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REVIEW

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Mechanisms and strategies for safe chimeric antigen receptor T-cell activity control

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Abstract

The clinical application of chimeric antigen receptor (CAR) T-cell therapy has rapidly changed the treatment options for terminally ill patients with defined blood-borne cancer types. However, CAR T-cell therapy can lead to severe therapy-associated toxicities including CAR-related hematotoxicity, ON-target OFF-tumor toxicity, cytokine release syndrome (CRS) or immune effector cell-associated neurotoxicity syndrome (ICANS). Just as CAR T-cell therapy has evolved regarding receptor design, gene transfer systems and production protocols, the management of side effects has also improved. However, because of measures taken to abrogate adverse events, CAR T-cell viability and persistence might be impaired before complete remission can be achieved. This has fueled efforts for the development of extrinsic and intrinsic strategies for better control of CAR T-cell activity. These approaches can mediate a reversible resting state or irreversible T-cell elimination, depending on the route chosen. Control can be passive or active. By combination of CAR T-cells with T-cell inhibiting compounds, pharmacologic control, mostly independent of the CAR construct design used, can be achieved. Other strategies involve the genetic modification of T-cells or further development of the CAR construct by integration of molecular ON/OFF switches such as suicide genes. Alternatively, CAR T-cell activity can be

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; AvidCAR, avidity-controlled CAR; BD, bromodomain; biAb, bispecific antibody; BsCAR, barstar-based CAR; CAR, chimeric antigen receptor; CARD, caspase recruitment domain; CCR, chimeric costimulatory receptor; CDC, complement-dependent cytotoxicity; CID, chemical inducer of dimerization; CRS, cytokine release syndrome; CTLA-4, cytotoxic T lymphocyte-associated protein-4; DARIC, dimerizing agent-regulated immunoreceptor complex; DARPin, designed ankyrin repeat proteins; EGFRt, truncated epidermal growth factor receptor; EMA, European Medicines Agency; FBP, folate binding protein; FDA, Food and Drug Administration; FITC, fluorescein isothiocyanate; FKBP, FK506-binding protein; FRB, FKBP-rapamycin binding domain; HSV-TK, herpes simplex virus-thymidine kinase; ICANS, immune effector cell-associated neurotoxicity syndrome; iCAR, inhibitory CAR; iCas9, inducible Caspase9; iCO, inducible costimulatory; iNKG2D, inert form of the human NKG2D extracellular domain; iTurbo, inducible Turbo; LiCAR, light-switchable CAR T-cells; LID, ligand-induced degradation; MCSP, melanoma-associated chondroitin sulfate proteoglycan; MM, multiple myeloma; MSLN, mesothelin; MUC1, mucin-1; NIR, near infrared; NK, natural killer; PD-1, programmed cell death-1; PROTAC, proteolysis-targeting chimaera; PSCA, prostate stem cell antigen; PSMA, prostate-specific membrane antigen; revCAR, reversed CAR; SAR, synthetic agonistic receptor; scFv, single chain variable fragment; SNIP, signal neutralization by an inhibitable protease; TAA, tumor-associated antigens; TCR, T-cell receptor; TET, tetracycline; TIL, tumor-infiltrating lymphocytes; TKI, tyrosine kinase inhibitor; TLS, tumor lysis syndrome; TM, transmembrane; TME, tumor microenvironment; TMPK, human thymidylate kinase; UCNP, upconversion nanoplate; VIPER, versatile protease regulatable; αFR, C4 folate receptor-alpha.

[Correction added after first online publication on 13 July 2023. Figure 1 and Table 2 Placement Changed]

regulated intracellularly through a self-regulation function or extracellularly through titration of a CAR adaptor or of a priming small molecule. In this work, we review the current strategies and mechanisms to control activity of CAR T-cells reversibly or irreversibly for preventing and for managing therapy-associated toxicities.

KEYWORDS

adoptive T-cell therapy, CAR T-cells, immunotherapy

1 | INTRODUCTION

Immunotherapy has become an indispensable component of a multimodal therapy concept for cancer patients in the last decade. Most of the strategies interact with or include T-cells to overcome resistance to treatment.[1] Strategies have been developed to isolate, expand and redirect primary human T-cells against tumors.[2] As "living drugs," a single administration of a T-cell product is typically needed for continuous therapeutic efficacy, which stands as a compelling argument in comparison to other treatment modalities.[2] There are three major types of T-cell-based therapies: tumor-infiltrating lymphocytes (TILs), T-cell receptor (TCR) and chimeric antigen receptor (CAR)-modified T-cells.[1] The most promising strategy with proven clinical efficacy is CAR T-cell therapy. CAR design has continuously evolved but the main components remain: single chain variable fragment (scFv) of an antibody as an extracellular binding domain, non-signaling extracellular spacer and transmembrane (TM) domain, costimulatory domain(s) and intracellular CD3ζ signaling domain.[2-4] Anti-CD19 and anti-BCMA CAR T-cell products have already been approved by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the therapy of hematological diseases, including various B-cell lymphomas, B-cell leukemias and multiple myeloma (MM).[5] Clinical efficacy in solid tumors is limited because of antigen heterogeneity, poor migration and infiltration of T-cells into tumor sites and the immunosuppressive tumor microenvironment (TME).[6,7] After administration of an ex vivo genetically modified and expanded CAR T–cell product, CAR T-cells are activated by antigen-positive malignant cells, leading to proliferation, cytokine production and tumor-directed cytotoxicity.[2] However, antigen-positive healthy tissues can also activate CAR T-cells, causing unwanted side effects.

2 | CAR T-CELL-ASSOCIATED TOXICITIES

Even though commercial CAR T-cell products reached the clinic and achieved sufficient clinical results in approval studies, therapeutic failure and relapse can still be observed. Therapeutic success is limited due to insufficient effector function of the CAR T-cells as well as CAR T-cell-associated toxicities. These adverse events include unspecific side effects such as tumor lysis syndrome (TLS)[8,9] and hematotoxicity[10] as well as immunotherapy-specific side effects such as ON-target OFF-tumor toxicity, antigen escape, cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS).[6,7,11-14] Clinical management of these side effects is difficile and positively correlates with clinical experience of the treating center.[15] Treatment options for CRS and ICANS include the IL-1 receptor antagonist anakinra, the IL-6 receptor antagonist tocilizumab and corticosteroids.[15-17] However, corticosteroids have to be applied for the shortest duration, at the lowest dose and as late as clinically feasible to avoid a negative impact on overall survival.[18] Along these lines, current drug interventions have a narrow window of opportunity to mitigate toxicities while preserving efficacy, which stresses the need for better and at times transient CAR T–cell control. Consequently, strategies for better control of CAR T–cell activity are being developed.

3 | STRATEGIES FOR CONTROLLING CAR T-CELL ACTIVITY

These approaches for better control of the activity of CAR T-cells can mediate either a reversible resting state of CAR T-cells or irreversible T cell elimination. The aim is to either (1) prevent treatment-associated toxicities or to (2) manage these toxicities by temporarily deactivating or permanently eliminating CAR T-cells. Genetic T-cell modifications or further development of the CAR construct by integration of a molecular ON-OFF switch or intracellular self-regulation function can be performed. This review article highlights mechanisms and strategies for safe CAR T–cell activity control.

3.1 | Prevention of treatment-associated toxicity

Strategies to prevent treatment-associated toxicities have the goal of heightening the threshold of CAR T–cell activation (Table 1 and Figure 1). They can either increase specificity of CAR T-cell activation by more selective target selection or by combinatorial target antigen recognition. Modulation of intensity of activation could be achieved by including more than one activation step or by regulating CAR expression through mRNA transfection.

