EA, Li YY, Herter-Sprie GS, Buczkowski KA, Richards WG et al (2016) Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. Nat Commun 17(7):10501

Krebs K, Böttinger N, Huang L-R, Chmielewski M, Arzberger S, Gasteiger G et al (2013) T cells expressing a chimeric antigen receptor that binds hepatitis B virus envelope proteins control virus replication in mice. Gastroenterology 145(2):456–465

Krebs S, Chow KKH, Yi Z, Rodriguez-Cruz T, Hegde M, Gerken C et al (2014) T cells redirected to interleukin-13Rα2 with interleukin-13 mutein–chimeric antigen receptors have anti-glioma activity but also recognize interleukin-13Rα1. Cytotherapy 16(8):1121–1131

Kruschinski A, Moosmann A, Poschke I, Norell H, Chmielewski M, Seliger B et al (2008) Engineering antigen-specific primary human NK cells against HER-2 positive carcinomas. Proc Natl Acad Sci U S A 105(45):17481–17486

Kudo K, Imai C, Lorenzini P, Kamiya T, Kono K, Davidoff AM et al (2014) T lymphocytes expressing a CD16 signaling receptor exert antibody-dependent cancer cell killing. Cancer Res 74(1):93–103

Kumaresan PR, Manuri PR, Albert ND, Maiti S, Singh H, Mi T et al (2014) Bioengineering T cells to target carbohydrate to treat opportunistic fungal infection. Proc Natl Acad Sci U S A 111 (29):10660–10665

Kunert A, Chmielewski M, Wijers R, Berrevoets C, Abken H, Debets R (2017) Intra-tumoral production of IL18, but not IL12, by TCR-engineered T cells is non-toxic and counteracts immune evasion of solid tumors. Oncoimmunology (in press)

Lamers CHJ, Sleijfer S, Vulto AG, Kruit WHJ, Kliffen M, Debets R et al (2006) Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. J Clin Oncol Off J Am Soc Clin Oncol 24(13): e20–e22

Lanitis E, Poussin M, Klattenhoff AW, Song D, Sandaltzopoulos R, June CH et al (2013) Chimeric antigen receptor T Cells with dissociated signaling domains exhibit focused antitumor activity with reduced potential for toxicity in vivo. Cancer Immunol Res 1(1):43–53

Lee DW, Gardner R, Porter DL, Louis CU, Ahmed N, Jensen M et al (2014) Current concepts in the diagnosis and management of cytokine release syndrome. Blood 124(2):188–195

Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA et al (2015) T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet Lond Engl 385(9967):517–528

Li Y, Bleakley M, Yee C (2005) IL-21 influences the frequency, phenotype, and affinity of the antigen-specific CD8 T cell response. J Immunol Baltim Md 1950 175(4):2261–2269

Ligtenberg MA, Mougiakakos D, Mukhopadhyay M, Witt K, Lladser A, Chmielewski M et al (2016) Coexpressed catalase protects chimeric antigen receptor-redirected T cells as well as bystander cells from oxidative stress-induced loss of antitumor activity. J Immunol Baltim Md 1950 196(2):759–766

Liu X, Jiang S, Fang C, Yang S, Olalere D, Pequignot EC et al (2015) Affinity-tuned ErbB2 or EGFR chimeric antigen receptor T cells exhibit an increased therapeutic index against tumors in mice. Cancer Res 75(17):3596–3607

Liu X, Ranganathan R, Jiang S, Fang C, Sun J, Kim S et al (2016) A chimeric switch-receptor targeting PD1 augments the efficacy of second-generation CAR T cells in advanced solid tumors. Cancer Res 76(6):1578–1590

Louis CU, Savoldo B, Dotti G, Pule M, Yvon E, Myers GD et al (2011) Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. Blood 118(23):6050–6056

