CAR-T cell and Personalized Medicine

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Abstract

Adoptive T cell transfer (ACT) is a new era for cancer treatment, involving infusion of autologous lymphocytes. Chimeric antigen receptors (CAR) on the surface of T cells are emerging as a novel therapeutic that is giving other direction to T-cell specificity and precision medicine. T cells are engineered modification to recognize specific target antigen and are co-stimulated with intracellular signal to increase the T cell response. CAR-T cells have impressive involvement in outcome on hematological malignancies; however severe toxicities as cytokine release syndrome or neurotoxicity are a challenge to face. Solid tumors have heterogeneous antigens and tumor microenvironment that hinder CAR-T cell efficacy and increase the risk of on-target/off-tumor. Novel strategies to increase CAR-Ts specificity, safety and efficacy are ongoing in clinical trials to improve clinical outcomes in hematological and solid malignances.

Keywords

Adaptive immune system · Chimeric antigen receptor (CAR) · Hematological malignancies · Solid tumors · Target antigen

9.1 Introduction

Diverse are the efforts to find new therapeutic options to treat malignancies. For long time several immunotherapy approaches have been tested in cancer to strength the patient's immune system against tumor. Now we have improved our understanding about tumor immunosurveillance and molecular biology tools, increasing our capacity to personalize immune therapy options with clinical efficacy and safety. An emerging immunotherapy approach is adoptive cell transfer (ACT) that consists in the collection of autologous or allogenic T cells with high affinity to tumor antigens (TA) to fight against patient's cancer. The most common types of ACT are: tumor infiltrating lymphocytes (TILs) from the tumor microenvironment that are isolated from surgically resected patient's tumor and are *ex vivo* propagated to be re-infused back into the same patient [1]. The others ACT types are genetically engineered T cells expressing high affinity receptors (TCRs) to tumour-specific antigen or chimeric antigen receptors (CAR-Ts) that consist of an extracellular antigen-recognition domain and an intracellular signaling domain [2].

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9.1.1 CARs Structure Designs

Chimeric antigen receptors are formed by an extracellular antigen-recognition domain usually an antibody single chain variable fragment (scFv) specific for a TA, or less frequently a peptide or protein and an intracellular signaling domain, which usually consists in TCR-associated CD3ζ (CD3 zeta) chain.

The external domain of CAR allows the specific antigen recognition by T cell and, posterior stimulation of intracellular domain that stimulates T cell proliferation, cytolysis and cytokine secretion to eliminate target cell. To generate this T modified cells, is necessary to isolate own patients' T cells, to activate and genetically modify them using retroviral or lentiviral vectors or non-viral methods such as transposon, and finally to reinfuse back into the same patient. This strategy carries low risk of graft-versus host disease and enables lipid, protein and carbohydrate antigens to be targeted by T cells in a MHC non dependent fashion [3] (Fig. 9.1).

The antigen-recognition domain is anchored to the cell by a flexible spacer/hinge region and a transmembrane domain. The intracellular domain consists of termed signaling domains necessary for T cell activation [4]. There are three CAR-Ts generation. First generation contains CD3ζ signaling chains, as termed signal 1. This CAR-T has limited efficacy in clinical trials, one possibility could be the activation-induced cell death of the transplanted T cells or lack to maintain long-term T cell expansion [5, 6]. To avoid these difficulties second generation CAR-Ts includes in their structure a first generation backbone and two co-stimulatory signaling domains to provide a second activation signal. For example, second generation CD19-CAR-T cells include a CD3ζ chain and CD28 signaling domain, this structure enhances persistence a proliferation of CAR-Ts compared with first-generation CD19-specific CAR-T. In acute lymphoblastic leukemia B (B-ALL) second generation CAR-Ts includes CD28 or 4-1BB (CD137) co-stimulatory signaling domains to enhance the response. Third generation CAR-Ts contains a CD3ζ domain and two co-stimulatory domains that could include CD28, 4.1BB or OX40 (CD143), showing higher antitumor efficacy than second-generation CAR-T cells [7, 8] (Fig. 9.2).