TABLE 1 Overview of strategies preventing toxicity of CAR T–cell therapy.

Strategy	Details	T-cell product	Model/target	References
Increase of the specificity of activation				
Passive control	Affinity tuning	Affinity-tuned CD19 CAR	ALL CLL	19-21
		Affinity-tuned CD229 CAR	MM	21
		Affinity-tuned EGFR CAR	Glioma	22
		Affinity-tuned EGFR CAR	Breast cancer Ovarian carcinoma	23,24
		Affinity-tuned HER2/neu CAR	Breast cancer Ovarian carcinoma Mesothelioma Lung cancer Melanoma Prostate cancer	
		Affinity-tuned αFR CAR	Ovarian carcinoma	25
		Affinity-tuned GD2 CAR	Neuroblastoma	26
		Affinity-tuned GPC2 CAR	Neuroblastoma	27
		Affinity-tuned CD38 CAR	MM	28
OR-gate CAR	Co-administration	CD19 CAR + CD22 CAR	ALL	29
		CD19 CAR + CD123 CAR	ALL	30
	Dual CAR	CD19 CAR + CD123 CAR	ALL	30
		CD19 CAR + CD22 CAR	ALL Lymphoma	31
		GPC3 CAR + CD133 CAR	HCC	32
		GD2 CAR + Vγγ9Vδ2 TCR	Neuroblastoma	33
	(Looped) Tandem CAR	CD19-CD20 CAR CD20-CD19 CAR	Leukemia	34
		CD19-HER2/neu CAR	Lymphoma Breast cancer	35
		HER2/neu-IL13Rα2 CAR	Glioblastoma	36
		Bivalent CD19VL-CD22VH-CD22VL-CD19VH. CD8.41BBz CAR	ALL	37
		Bicistronic CD22.CD8.41BBz-CD19.CD28.CD28z CAR		
		Bicistronic CD19-CD22 CAR (AUTO3)	ALL Lymphoma	38,39
		Bicistronic CD19-CD20 CAR	ALL Lymphoma	40
		CD19-CD22 CAR	ALL Lymphoma	41
		CD19-CD20 CAR	Lymphoma	42
		CD20-CD19 CAR	B-cell malignancies	37,43
		CD19-CD20 CAR CD20-CD19 CAR	B-cell malignancies	44
CAR exosome delivery	CAR extracellular vesicles	CD19 CAR	ALL	45
		HER2 CAR EGFR CAR	Breast cancer	46
		HER2 CAR	HER2+ tumor cells	47
NOT-gate CAR	Inhibitory CAR (iCAR)	PD-1- and CTLA-4-based iCAR	PSMA+ and/or CD19+ tumor cells	48
	Inhibitory receptor	MSLN CAR + HLA-A*02-gated inhibitory receptor	Cervical carcinoma	49
		CEA CAR + HLA-A*02-gated inhibitory receptor	Colorectal cancer Pancreatic cancer Lung cancer	50

TABLE 1 (Continued)

Strategy	Details	T-cell product	Model/target	References
	Signal-CAR/ Scissors-CAR	CD19-Signal-CAR + HER2/neu-Scissors-CAR	ALL Lymphoma CML Breast cancer	51
Modulation of the intensity of activation				
AND-gate CAR	Trans signaling CAR	MSLN CAR + αFR CAR	Ovarian carcinoma	52
	Dual transduced CAR	HER2/neu CAR + Folate binding protein (FBP) CAR	Breast cancer Sarcoma	53
		HER2/neu CAR + Mucin-1 (MUC1) CAR	Antigen-expressing Jurkat cells	54
	Split-CAR	CD19.41BB-CAR + 41BB.CD3z domain Dimerizing molecule: AP21967 (rapalog)	CML B cell malignancies	55
		CD19 CAR + 41BB + CD3z domains (FcεRI receptor scaffold with alpha, beta and gamma chains) Dimerizing molecule: rapamycin, AP21967, tacrolimus	B cell lymphoma	56
		HER2/neu CAR + MyD88/CD40 iCO molecule Dimerizing molecule: AP20187	Osteosarcoma NSCLC	57
		CD19 CAR +41BB.CD3z domain (CD19-DARIC T-cells) Dimerizing molecule: rapamycin, AP21967	ALL CML	58
		CD19-DARIC T-cells + BCMA DARIC plug-in Dimerizing molecule: rapamycin, AP21967		
		BCMA-DARIC T-cells + CD19 DARIC plug-in Dimerizing molecule: rapamycin, AP21967		
		Not evaluated for T cell therapy Dimerizing molecule: GA_3-AM + rapamycin	/	59
		PSCA.CD3z-CAR + MyD88-CD40 (iMC) Dimerizing molecule: rimiducid/AP1903	Pancreatic carcinoma	60
		CD123.CD3z-CAR + MyD88-CD40 (iMC) Dimerizing molecule: rimiducid/AP1903	AML	
		GD2.CD3z-CAR + MyD88-CD40 (iMC) Dimerizing molecule: rimiducid/AP1903	Malignant melanoma	
		CD19.CD28-CAR + CD28.CD3z dimerizing domain (ON SWITCH CAR) Dimerizing molecule: lenalidomide/pomalidomide	Mantle cell lymphoma ALL	61
		CAR + Inducible Turbo (iTurbo) cytokine signaling domain CAR Dimerizing molecule: AP1903	Antigen-positive tumor cells	62,63
		CD19 light-switchable CAR (LiCAR) Activating molecule: surgically removable upconversion nanoplates (UCNPs)	CML Lymphoma CD19+ melanoma cells	64
		EGFR AvidCAR HER2/neu AvidCAR Dimerizing molecule: AP20187	ALL ± hEGFRt ALL ± hHER2t	65
		CD19 LINK CAR HER2/neu LINK CAR Dimerizing molecule: GADS	ALL	66
	Oxygen sensitivity	CD19 HIF-CAR Trigger: hypoxia	Lymphoma	67

(Continues)

TABLE 1 (Continued)

Strategy	Details	T-cell product	Model/target	References
IF-THEN-gate CAR	synNotch	GFP synNotch CAR	CML	68
		HER2/neu synNotch CAR	Breast cancer	
		CD19 synNotch CAR	CML Lymphoma	
			CML Lymphoma	69
			CML Fibrosarcoma	70
		EGFRvIII synNotch CAR	Glioblastoma	71
IF-BETTER-gate CAR	CAR + chimeric costimulatory receptor (CCR)	PSCA CAR + PSMA-specific CCR	Prostate carcinoma	72
		ADCLEC.syn1: ADGRE2- targeting 28z1XX-CAR + CLEC12A-targeting CCR	AML	73
		BCMA- or CD19-directed CAR + CD38-directed CCR	ALL Multiple myeloma	74
Adaptor CAR	Fc-binding	CD16 CAR		75
		+ EGFR antibody (cetuximab)	Pancreatic carcinoma	
		+ CD20 antibody (GA101, glycoengineered)	Lymphoma	
		+ MCSP antibody (LC007, glycoengineered)	Malignant melanoma	
		CD16 CAR		76
		+ CD20 antibody (rituximab)	Lymphoma	
		+ HER2/neu antibody (trastuzumab)	Breast cancer Gastric carcinoma	
		+ GD2 antibody (hu14.18K322A)	Osteosarcoma Neuroblastoma	
	Tag-binding	FITC-tag	Colon carcinoma Breast cancer Pancreatic carcinoma Lymphoma Mastocytoma	77
			Cervical carcinoma Lung carcinoma	78
			ALL	79
			Breast cancer	80
			Breast cancer Epidermoid carcinoma	81
		Biotin-tag	Ovarian cancer Mesothelioma	82
			Lymphoma CML	83
			Lymphoma	84
			AML	85
			Pancreatic cancer TGF-β	86
		Peptide-tag	Breast cancer	80
			Prostate cancer	87,88
			AML	89-91
			ALL	92
			Epidermoid carcinoma Pharynx carcinoma	93
	BiAb-binding/ bispecific	SAR T–cells	AML	94
			Pancreatic carcinoma mesothelioma	95