Ma Q, Garber HR, Lu S, He H, Tallis E, Ding X et al (2016) A novel TCR-like CAR with specificity for PR1/HLA-A2 effectively targets myeloid leukemia in vitro when expressed in human adult peripheral blood and cord blood T cells. Cytotherapy 18(8):985–994

MacDonald KG, Hoeppli RE, Huang Q, Gillies J, Luciani DS, Orban PC et al (2016) Alloantigen-specific regulatory T cells generated with a chimeric antigen receptor. J Clin Invest 126(4):1413–1424

Mackall CL, Miklos DB (2017) CNS endothelial cell activation emerges as a driver of CAR T cell-associated neurotoxicity. Cancer Discov 7(12):1371–1373

Manuri PVR, Wilson MH, Maiti SN, Mi T, Singh H, Olivares S et al (2010) piggyBac transposon/transposase system to generate CD19-specific T cells for the treatment of B-lineage malignancies. Hum Gene Ther 21(4):427–437

Mardiana S, John LB, Henderson MA, Slaney CY, von Scheidt B, Giuffrida L et al (2017) A multifunctional role for adjuvant anti-4-1BB therapy in augmenting antitumor response by chimeric antigen receptor T cells. Cancer Res 77(6):1296–1309

Margalit A, Fishman S, Berko D, Engberg J, Gross G (2003) Chimeric beta2 microglobulin/CD3zeta polypeptides expressed in T cells convert MHC class I peptide ligands into T cell activation receptors: a potential tool for specific targeting of pathogenic CD8 (+) T cells. Int Immunol 15(11):1379–1387

Martyniszyn A, Krahl A-C, André MC, Hombach AA, Abken H (2017) CD20-CD19 bispecific CAR T cells for the treatment of B cell malignancies. Hum Gene Ther

Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ et al (2014a) Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med 371(16):1507–1517

Maude SL, Barrett D, Teachey DT, Grupp SA (2014b) Managing cytokine release syndrome associated with novel T cell-engaging therapies. Cancer J Sudbury Mass 20(2):119–122

Maus MV, Haas AR, Beatty GL, Albelda SM, Levine BL, Liu X et al (2013) T cells expressing chimeric antigen receptors can cause anaphylaxis in humans. Cancer Immunol Res 1(1):26–31

Mohammed S, Sukumaran S, Bajgain P, Watanabe N, Heslop HE, Rooney CM et al (2017) Improving chimeric antigen receptor-modified T cell function by reversing the immunosuppressive tumor microenvironment of pancreatic cancer. Mol Ther J Am Soc Gene Ther 25 (1):249–258

Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA (2010) Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. Mol Ther J Am Soc Gene Ther 18(4):843–851

Morsut L, Roybal KT, Xiong X, Gordley RM, Coyle SM, Thomson M et al (2016) Engineering customized cell sensing and response behaviors using synthetic notch receptors. Cell 164 (4):780–791

Newick K, O'Brien S, Sun J, Kapoor V, Maceyko S, Lo A et al (2016) Augmentation of CAR T cell trafficking and antitumor efficacy by blocking protein kinase A (PKA) localization. Cancer Immunol Res

Ninomiya S, Narala N, Huye L, Yagyu S, Savoldo B, Dotti G et al (2015) Tumor indoleamine 2,3-dioxygenase (IDO) inhibits CD19-CAR T cells and is downregulated by lymphodepleting drugs. Blood 125(25):3905–3916

Noyan F, Zimmermann K, Hardtke-Wolenski M, Knoefel A, Schulde E, Geffers R et al (2017) Prevention of Allograft rejection by use of regulatory T cells with an MHC-specific chimeric antigen receptor. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg 17(4):917–930

O'Rourke DM, Nasrallah MP, Desai A, Melenhorst JJ, Mansfield K, Morrissette JJD et al (2017) 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(399)

Osborn MJ, Webber BR, Knipping F, Lonetree C, Tennis N, DeFeo AP et al (2016) Evaluation of TCR gene editing achieved by TALENs, CRISPR/Cas9, and megaTAL nucleases. Mol Ther J Am Soc Gene Ther 24(3):570–581