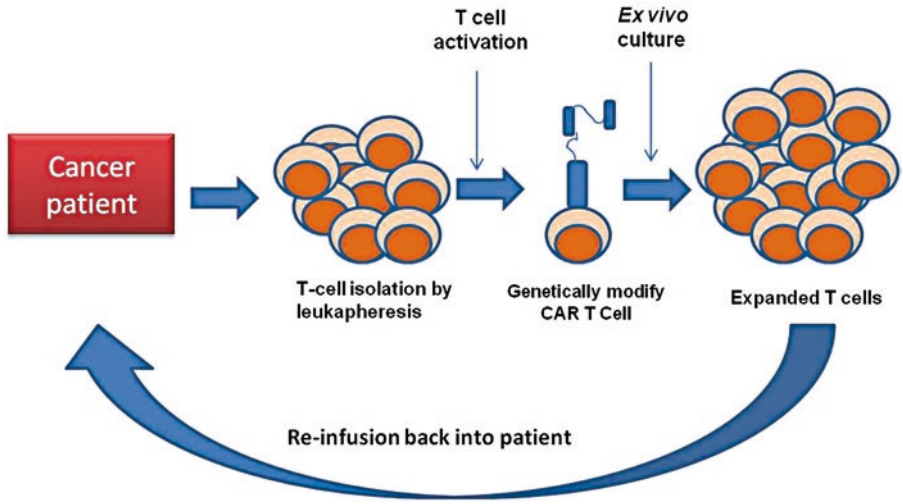

Fig. 9.1 CAR T cell manufacturing and treatment process.
Patient T cells are isolated by leukapheresis, then are *in vitro* activated by stimulation of T cell by magnetic beads or artificial antigen presenting cells. Cells are genetically engineered CARs are delivered by lenti-viral or retroviral and transposon method. The cells are expanded in culture divices. The patient undergo to prior infusion treatment, and then CARs are re-infusion back