TABLE 1 (Continued)

Strategy	Details	T-cell product	Model/target	References
	molecule-binding		Melanoma	96
		P329G CAR T–cells	Breast cancer Pancreas cancer Mesothelioma	97
		iNKG2D CAR T–cells	Lymphoma Colon carcinoma	98
		RevCAR T–cells	Prostate cancer	99
	Ligand-binding	SpyCatcher immune receptor + SpyTag-labeled targeting ligands	Breast cancer Ovarian cancer Lymphoma NSCLC	100
		Barstar-based CAR (BsCAR) + ankyrin repeat (DARPin)-barnase proteins	Breast cancer	101
	Zipper-binding	zipCAR with an extracellular leucine zipper + scFv fused to cognate leucine zipper	Breast cancer CML	102
Passive control	Transient transfection	CD19 CAR	ALL	103
		MSLN CAR	Pancreatic cancer Mesothelioma CML	104
		MSLN CAR	Pancreatic cancer	105
		CD19 CAR	Leukemia	106
		NY-ESO-1-specific redirected T–cells MART-1-specific redirected T–cells p53 antigen-specific redirected T–cells	Melanoma	107
		CD20 CAR	Lymphoma	108
		GPC2 CAR	Medulloblastoma Glioma	109
		CD5 CAR	Fibrosis	110
TET-CAR		CD38 CAR Activating drug: doxycycline	Multiple myeloma	111
		CD19 CAR Activating drug: doxycycline	Lymphoma CML	112
		CD147 CAR Activating drug: doxycycline		113
		CD19 CAR Activating drug: doxycycline	Lymphoma CML	114

3.1.1 | Increase of the specificity of activation

CAR T-cells are mostly directed against tumor-associated antigens (TAA), which are upregulated compared to healthy tissues, but are not unique to cancer cells.[115] This explains the inherent risk of ON-target OFF-tumor toxicity.[7] Novel strategies to identify safe targets for CAR T cell therapy are required.[116] By targeting two antigens simultaneously (Figure 1 and Table 1), this risk can be reduced,[117,118] while eventually preventing antigen-negative escape and improving antitumor efficacy.

(a) Decrease of OFF-tumor activation

Affinity tuning. For now, high-affinity CAR-binding domains are mostly used and tested.[119] To avoid ON-target OFF-tumor toxicity, the CAR's binding domain affinity towards the target antigen can be reduced, leading to passive toxicity control (Table 1).[19-28] Tumor cells would still be targeted due to their high antigen expression; but non-malignant cells with low antigen expression less frequently. Generation of optimal low-affinity binders derived from existing antibodies might facilitate the development of more functional and selective CAR binding domains.[119] The strategy was evaluated with anti-CD19 CAR T-cells for hematological diseases.[19-21] The approach is better suitable for antigens whose expression on healthy tissues is low but upregulated on malignant tissues such as HER2/neu (ErbB2), EGFR, C4 folate receptor-alpha (αFR), GD2, GPC2 and CD38.[22-28] On the other hand, if tumor cells express only low levels of the antigen, tumor escape might occur.[120]

(b) Increase of ON-tumor activation

OR-gate CAR. OR-gate CAR (1 or 2 strategy) target multiples tumor antigens (Table 1) and have been developed to increase specificity of

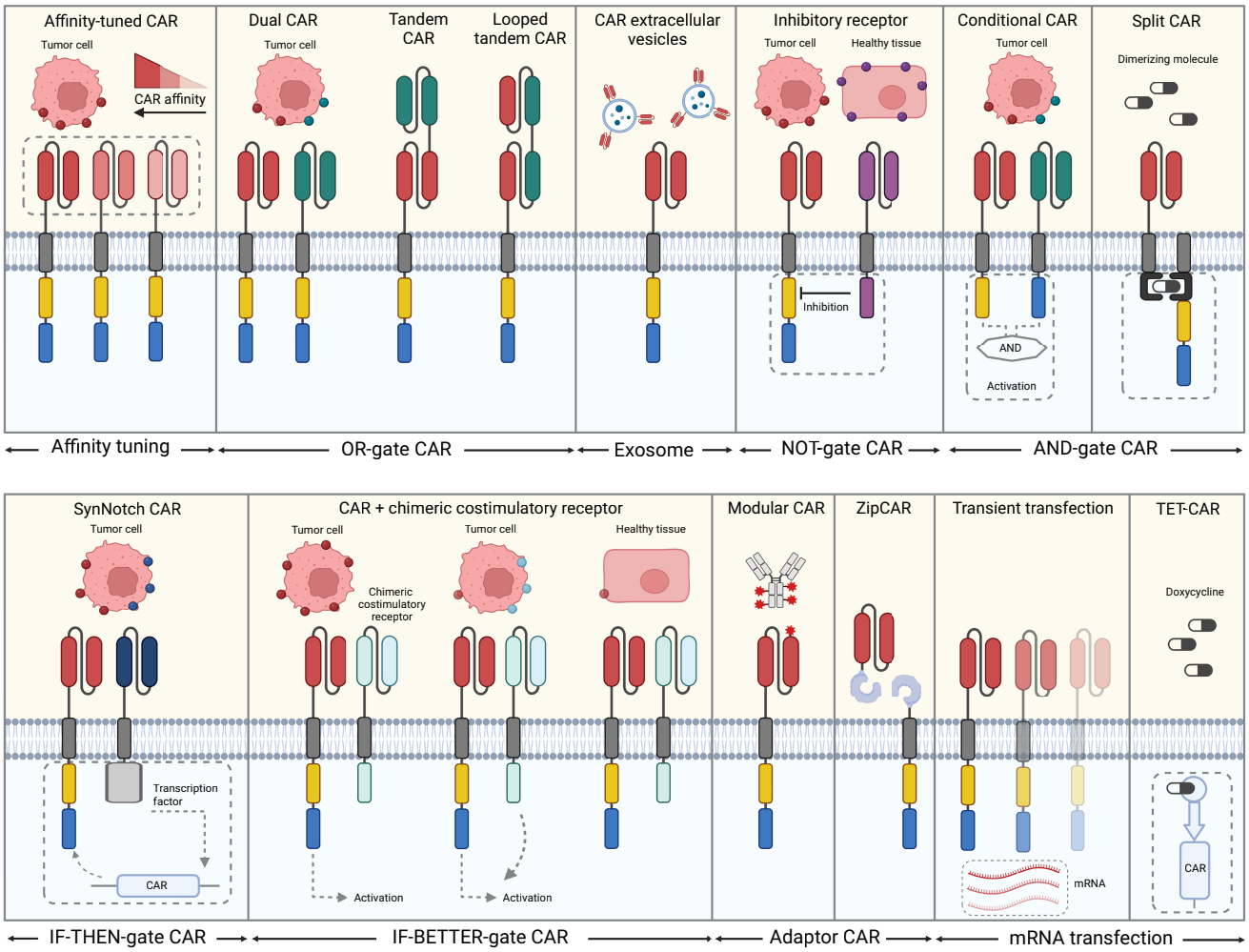

FIGURE 1 Strategies preventing toxicity of CAR T-cell therapy.