Parente-Pereira AC, Burnet J, Ellison D, Foster J, Davies DM, van der Stegen S et al (2011) Trafficking of CAR-engineered human T cells following regional or systemic adoptive transfer in SCID beige mice. J Clin Immunol 31(4):710–718

Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang L-S et al (2009) Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature 460(7251):103–107

Pegram HJ, Lee JC, Hayman EG, Imperato GH, Tedder TF, Sadelain M et al (2012) Tumor-targeted T cells modified to secrete IL-12 eradicate systemic tumors without need for prior conditioning. Blood 119(18):4133–4141

Pegram HJ, Park JH, Brentjens RJ (2014) CD28z CARs and armored CARs. Cancer J Sudbury Mass 20(2):127–133

Peng W, Ye Y, Rabinovich BA, Liu C, Lou Y, Zhang M et al (2010) Transduction of tumor-specific T cells with CXCR208 chemokine receptor improves migration to tumor and antitumor immune responses. Clin Cancer Res Off J Am Assoc Cancer Res 16(22):5458–5468

Perna SK, Pagliara D, Mahendravada A, Liu H, Brenner MK, Savoldo B et al (2014) Interleukin-7 mediates selective expansion of tumor-redirected cytotoxic T lymphocytes (CTLs) without enhancement of regulatory T-cell inhibition. Clin Cancer Res Off J Am Assoc Cancer Res 20 (1):131–139

Philip B, Kokalaki E, Mekkaoui L, Thomas S, Straathof K, Flutter B et al (2014) A highly compact epitope-based marker/suicide gene for easier and safer T-cell therapy. Blood 124 (8):1277–1287

Pipkin ME, Sacks JA, Cruz-Guilloty F, Lichtenheld MG, Bevan MJ, Rao A (2010) Interleukin-2 and inflammation induce distinct transcriptional programs that promote the differentiation of effector cytolytic T cells. Immunity 32(1):79–90

Poirot L, Philip B, Schiffer-Mannioui C, Le Clerre D, Chion-Sotinel I, Derniame S et al (2015) Multiplex genome-edited T-cell manufacturing platform for "off-the-shelf" adoptive T-cell immunotherapies. Cancer Res 75(18):3853–3864

Porter DL, Levine BL, Kalos M, Bagg A, June CH (2011) Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med 365(8):725–733

Porter DL, Hwang W-T, Frey NV, Lacey SF, Shaw PA, Loren AW et al (2015) Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. Sci Transl Med 7(303):303ra139

Posey AD, Schwab RD, Boesteanu AC, Steentoft C, Mandel U, Engels B et al (2016) Engineered CAR T cells targeting the cancer-associated Tn-glycoform of the membrane mucin MUC1 control adenocarcinoma. Immunity 44(6):1444–1454

Prosser ME, Brown CE, Shami AF, Forman SJ, Jensen MC (2012) Tumor PD-L1 co-stimulates primary human CD8(+) cytotoxic T cells modified to express a PD1:CD28 chimeric receptor. Mol Immunol 51(3–4):263–272

Provasi E, Genovese P, Lombardo A, Magnani Z, Liu P-Q, Reik A et al (2012) Editing T cell specificity towards leukemia by zinc finger nucleases and lentiviral gene transfer. Nat Med 18 (5):807–815

Pule MA, Savoldo B, Myers GD, Rossig C, Russell HV, Dotti G et al (2008) Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. Nat Med 14(11):1264–1270

Qasim W, Zhan H, Samarasinghe S, Adams S, Amrolia P, Stafford S et al (2017) Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. Sci Transl Med 9(374)

Ren J, Zhang X, Liu X, Fang C, Jiang S, June CH et al (2017a) A versatile system for rapid multiplex genome-edited CAR T cell generation. Oncotarget 8(10):17002–17011

Ren J, Liu X, Fang C, Jiang S, June CH, Zhao Y (2017b) Multiplex genome editing to generate universal CAR T cells resistant to PD1 inhibition. Clin Cancer Res Off J Am Assoc Cancer Res 23(9):2255–2266

Riegler LL, Jones GP, Lee DW (2019) Current approaches in the grading and management of cytokine release syndrome after chimeric antigen receptor T-cell therapy. Ther Clin Risk Manag 15:323–335

Rodgers DT, Mazagova M, Hampton EN, Cao Y, Ramadoss NS, Hardy IR et al (2016) Switch-mediated activation and retargeting of CAR-T cells for B-cell malignancies. Proc Natl Acad Sci U S A 113(4):E459–E468

Rodriguez PC, Zea AH, Culotta KS, Zabaleta J, Ochoa JB, Ochoa AC (2002) Regulation of T cell receptor CD3zeta chain expression by L-arginine. J Biol Chem 277(24):21123–21129

Rodriguez PC, Quiceno DG, Ochoa AC (2007) l-arginine availability regulates T-lymphocyte cell-cycle progression. Blood 109(4):1568–1573

Romeo C, Seed B (1991) Cellular immunity to HIV activated by CD4 fused to T cell or Fc receptor polypeptides. Cell 64(5):1037–1046

Roybal KT, Rupp LJ, Morsut L, Walker WJ, McNally KA, Park JS et al (2016a) Precision tumor recognition by T cells with combinatorial antigen-sensing circuits. Cell 164(4):770–779

Roybal KT, Williams JZ, Morsut L, Rupp LJ, Kolinko I, Choe JH et al (2016b) Engineering T cells with customized therapeutic response programs using synthetic notch receptors. Cell 167 (2):419–432.e16

Ruella M, Barrett DM, Kenderian SS, Shestova O, Hofmann TJ, Perazzelli J et al (2016a) Dual CD19 and CD123 targeting prevents antigen-loss relapses after CD19-directed immunotherapies. J Clin Invest 126(10):3814–3826

Ruella M, Kenderian SS, Shestova O, Fraietta JA, Qayyum S, Zhang Q et al (2016b) The addition of the btk inhibitor ibrutinib to anti-CD19 chimeric antigen receptor T cells (CART19) improves responses against mantle cell lymphoma. Clin Cancer Res Off J Am Assoc Cancer Res 22(11):2684–2696

Ruella M, Xu J, Barrett DM, Fraietta JA, Reich TJ, Ambrose DE, Klichinsky M, Shestova O, Patel PR, Kulikovskaya I, Nazimuddin F, Bhoj VG, Orlando EJ, Fry TJ, Bitter H, Maude SL, Levine BL, Nobles CL, Bushman FD, Young RM, Scholler J, Gill SL, June CH, Grupp SA, Lacey SF, Melenhorst JJ (2018) Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell. Nat Med 24(10):1499–1503

Sabatino M, Hu J, Sommariva M, Gautam S, Fellowes V, Hocker JD et al (2016) Generation of clinical-grade CD19-specific CAR-modified CD8+ memory stem cells for the treatment of human B-cell malignancies. Blood 128(4):519–528

Sampson JH, Choi BD, Sanchez-Perez L, Suryadevara CM, Snyder DJ, Flores CT et al (2014) EGFRvIII mCAR-modified T-cell therapy cures mice with established intracerebral glioma and generates host immunity against tumor-antigen loss. Clin Cancer Res Off J Am Assoc Cancer Res 20(4):972–984

Sautto GA, Wisskirchen K, Clementi N, Castelli M, Diotti RA, Graf J et al (2016) Chimeric antigen receptor (CAR)-engineered T cells redirected against hepatitis C virus (HCV) E2 glycoprotein. Gut 65(3):512–523