CAR T-cells toward tumor cells. To achieve combinatorial target antigen recognition, multiple CAR are used.[117,118] This can be performed by administering a combination of two different CAR-transduced T-cell populations in a specific ratio (NCT03620058).[29,30] Another possible approach is the transduction of T-cells with two viral vectors encoding for two different CAR[30-32] or the transduction of TCR-specific T-cells with a CAR to generate dual-targeting T-cells.[33] In such situations, each CAR/TCR is intact and can mediate full activation upon antigen contact. Another strategy is the use of a (looped) tandem CAR, which contain two scFv domains connected by a linker in a single CAR molecule.[117,118] These CAR T-cells can mediate cytotoxicity against tumor cells expressing either the first antigen, the second antigen or both antigens.[34-37,44] *Bivalent* CD19/CD22 CAR T-cells with a looped tandem CAR composition[121] are currently already under clinical investigation (NCT03448393).[37] Analysis of the clinical outcome led to the development of a novel *bicistronic* CD19.28ζ/CD22.BBζ construct with enhanced cytokine production against CD22 in preclinical models.[37] Another strategy targeting CD19 and CD22 with *bicistronic* CAR T-cells (AUTO3) was tested in patients with ALL[38] (AMELIA trial: NCT03289455) as well as in relapsed/refractory DLBCL[39] (ALEXANDER trial: NCT03287817) and convincing primary results have been reported. As the position of the two scFv domains has an influence on their functionality, these CAR T-cells still require optimization.[117] While such approaches reduce the risk of antigen-negative escape, they do not address the problem of ON-target OFF-tumor toxicity. Ongoing clinical trials are currently testing dual or tandem CAR T-cells (NCT03330691, NCT02443831,[31] NCT03185494, NCT03097770, NCT03019055, NCT04844866, NCT04160195, NCT03375619, NCT05442515, NCT05797233, NCT03233854,[41] NCT05507827, NCT04029038, NCT04007029[42] and NCT04186520[43]) simultaneously targeting B–cell-associated target antigens (CD19, CD20 and/or CD22) for the treatment of Bcell malignancies. Additionally, there are several clinical phase 1 or 2 trials testing BCMA as target combined with CD19 like the MCARTY study (NCT04795882), CD38, CS1 or PD1 for MM, and some already reported enhanced clinical activity.[122] Another

strategy is to use scFv for CAR, which has been derived from an antibody targeting CD19, CD20 and CD22, the so-called "TriCAR" (NCT05010564, NCT05094206).

CAR exosome delivery. Extracellular vesicles derived from CAR T-cells have the potential to overcome current limitations of CAR T-cell therapy (Table 1). CAR T-cells release extracellular vesicles, mostly in the form of exosomes that carry the CAR on their surface and contain highly cytotoxic molecules that, when applied can reduce tumor growth.[45-47] CAR exosomes target antigens on malignant cells and can induce contact-dependent cytotoxicity. CAR exosomes can be derived from any parent cells in vitro to develop an "off-the-shelf" product.[45] The approach was preclinically tested in CD19 CAR,[45] in HER2 CAR and EGFR CAR.[46,47]

NOT-gate CAR. NOT-gate CAR (1 not 2 strategy) inhibit OFF-tumor CAR T-cell activation by introducing two CAR constructs with opposing effects: a stimulatory CAR recognizing TAAs as well as an inhibitory CAR (iCAR) recognizing healthy tissue antigens, which is coupled to the signaling domain of an inhibitory co-receptor (Table 1).[117,118] Once an iCAR is activated, it temporarily inhibits CAR T-cells by either activating intracellular inhibitory signaling pathways or by cleaving the stimulatory CAR. The first approach relies on the incorporation of intracellular domains of cytotoxic T lymphocyte-associated protein-4 (CTLA-4) or programmed cell death-1 (PD-1) in an iCAR, leading to a temporary and reversible inhibition of T-cells upon iCAR activation.[48] Thus CAR T-cells only mediate cytotoxicity when the tumor antigen is present and the iCAR-targeting antigen is absent.[48] This approach can also be used to target healthy tissue antigens that have been downregulated on tumor tissue through heterozygous gene loss, as exemplified by the combination of an anti-mesothelin (anti-MSLN) CAR T-cells[49] or anti-CEA CAR T-cells[50] (EVEREST-1 trial: NCT05736731) and an HLA-A*02-gated inhibitory receptor on CAR T-cells. Another strategy is substantiated by the "*Signal-CAR*" and "*Scissors-CAR*" platform. The "*Signal-CAR*" recognizes a protein on tumor cells, while the "*Scissors-CAR*" recognizes another protein on normal cells which then cleaves and inhibits the "*Signal-CAR*" upon activation.[51] Thus again, CAR T-cells only mediate cytotoxicity in the presence of the tumor antigen and in the absence of healthy tissue.[51]

3.1.2 | Modulation of the intensity of activation

CAR T cell activation can also be controlled by modulating the intensity of the activation signal. This can be achieved through a second controllable stimulus for CAR T–cell activation or through adjustable/controllable CAR surface expression (Figure 1 and Table 1).

(a) ON-switch CAR T-cell systems

AND-gate CAR. Additional activation steps for CAR T-cell activation might be achieved by using so-called *AND-gate CAR* (1 and 2 strategy) which include two different signaling components needed for activation (Table 1). For this trans-signaling or combinatorial CAR strategy, T-cell activation signal 1 (CD3ζ) is physically dissociated from the costimulatory domain (CD28 or 4-1BB) in two CAR with different antigen specifity.[117] The optimal effector function of these CAR T-cells is only achieved when both target antigens are bound. However, cells expressing only one of the antigens might still be eliminated, albeit not efficiently, leading to certain ON-target OFF-tumor toxicity.[52-54]

A so-called Split-CAR consists of two separate parts: an extracellular scFv attached to intracellular costimulatory domains and an intracellular down-stream signaling element containing the ITAMs of the TCR CD3ζ subunit.[117,118] Both elements contain heterodimerization domains, which interact with each other only after addition of a chemical inducer of dimerization (CID), leading to CAR T-cell activation.[117,118] The dosage, duration of application and half-life of the CID allow for external control of CAR T-cell activity.[117,118] One approach includes a scFv fused to FK506-binding protein (FKBP), allowing for rapamycin-induced heterodimerization with membrane-bound signaling domains fused to FKBP-rapamycin binding domain (FRB).[55-58] Extracellular heterodimerization of soluble scFv with membrane-anchored costimulatory domains was also shown for a sub-immunosuppressive dose of rapamycin and FKBP/FRB domains.[58] Such called dimerizing agent-regulated immunoreceptor complex (DARIC) T-cells can be re-directed against a second antigen by using DARIC plug-in targeting a second antigen.[58] Rapamycin has a certain toxicity and must be applied with caution. Further development was made by switching from the human FKBP/FRB combination to the structurally unrelated Arabidopsis gibberellin-induced dimerization domains (GID1/GAI).[59] However, Gibberellic acid is plant-derived and therefore likely immunogenic.

The lipid-permeable tacrolimus analog rimiducid can also be used to mediate inducible dimerization of MyD88/CD40 (iMC) to activate downstream Toll-like receptor (TLR) and CD40 signaling.[60] The iMC molecule is composed of truncated MyD88 and CD40 fused in frame to tandem FKBP12v36 domains.[60] The iMC is then co-expressed with a CAR.[60] BPX-601 CAR T-cells targeting PSCA and BPX-603 CAR T-cells targeting HER2/neu were engineered to express a rimiducid-inducible signaling domain which functions as a molecular "go-switch" to enhance activation and proliferation and were tested in clinical trials (NCT02744287, NCT04650451).

However, ON-target OFF-tumor toxicity is not prevented with this system, as the distribution of the drug is hard to control. Another approach harnessed a mutated E3 ubiquitin ligase domain ($CRL4^{CRBN}$) in combination with a Cys^2-His^2 (C2H2) zinc finger degron motif, which homodimerize upon lenalidomide administration and thus induce a functional ON-state in CAR T-cells.[61] The here described lenalidomide ON-switch split CAR uses CRBN and the zinc finger degron from IKZF3.[61]

Novel designs include CAR T-cells with an inducible Turbo (iTurbo) cytokine signaling domain[62,63] and light-switchable CAR (LiCAR) T-cells.[64] The iTurbo domain can be activated by the dimerizer AP1903, and different iTurbo domains program iTurboCAR T-cells towards different phenotypes.[62] LiCAR T-cells were created by splitting the intracellular functional domains of the CAR and installing

photo-responsive modules into each half of a split CAR.[64] The T-cells could only be activated by the presence of blue light.[64] These LiCAR T-cells were combined with surgically removable upconversion nanoplates (UCNPs) that have enhanced near infrared (NIR)-to-blue upconversion luminescence, thus serving as a miniature light transducer.[64] The UCNPs mediate inducible CAR T-cell activation upon stimulation with deep tissue-penetrable NIR light.[64] Furthermore, the fact that the tumor microenvironment is hypoxic can be exploited for CAR T-cell therapy. For example, a CAR fused to the oxygen-sensitive subdomain of HIF1α (HIF CAR) is only effective in the hypoxic tumor microenvironment, thus limiting off-tumor activation.[67]

The avidity-controlled CAR (AvidCAR) platform is a combination of different strategies. It combines a CAR dimerization domain and at least two low-affinity antigen-binding domains.[65] There are different variants of this AvidCAR. It can be an ON-switch AvidCAR that only mediates activation when both antigen-binding domains bind their target antigen and when a dimerization molecule is added. Or it can be an AND-gate AvidCAR in which both antigen-binding domains recognize two different target antigens to induce CAR T−cell activation with no need for a dimerization molecule.[65]

Co-opting signaling molecules can enable logic-gated control of CAR T-cells, in which CD3ζ was replaced with intracellular proximal T-cell signaling molecules.[66] ZAP-70 CAR showed sufficient efficacy, while bypassing upstream signaling proteins.[66] LAT and SLP-76, phosphorylated by ZAP-70, were used to an engineer logic-gated intracellular network CAR (LINK CAR).[66]

IF-THEN-gate CAR. The optimal effector function of these AND-gate CAR T-cells is only achieved when both target antigens are present on a tumor cell. Cells expressing only one of the target antigens might still be eliminated, albeit not efficiently, leading to certain ON-target OFF-tumor toxicity.[52-54] To reduce this risk, another dual-receptor AND-gate approach was developed with an exclusively priming signaling receptor without activating signaling capacity. Upon recognition of a tumor antigen, these synNotch receptors undergo induced trans-membrane cleavage like the wild-type Notch receptor[123] to release an intracellular transcription factor, which in turn induces the expression of a CAR targeting another tumor antigen (Table 1).[68-71,123] These synNotch CAR T-cells can mediate cytotoxicity against tumor cells expressing both antigens and leave single antigen-expressing tumor cells out (*IF-THEN-gate CAR*). The technology was tested with anti-GFP, anti-HER2/neu, anti-CD19 and anti-EGFRvIII synNotch CAR T-cells.[68-71,123]

IF-BETTER-gate CAR. The *IF-BETTER-gate CAR* can mediate cytotoxicity only when the target antigen is highly expressed. If the target antigen is expressed at low levels, killing is only initiated if another antigen recognized by a chimeric costimulatory receptor (CCR) is present (Table 1).[118] Avidity and costimulation is increased by interaction of the CCR with the specific target antigen. In case of healthy tissue only expressing the CAR-antigen in low levels but not the CCR-antigen, no cytotoxicity is mediated. With this concept CAR sensitivity is focused on malignant cells which express both selected antigens. Transducing T-cells with a CAR providing suboptimal activation upon binding of the first antigen and a CCR recognizing a second antigen revealed cytotoxicity against prostate cancer cells only if expressing both PSCA and PSMA.[72] The combinatorial CAR construct ADCLEC.syn1 consists of an ADGRE2- targeting 28z1XX-CAR and a CLEC12A-targeting CCR for application against AML.[73] The killing capacity of anti-BCMA CAR and anti-CD19 CAR T-cells against multiple myeloma and acute lymphoblastic leukemia with low antigen density was enhanced by concomitant high-affinity engagement of a CD38-binding CCR.[74] These *IF-BETTER-gate* CAR T-cells can reduce toxicity against healthy tissue.

Adaptor CAR T-cell platforms. Modular (universal) CAR T-cell platforms (Table 1) comprise both a T-cell product and an intracellular or extracellular CAR-adaptor molecule necessary for CAR activation.[124-126] Therefore they can be considered as *AND and OR logic gating* strategies. The major advantages of this system are that the administration of the adaptor molecule can be paused in case of adverse events and that multiple antigens can be targeted by applying different adaptor molecules.[124-126] Therefore, only one universal T-cell product has to be engineered and could then be combined with various adaptor molecules. This reduces labor-intensive and cost-intensive CAR development.

Modular (universal) CAR T-cell platforms can be subdivided into Fc-binding CAR, Tag-specific CAR, antibody (Ab)-binding/bispecific molecule-binding CAR and ligand-/zipper-binding CAR. The pharmacokinetics and biodistribution of the adaptor molecule influence the therapeutic efficacy. The clear advantage of Fc-binding CAR, which bind the Fc part of IgG antibodies mostly through a CD16 (FcγRIII) extracellular binding domain, is that they can be combined with clinically approved monoclonal antibodies like rituximab, trastuzumab, or cetuximab.[75,76] However, these CAR T-cells cannot discriminate between administered monoclonal and endogenous antibodies, raising concerns of autoimmune side effects.[75,97]

Tag-specific or anti-tag CAR T-cells recognize a tag which is chemically, enzymatically, or genetically coupled to a tumor-targeting moiety[124-126] such as the synthetic dye fluorescein isothiocyanate (FITC),[77-81] biotin[82-86] or peptide tags.[80,87-93] The introduction of these tags by genetic fusion requires additional quality control steps of the adaptor molecule and increases the risk of immunogenicity. FITC-specific CAR T-cells are currently evaluated in a Phase I trial in osteogenic sarcoma (NCT05312411). The so-called AdCAR T-cell system is redirected to surface antigens via biotin-labeled adapter molecules and was tested in aggressive lymphoma models,[84] in AML[85] and for the detection of soluble latent TGF-β within the TME of a pancreatic tumor model.[86] Another well-established system is the UniCAR strategy, which is based on an anti-epitope scFv used in the modular BiTE format UniMab and UniCAR T-cells recognizing the peptide epitope.[127] The epitope is derived from human nuclear autoantigen La/SS-B.[127] The system was evaluated with CD123 (Phase 1 trial: NCT04230265) and CD33 in AML[90,91] and with PSCA and PSMA (Phase 1 trial: NCT04633148) in prostate cancer[87,88] and EGFR-positive tumor models.[93]