Savoldo B, Rooney CM, Di Stasi A, Abken H, Hombach A, Foster AE et al (2007) Epstein Barr virus specific cytotoxic T lymphocytes expressing the anti-CD30zeta artificial chimeric T-cell receptor for immunotherapy of Hodgkin disease. Blood 110(7):2620–2630

Schönfeld K, Sahm C, Zhang C, Naundorf S, Brendel C, Odendahl M et al (2015) Selective inhibition of tumor growth by clonal NK cells expressing an ErbB2/HER2-specific chimeric antigen receptor. Mol Ther J Am Soc Gene Ther 23(2):330–338

Serafini M, Manganini M, Borleri G, Bonamino M, Imberti L, Biondi A et al (2004) Characterization of CD20-transduced T lymphocytes as an alternative suicide gene therapy approach for the treatment of graft-versus-host disease. Hum Gene Ther 15(1):63–76

Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Mikkelson T et al (2014) Genome-scale CRISPR-Cas9 knockout screening in human cells. Science 343(6166):84–87

Shen C-J, Yang Y-X, Han EQ, Cao N, Wang Y-F, Wang Y et al (2013) Chimeric antigen receptor containing ICOS signaling domain mediates specific and efficient antitumor effect of T cells against EGFRvIII expressing glioma. J Hematol Oncol 6:33

Singh H, Figliola MJ, Dawson MJ, Olivares S, Zhang L, Yang G et al (2013) Manufacture of clinical-grade CD19-specific T cells stably expressing chimeric antigen receptor using Sleeping Beauty system and artificial antigen presenting cells. PLoS ONE 8(5):e64138

Singh H, Moyes JSE, Huls MH, Cooper LJN (2015) Manufacture of T cells using the Sleeping Beauty system to enforce expression of a CD19-specific chimeric antigen receptor. Cancer Gene Ther 22(2):95–100

Singh N, Perazzelli J, Grupp SA, Barrett DM (2016) Early memory phenotypes drive T cell proliferation in patients with pediatric malignancies. Sci Transl Med 8(320):320ra3

Skuljec J, Chmielewski M, Happle C, Habener A, Busse M, Abken H et al (2017) Chimeric antigen receptor-redirected regulatory T cells suppress experimental allergic airway inflammation, a model of asthma. Front Immunol 8:1125

Slaney CY, von Scheidt B, Davenport AJ, Beavis PA, Westwood JA, Mardiana S et al (2017) Dual-specific chimeric antigen receptor T cells and an indirect vaccine eradicate a variety of large solid tumors in an immunocompetent, self-antigen setting. Clin Cancer Res Off J Am Assoc Cancer Res 23(10):2478–2490

Song D-G, Ye Q, Poussin M, Harms GM, Figini M, Powell DJ (2012) CD27 costimulation augments the survival and antitumor activity of redirected human T cells in vivo. Blood 119 (3):696–706

Sotillo E, Barrett DM, Black KL, Bagashev A, Oldridge D, Wu G et al (2015) Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. Cancer Discov 5(12):1282–1295

Srivastava S, Riddell SR (2015) Engineering CAR-T cells: design concepts. Trends Immunol 36 (8):494–502

Stewart-Jones G, Wadle A, Hombach A, Shenderov E, Held G, Fischer E et al (2009) Rational development of high-affinity T-cell receptor-like antibodies. Proc Natl Acad Sci U S A 106 (14):5784–5788

Straathof KC, Pulè MA, Yotnda P, Dotti G, Vanin EF, Brenner MK et al (2005) An inducible caspase 9 safety switch for T-cell therapy. Blood 105(11):4247–4254

Sukumar M, Liu J, Ji Y, Subramanian M, Crompton JG, Yu Z et al (2013) Inhibiting glycolytic metabolism enhances CD8+ T cell memory and antitumor function. J Clin Invest 123 (10):4479–4488