Bispecific antibodies (BiAb) or bispecific T-cell engagers can effectively redirect T-cells by targeting both tumor cells and a

synthetic receptor on T-cells.[124] Promising results were shown with BiAb and synthetic agonistic receptor (SAR)-transduced T-cells.[94-96] However, since SAR T-cells have not been as extensively studied as CAR T-cells, the hurdles to getting a SAR-based concept approved are anticipated to be higher than for CAR T-cells. A novel development in antibody technologies are effector-silenced antibodies generated by the introduction of P329G mutations in their Fc part,[128] which can be targeted by P329G-directed CAR T-cells (NCT05199519).[97,129] Also other bispecific molecules can also be used as CAR adaptor molecules. The humanized artificial receptor platform termed RevCAR reduces the size of the CAR, minimizes nonspecific antigen binding and antigen-independent tonic signaling caused by scFv dimerization.[99] RevCAR are inactive and only become active in the presence of bispecific antibody-based target modules (RevTM) consisting of two scFv. One recognizes the target antigen, and the other binds the short peptide epitode of the RevCAR.[99]

Another system is the T-cell-directed SpyCatcher immune receptor combined with SpyTag-labeled targeting ligands (SpyCatcher-SpyTag chemistry).[100] An inert form of the human NKG2D extracellular domain (iNKG2D)-specific CAR T-cells can be combined with a bispecific adapter comprised of an iNKG2D-exclusive ULBP2-based ligand fused to an antigen-targeting antibody.[98] Another modular platform combines ankyrin repeat (DARPin)-barnase proteins and a barstar-based CAR (BsCAR).[101] Multiple tumor antigens can be targeted with a single BsCAR by changing the DARPin-barnase switches.[101]

Another universal CAR system is the split, universal, and programmable (SUPRA) CAR system.[102] The system is composed of a zipCAR consisting of a leucine zipper as extracellular domain combined with a soluble zipFv which is a scFv fused to a cognate leucine zipper that can bind to the leucine zipper on the zipCAR.[102] This SUPRA CAR concept responds to combinatorial antigens in target cells (AND-gate strategy) and enables ON/OFF switching for fine-tuning of T-cell activation.

(b) Modulation of CAR expression by mRNA transfection

Transient transfection. Conventional CAR T-cells undergo transduction with a viral or nonviral vector encoding for the CAR, leading to the genomic integration of this information.[2] Another approach is the use of CAR-encoding mRNA to generate CAR T-cells (Table 1).[103-110] CAR expression is thereby limited by T-cell division and mRNA degradation and thus inherently transient.[103-110] When no more T-cells are infused, the number of T-cells will decrease over time, leading to the need of repeated infusions to maintain an ongoing antitumor attack and the risk of a reduced antileukemic effect.[108]

TET-ON/TET-OFF CAR. CAR T-cell regulation can occur transcriptionally with the Tetracycline (TET)-ON/TET-OFF system, in which a tetracycline analog can be used to activate CAR T-cells.[111-114] The system relies on doxycycline-dependent production of CAR mRNA and thus of CAR expression.[112] A certain risk of CAR T–cell elimination exists as the system is bacteria- and virus-derived, leading to potential immunogenicity. Like transient transfection, this strategy shows limited clinical applicability as the system relies on RNA degradation and thus cannot react rapidly.

3.2 | Management of CAR T-cell-mediated toxicity by systemic T-cell inhibition

In clinical settings, CAR T-cell-associated toxicities are currently managed symptomatically, underlining the need for approaches which directly target and inactivate CAR T-cells either transiently or permanently (Table 2 and Figure 2). Such exogenous, CAR-independent strategies rely on the administration of small molecules, which activate a reversible OFF-switch in CAR T-cells or induce their transient or permanent elimination. These strategies depend on the pharmacokinetics and pharmacodynamics, tissue distribution and availability as well as drug-associated side effects of the administered molecule.

3.2.1 | Transient

Antibodies

Current management of adverse events like CRS and ICANS relies on the mitigation of the cytokine response involved in these events (Table 2). At the moment, this includes CAR-independent approaches such as corticosteroids (ZUMA-24 trial: NCT05459571), tocilizumab and anakinra (NCT04205838, NCT04148430, NCT04359784, NCT03430011).[15-17] A more novel monoclonal antibody is siltuximab, which can also be used to treat CRS (NCT04975555, NCT05665725).[130] Analysis revealed that CAR T cell patients affected by adverse events have high GM-CSF serum levels.[184] The use of the GM-CSF neutralizing antibody lenzilumab in combination with CAR T-cells has the potential to prevent CRS and ICS[131,132] and was tested in the ZUMA-19 trial (NCT04314843).[133] Other strategies include targeting IFN-γ, TNF-α or IL-6.[185,186]

Kinase inhibitors

Another approach to reduce CAR-mediated toxicities is the inhibition of important signaling pathways for T-cell survival (Table 2).[91,136-138] This can be achieved using the tyrosine kinase inhibitor (TKI) dasatinib, which induces a functional OFF state in T-cells without decreasing their viability.[134-138] Addition of dasatinib suppresses effector functions like cytotoxicity, cytokine production as well as proliferation and was shown to prevent fatal CRS in a mouse model.[136,137] Importantly, due to its short half-life, the switch between ON and OFF states can be performed quite easily and is clinically feasible.[187] Similar approaches such as other SRC-inhibiting TKI (ponatinib, saracatinib),[138] MAPK pathway inhibitors (refametinib, trametinib),[138] calcineurin inhibitors (tacrolimus)[138] and FLT3-inhibiting TKIs (midostaurin)[91] also demonstrated a suppressive effect on the cytotoxic capacity of CAR T-cells. Itacitinib (INCB039110), a JAK1 Inhibitor, can

TABLE 2 Overview about strategies managing CAR T–cell treatment-associated toxicity.

Strategy	Details	T-cell product	Model/target	References
Transient				
Antibodies	IL-6 antagonist	CD19 CAR BCMA CAR Antibody: siltuximab	Lymphoma ALL MM	130
	CSF2/GM-CSF-targeting	CD19 CAR Antibody: lenzilumab	ALL	131
		CD22 CAR Antibody: lenzilumab	Lymphoma	132
		CD19 CAR Antibody: lenzilumab	Lymphoma	133
Kinase inhibitors	TKI	T–cells Inhibitor: dasatinib	/	134,135
		CD19 CAR Inhibitor: dasatinib	CML Lymphoma ALL	136,137
	Calcineurin inhibitor	CD19 CAR Inhibitor: tacrolimus	ALL Lymphoma	138
	MAPK pathway inhibitor	CD19 CAR Inhibitor: refametinib, trametinib		
	TKI	CD19 CAR Inhibitor: ponatinib, dasatinib, saracatinib		
	FLT3-inhibiting TKI	CD33 CAR Inhibitor: midostaurin	AML	91
	JAK1 inhibitor	CD19 CAR Inhibitor: itacitinib	ALL Lymphoma	139
Enzyme inhibitors	Inducer of intracellular proteolysis	Bromodomain-fused CAR Compound: proteolysis-targeting chimaera (PROTAC)	ALL NK cell leukemia	140
	Protease inhibitor/ proteasomal degradation	SNIP B7H3.BBz CAR Compound: grazoprevir	ALL Medulloblastoma	141
		SNIP HER2/neu CAR Compound: grazoprevir	Osteosarcoma	141
		SNIP GD2.BBz CAR Compound: grazoprevir	Neuroblastoma	141
		SWIFF-CAR Compound: asunaprevir	Lymphoma	142
		VIPER CAR Compound: antiviral protease inhibitor	Xenograft tumor model	143
		FKBP/FRB pair + GD2 CAR Compound: shield-1	Mesothelioma Neuroblastoma	144,145
		CD19.41BB.CD3z CAR + IKZF3 zinc finger degron tag Compound: lenalidomide/ pomalidomide	Mantle cell lymphoma ALL	61
Fibrinolytic	Endothelial cell protection	CD19 CAR Compound: defibrotide	Lymphoma	146
Permanent				
Suicide genes	Metabolic/enzymatic	T-cells (in context of allogeneic stem cell transplantation) Suicide gene: HSV-TK	AML ALL CML Lymphoma	147-152
		PBMCs Suicide gene: HSV-TK	AML CML NHL	153
		T–cells	CML	154