Tamada K, Geng D, Sakoda Y, Bansal N, Srivastava R, Li Z et al (2012) Redirecting gene-modified T cells toward various cancer types using tagged antibodies. Clin Cancer Res Off J Am Assoc Cancer Res 18(23):6436–6445

Tanoue K, Rosewell Shaw A, Watanabe N, Porter C, Rana B, Gottschalk S et al (2017) Armed oncolytic adenovirus-expressing PD-L1 mini-body enhances antitumor effects of chimeric antigen receptor T cells in solid tumors. Cancer Res 77(8):2040–2051

Tchou J, Zhao Y, Levine BL, Zhang PJ, Davis MM, Melenhorst JJ et al (2017) Safety and efficacy of intratumoral injections of chimeric antigen receptor (CAR) T cells in metastatic breast cancer. Cancer Immunol Res 5(12):1152–1161

Teachey DT, Lacey SF, Shaw PA, Melenhorst JJ, Maude SL, Frey N et al (2016) Identification of predictive biomarkers for cytokine release syndrome after chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. Cancer Discov 6(6):664–679

Textor A, Listopad JJ, Wührmann LL, Perez C, Kruschinski A, Chmielewski M et al (2014) Efficacy of CAR T-cell therapy in large tumors relies upon stromal targeting by IFNγ. Cancer Res 74(23):6796–6805

Tey S-K, Dotti G, Rooney CM, Heslop HE, Brenner MK (2007) Inducible caspase 9 suicide gene to improve the safety of allodepleted T cells after haploidentical stem cell transplantation. Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant 13(8):913–924

Thistlethwaite FC, Gilham DE, Guest RD, Rothwell DG, Pillai M, Burt DJ et al (2017) The clinical efficacy of first-generation carcinoembryonic antigen (CEACAM5)-specific CAR T cells is limited by poor persistence and transient pre-conditioning-dependent respiratory toxicity. Cancer Immunol Immunother CII

Thomis DC, Marktel S, Bonini C, Traversari C, Gilman M, Bordignon C et al (2001) A Fas-based suicide switch in human T cells for the treatment of graft-versus-host disease. Blood 97 (5):1249–1257

Torikai H, Reik A, Liu P-Q, Zhou Y, Zhang L, Maiti S et al (2012) A foundation for universal T-cell based immunotherapy: T cells engineered to express a CD19-specific chimeric-antigen-receptor and eliminate expression of endogenous TCR. Blood 119 (24):5697–5705

Urbanska K, Lanitis E, Poussin M, Lynn RC, Gavin BP, Kelderman S et al (2012) A universal strategy for adoptive immunotherapy of cancer through use of a novel T-cell antigen receptor. Cancer Res 72(7):1844–1852

van der Waart AB, van de Weem NMP, Maas F, Kramer CSM, Kester MGD, Falkenburg JHF et al (2014) Inhibition of Akt signaling promotes the generation of superior tumor-reactive T cells for adoptive immunotherapy. Blood 124(23):3490–3500

van der Windt GJW, Pearce EL (2012) Metabolic switching and fuel choice during T-cell differentiation and memory development. Immunol Rev 249(1):27–42

van der Windt GJW, Everts B, Chang C-H, Curtis JD, Freitas TC, Amiel E et al (2012) Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. Immunity 36(1):68–78

Vera J, Savoldo B, Vigouroux S, Biagi E, Pule M, Rossig C et al (2006) T lymphocytes redirected against the kappa light chain of human immunoglobulin efficiently kill mature B lymphocyte-derived malignant cells. Blood 108(12):3890–3897

Vera JF, Hoyos V, Savoldo B, Quintarelli C, Giordano Attianese GMP, Leen AM et al (2009) Genetic manipulation of tumor-specific cytotoxic T lymphocytes to restore responsiveness to IL-7. Mol Ther J Am Soc Gene Ther 17(5):880–888