TABLE 2 (Continued)

Strategy	Details	T-cell product	Model/target	References
		Suicide gene: TMPK		
		Tumor cells Suicide gene: TMPK	Prostate cancer	155
		EBV-T–cells Suicide gene: TMPK, HSV-TK, iCasp9, CD20	B cell malignancies	156
		E. coli Suicide gene: cytosine deaminase (CD) converts 5-fluorocytosine (5-FC) to cytotoxic 5-fluorouracil (5-FU)	/	157
	Dimerization inducing	Tumor cells Dimerization domain: iFAS Elimination: AP1903 (rimiducid)	Fibrosarcoma	158
		GD2 CAR Dimerization domain: Casp9 Elimination: AP1903 (rimiducid)	Melanoma	159
		CD33 CAR Dimerization domain: Casp9 Elimination: AP 1903 (rimiducid) + additive effect with BCL-2 inhibitor ABT-199, the pan-BCL inhibitor ABT-737, or mafosfamide	AML	160,161
		CD19 CAR Dimerization domain: Casp9 Elimination: AP1903 (rimiducid), AP20187	Lymphoma	162,163
		CD123 CAR Dimerization domain: Casp9 Elimination: rapamycin	AML	164
		Allodepleted T–cells Dimerization domain: Casp9 Elimination: AP1903 (rimiducid)	GvHD	165,166
		CD20 CAR Dimerization domain: Casp9 Elimination: AP1903 (rimiducid), AP20187	Lymphoma	167
		EBV-CTLs Dimerization domain: Casp9 Elimination: AP20187	Lymphoblastoid cell lines	168,169
		SLAMF7 CAR Dimerization domain: Casp9 Elimation: AP1903 (rimiducid)	Multiple myeloma	170
Elimination marker targeted by monoclonal antibodies	CD52	CD123 CAR Elimination: alemtuzumab	AML	171,172
		CD4 CAR Elimination: alemtuzumab	ALL	173
	CD20	Cytotoxic T lymphocytes Elimination: rituximab	CD20-transduced T-cells	174-177
		CD123 CAR Elimination: rituximab		171,172
		GD2 CAR Elimination: rituximab		178
	c-myc	gp100 TCR T–cells Elimination: tag-specific antibody	Myc-tagged T-cells	179
	EGFRt	CD19 CAR Elimination: cetuximab	ALL Lymphoma Epidermoid carcinoma	180-182
	FR806 (EGFR/FOLR1)	CD19 CAR Elimination: CH12	Lymphoma	183

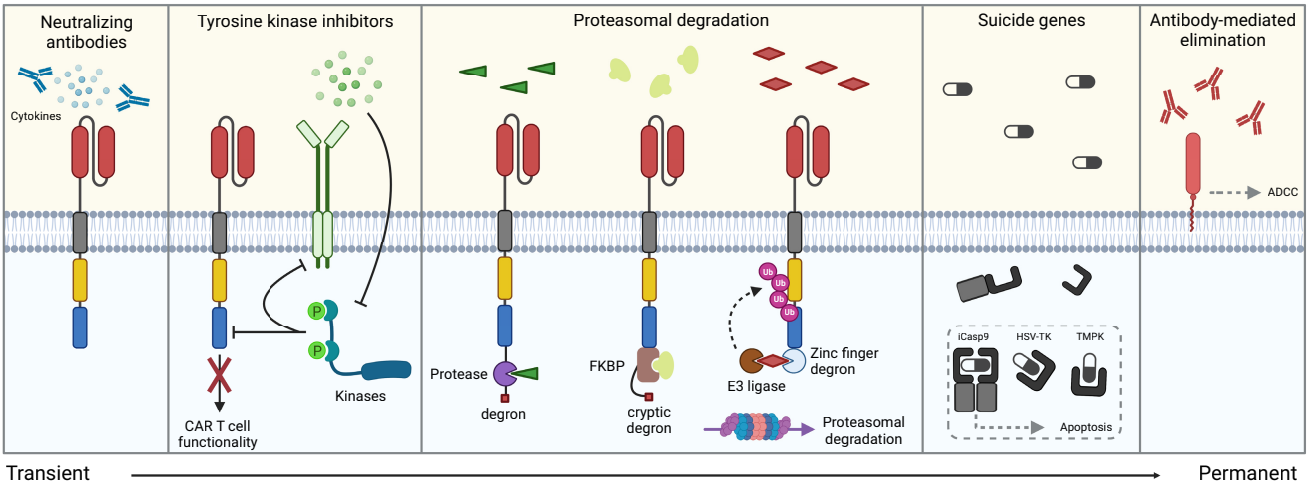

FIGURE 2 Strategies treating toxicity of CAR T-cell therapy.

reduce CRS and is being tested in a phase II clinical trial (NCT04071366).[139]

Enzyme inhibitors

Another strategy for reversible CAR T-cell inhibition involves enzyme inhibitors to induce a functional ON/OFF-state in CAR T-cells (Table 2). This can be achieved by adding a bromodomain (BD) to a CAR, which mediates the reversible degradation of the CAR upon interaction with a proteolysis-targeting chimaera (PROTAC) compound.[140] Protease/protease-inhibitor interactions can also be used to control CAR expression in a reversible manner. Signal neutralization by inhibitable protease (SNIP) CAR T-cells include a hepatitis-C-derived NS3 protease domain, which cleaves and inactivates CAR, thus inducing a functional OFF-state. The addition of a protease inhibitor (grazoprevir), however, prevents CAR cleavage, resulting in CAR activation.[141] A similar approach incorporates an auto-cleaving degradation moiety including the HCV-NS3 protease into the CAR construct under control of a protease/protease inhibitor pair (SWIFF-CAR). In contrast to the SNIP-CAR, activation of the protease of the SWIFF-CAR results in enzymatic cleavage of the degradation moiety (degron) and CAR expression.[142] Inhibition of CAR expression and CAR-mediated T–cell activation can be achieved by using the protease inhibitor asunaprevir, which inhibits the cleavage of the degradation moiety of the CAR.[142] Both approaches use the hepatitis-C-derived NS3 protease, which has the risk of immunogenicity and can limit these approaches. Whereas VIPER CARs (versatile protease regulatable CARs) are engineered with a viral protease domain and under the control of antiviral protease inhibitors.[143] Other degron-based approaches include a ligand-induced degradation (LID) domain in combination with Shield-1,[144,145] or a zinc finger degron in combination with lenalidomide which leads to lenalidomide-induced $CRL4^{CRBN}$-mediated ubiquitination and proteasomal degradation of the CAR.[61]

Fibrinolytic

Endothelial cell activation from systemic inflammation is a key driver of ICANS.[188] Defibrotide, an FDA-approved drug for the treatment of hepatic veno-occlusive disease,[189] can be used to prevent ICANS (Table 2). While there is no preclinical data available on the effects of defibrotide on CAR T cell-related toxicities, a phase 2 study (NCT03954106) is evaluating defibrotide for the prevention of CART-cell–associated neurotoxicity.[146] The study was terminated when its primary endpoint was unlikely to be met.[146]

3.2.2 | Permanent

(a) Suicide genes

Suicide genes are genetically encoded elements allowing the targeted elimination of cells through the application of an activating pharmaceutical agent (Table 2).[190,191] If integrated into CAR T-cells, these genes can be used as OFF-switches to control CAR T-cell activity.

Metabolic. Metabolic suicide gene systems represent a gene-directed enzyme prodrug therapy which relies on the conversion of a nontoxic drug to a toxic compound in gene-modified cells as a method for eliminating transduced cells (Table 2).[191] Well established suicide gene systems for cellular therapy are the herpes simplex virus-thymidine kinase (HSV-TK)[147-153] and human thymidylate kinase (TMPK)[154-156] systems. HSV-TK can produce cytotoxic tri-phosphorylated nucleoside analogs which interfere with DNA synthesis. Ganciclovir, aciclovir and brivudine can be used as pro-drugs for the system, of which ganciclovir is the most promising.[191,192] Disadvantages include the slow ganciclovir-mediated HSV-TK activation,[156] risk of intrinsic immunogenicity,[152,193] and impossibility to treat CMV infections in stem cell transplanted patients with ganciclovir without eliminating the transduced cells.[194-196] As TMPK is human, the risk of immunogenicity is low. Another metabolic suicide gene system is cytosine

deaminase (CD), which converts 5-fluorocytosine (5-FC) into cytotoxic 5-fluorouracil (5-FU).[157]

Dimerization inducing. Further strategies to eliminate CAR T-cells to mitigate toxic side effects are based on the administration of an exogeneous chemical inducer of dimerization (CID),[191] which induces the dimerization and activation of components of the apoptotic pathway (Table 2) such as inducible FAS (iFAS)[158] or inducible Caspase9 (iCasp9).[159-169] The iCasp9 gene contains the intracellular portion of the caspase 9 protein fused to a drug-binding domain derived from human FKBP.[169] AP1903 (rimiducid) or AP20187 as CIDs mediate crosslinking of the drug-binding domains, dimerization of Casp9 and apoptosis.[159,161,197] Another possible CID is the safe and easily accessible immunosuppressive drug rapamycin, which can lead to caspase 9 activation through heterodimerization of FKBP12 with the FRB fragment of mTOR in an adapted iCasp9 system.[190] Multiple preclinical and clinical studies have assessed suicide switches using rimiducid to control CAR T-cells, including anti-CD19 CAR T-cells (NCT03016377, NCT03594162, NCT03696784), anti-BCMA CAR T-cells (NCT04960579), anti-SLAMF7 CAR T-cells (NCT03958656),[170] anti-MSLN CAR T-cells (NCT02414269, NCT02792114), anti-PSMA CAR T-cells (NCT04249947) and anti-GD2 CAR T-cells (NCT04196413,[198] NCT01953900, NCT01822652, NCT03721068, NCT02107963). Early results of the NCT04196413 trial underscore the benefit of this treatment for patients with H3K27M-mutated diffuse intrinsic pontine glioma or spinal cord diffuse midline gliomas.[198]

(b) Elimination markers

Co-expression of a targetable marker on CAR T-cells enables antibody-mediated T-cell control and/or elimination (Table 2).[171-183] CAMPATH-1 antigen or CD52 is present on the surface of mature lymphocytes, but not on corresponding stem cells. Anti-CD52 antibodies like alemtuzumab can be used to deplete CAR T-cells (NCT05607420)[171-173]; however, this approach leads to the complete elimination of T-cells causing a relevant immunodeficiency. Other well-established targets include CD20, targetable by anti-CD20 antibodies such as rituximab,[174-178] and the truncated epidermal growth factor receptor (EGFRt), targetable by anti-EGFR antibodies such as cetuximab[180-182] or a EGFR/FOLR1 fusion receptor (designated as FR806) targetable by a monoclonal antibody recognizing the 806 epitope which cannot bind wild-type EGFR in healthy tissues.[183] EGFRt-mediated CAR T-cell elimination is incorporated in several CAR T-cell clinical trials targeting CD171 (NCT02311621), CD19 (NCT02028455, NCT01865617, NCT02146924, NCT02051257, NCT05625594, NCT03085173, NCT03103971), CD22 (NCT03244306), CD123 (NCT02159495), B7H3 (NCT04483778), EGFR (NCT03618381) and MUC16^{ecto} (NCT02498912). Other targets are a 10 amino acid tag of the c-myc protein.[179] However, the cytotoxic effect of this approach relies on complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC), which can be limited in heavily pretreated CAR T-cells patients.[199] Pharmacokinetics and pharmacodynamics, tissue distribution and availability of the antibody might also limit cytotoxicity. Cell-based elimination strategies, for instance using anti-CAR19 CAR T-cells to deplete anti-CD19 CAR T-cells, have been developed to overcome these limitations.[200]

4 | CONCLUSIONS

Modern cancer therapy has profited enormously from immunotherapies and particularly from CAR T–cell therapy. Patients with CD19+ and BCMA+ tumors are already being treated with CAR T-cell products on a regular basis. However, these advances have also created new challenges in terms of patient management concerning side effects and intensification of medical care. The medications used to treat adverse events endanger the still persisting and functioning CAR T-cells. Strategies are needed to bring CAR T-cells into a functional ON/OFF state to improve the general condition of patients without jeopardizing the therapeutic success of the treatment. In this review, we discuss several strategies that have been developed to address this dilemma. Pharmacological, CAR-independent or nonautonomous control strategies have been introduced which all rely on a small molecule or antibody being administered to either activate a suicide gene, leading to irreversible T–cell elimination, or to promote CAR T–cell activation in a controlled and reversible manner. Other strategies involve novel CAR T–cell constructs, which regulate themselves in an autonomous self-switch manner but whose activity cannot be controlled anymore after administration. These systems exploit the capacity of cells to integrate multiple signals into a coordinated response, essential for overcoming the current challenges of solid-tumor therapy.

The listed approaches target different aspects of CAR Tcell functionality to control their activity. For now, it is difficult to predict, which strategy will succeed on the long run. Ongoing clinical trials will provide more insights about the safety and feasibility of these novel strategies in the near future.

Even though all these strategies seem very promising, development costs of the products and additional regulatory burden might reduce the feasibility for a broad clinical application. Ultimately, clinical application of such modular approaches will rely heavily on an adequate ratio between enhanced efficacy and safety as well as feasibility in relation to costs. Such ratio and its adequacy for a given health care system remain to be defined.

These new strategies give hope for expanding the clinical application of T–cell-based products. However, the financial burden and regulatory steps needed to approve these novel strategies still limit the potential beneficial effects for patients already treated with CAR T-cells.

AUTHOR CONTRIBUTIONS

Sophia Stock, Anna-Kristina Klüver, Luisa Fertig and Vivien D. Menkhoff drafted the manuscript. Sophia Stock created the figures. Marion Subklewe, Stefan Endres and Sebastian Kobold critically reviewed the manuscript. All authors have read and agreed to the published version of the manuscript. The work reported in the paper

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DATA AVAILABILITY STATEMENT

Data are available on reasonable request. All data relevant to the study are included in the article or uploaded as online supplemental information.

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