Von Hoff DD, Ramanathan RK, Borad MJ, Laheru DA, Smith LS, Wood TE et al (2011) Gemcitabine plus nab-paclitaxel is an active regimen in patients with advanced pancreatic cancer: a phase I/II trial. J Clin Oncol Off J Am Soc Clin Oncol 29(34):4548–4554

Wang X, Chang W-C, Wong CW, Colcher D, Sherman M, Ostberg JR et al (2011) A transgene-encoded cell surface polypeptide for selection, in vivo tracking, and ablation of engineered cells. Blood 118(5):1255–1263

Wang E, Wang L-C, Tsai C-Y, Bhoj V, Gershenson Z, Moon E et al (2015a) Generation of potent T-cell immunotherapy for cancer using DAP12-based, multichain, chimeric immunoreceptors. Cancer Immunol Res 3(7):815–826

Wang X, Wong CW, Urak R, Mardiros A, Budde LE, Chang W-C et al (2015b) CMVpp65 vaccine enhances the antitumor efficacy of adoptively transferred CD19-redirected CMV-specific T cells. Clin Cancer Res Off J Am Assoc Cancer Res 21(13):2993–3002

Wang X, Popplewell LL, Wagner JR, Naranjo A, Blanchard MS, Mott MR et al (2016) Phase 1 studies of central memory-derived CD19 CAR T-cell therapy following autologous HSCT in patients with B-cell NHL. Blood 127(24):2980–2990

Wilkie S, van Schalkwyk MCI, Hobbs S, Davies DM, van der Stegen SJC, Pereira ACP et al (2012) Dual targeting of ErbB2 and MUC1 in breast cancer using chimeric antigen receptors engineered to provide complementary signaling. J Clin Immunol 32(5):1059–1070

Wofford JA, Wieman HL, Jacobs SR, Zhao Y, Rathmell JC (2008) IL-7 promotes Glut1 trafficking and glucose uptake via STAT5-mediated activation of Akt to support T-cell survival. Blood 111(4):2101–2111

Wu C-Y, Roybal KT, Puchner EM, Onuffer J, Lim WA (2015) Remote control of therapeutic T cells through a small molecule-gated chimeric receptor. Science

Xu A, Bhanumathy KK, Wu J, Ye Z, Freywald A, Leary SC et al (2016) IL-15 signaling promotes adoptive effector T-cell survival and memory formation in irradiation-induced lymphopenia. Cell Biosci 6:30

Zah E, Lin M-Y, Silva-Benedict A, Jensen MC, Chen YY (2016) T cells expressing CD19/CD20 bispecific chimeric antigen receptors prevent antigen escape by malignant B cells. Cancer Immunol Res 4(6):498–508

Zhang L, Yu Z, Muranski P, Palmer DC, Restifo NP, Rosenberg SA et al (2013) Inhibition of TGF-β signaling in genetically engineered tumor antigen-reactive T cells significantly enhances tumor treatment efficacy. Gene Ther 20(5):575–580

Zhang T, Cao L, Xie J, Shi N, Zhang Z, Luo Z et al (2015) Efficiency of CD19 chimeric antigen receptor-modified T cells for treatment of B cell malignancies in phase I clinical trials: a meta-analysis. Oncotarget 6(32):33961–33971

Zhang C, Burger MC, Jennewein L, Genßler S, Schönfeld K, Zeiner P et al (2016) ErbB2/HER2-specific NK cells for targeted therapy of glioblastoma. J Natl Cancer Inst 108(5)

Zhang C, Wang Z, Yang Z, Wang M, Li S, Li Y et al (2017) Phase I escalating-dose trial of CAR-T therapy targeting CEA(+) metastatic colorectal cancers. Mol Ther J Am Soc Gene Ther

Zhou X, Di Stasi A, Tey S-K, Krance RA, Martinez C, Leung KS et al (2014) Long-term outcome after haploidentical stem cell transplant and infusion of T cells expressing the inducible caspase 9 safety transgene. Blood 123(25):3895–3905