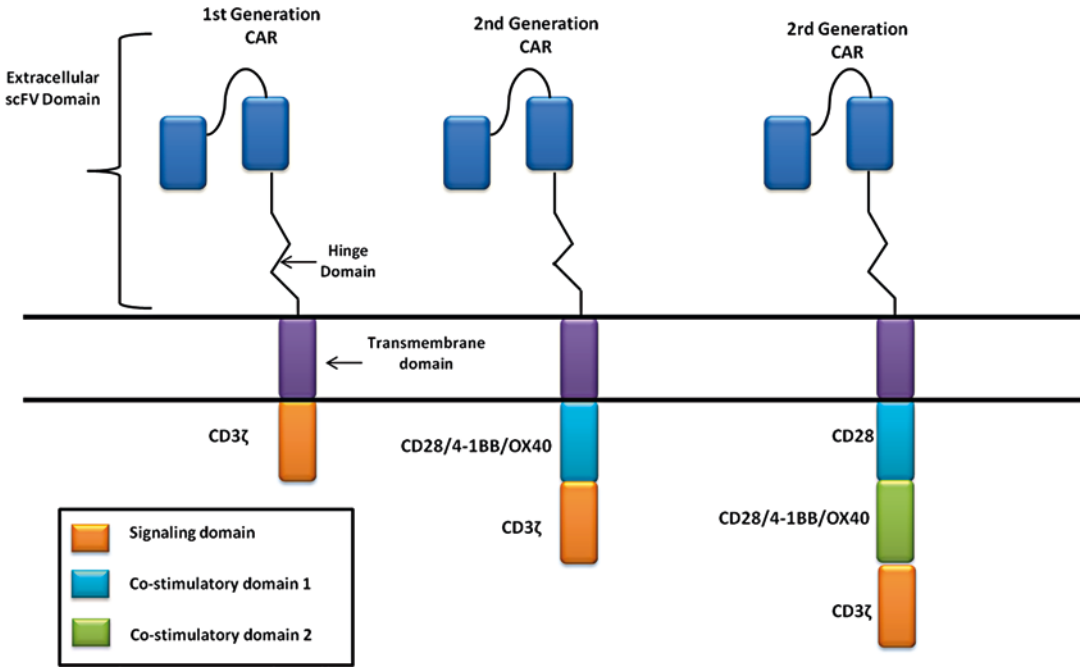

Fig. 9.2 Structure of CAR-T cells generation. First generation CAR consist in extracellular scFV domain with antigen recognition region, the hinge domain and intracellular activation domain. Second and third generation CAR-Ts had one or two co-stimulatory domain including signals such as CD28, 4-1BB or 0X40. *CAR* chemeric antigen receptor, *CD* cluster of differentation, *scFV* single chain variable fragment

9.2 CAR-Ts and Hematological Malignancies

CAR-Ts efficacy have been proved in hematological malignancies. Clinical trials of CARs-T therapy have mostly been conducted in patients with CD19-positive hematological diseases, such as acute and chronic B leukemia (B-ALL or CLL), follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL) and mantle-cell lymphoma. For example, CD19-targeted-CAR-Ts have achieved 70–90% response rate in B-ALL or CLL resulting in an important tool to treat these malignancies. Below the most important trials in hematological diseases are described. Table 9.1 summarizes CD19-specific published trials. Table 9.2 shows the ongoing clinical trials in hematological malignancies.

9.2.1 B-Cell Malignancies

Memorial Sloan Kettering Cancer Center (MSKCC) published their results of second generation CD19 specific-CAR-T (CD28/CD3ζ), called 19–28 z, in 33 relapsed-refractory adults with B-ALL. All patients received conditioning chemotherapy and then 1–3 × 10^6 19–28 z CAR-T cells/kg. Minimum residual disease (MRD-negative) was achieved in 81% and overall complete response rate (CRR) was 91%. The toxicities reported were cytokine-release syndrome (CRS) and neurological toxicities [9].

The University of Pennsylvania and Children's Hospital of Philadelphia conducted a trial in 57 pediatric and adult patients with B-ALL treated with CD19 transduced second-generation CAR-Ts cells (4-1BB/ CD3ζ) or CTL019 cells. Patients receive doses of 1–10 × 10^6 CAR-T

Table 9.1 Published clinical trials of CD19-specific-CAR-T cells in hematological malignancies

Institution	CAR structure	Patient characteristics	CR rate	Toxicities	Reference
UPenn/ CHOP	CD3ζ and 4-1BB	N = 30 children and young adults with B-ALL	90%	B-cell aplasia CRS	NCT01626495 [11, 21]
MSKCC	CD3ζ and CD28	N = 32 adults relapsed B-ALL	91%	B-cell aplasia CRS	NCT01044069 [22]
NCI	CD3ζ and CD28	N = 20 children and young adults B-LL	70%	B-cell aplasia CRS	NCT01593696 [13]
FHCRC	CD3ζ and 4-1BB	N = 32 adults B-LL	93%	CRS Neurotoxicity B-cell aplasia	NCT01865617 [17]
SCRI	CD3ζ and 4-1BB	N = 45 children and young adults	93%	CRS Neurotoxicity	NCT02028455 [23]
CHOP	CD3ζ and 4-1BB	N = 30 children and young adults ALL, DLCL	87%	CRS	NCT02374333 [24]
UK/ German	CD3ζ	N = 5	25%		[25]

ALL acute lymphoblastic lymphoma, *B-ALL* B cell-acute lymphoblastic leukaemia, *B-LL* B Lymphoma/leukaemia, *CHOP* Children's Hospital of Philadelphia, *CR* complete response, *CRS* cytokine-release syndrome, DLCL diffuse large cell lymphoma, *FHCRC* Fred Hutchinson Cancer Research Center, *MSKCC* Memorial Sloan Kettering Cancer Center, *NCI* National Cancer Institute, *SCRI* Seattle Children's Hospital, *UK* United Kindom, *UPenn* University of Penssylvania

Table 9.2 Ongoing Trials of CARs-T in Hematological Malignancies

Target	CAR Structure	Malignancy	Reference
CD19	KIR2DS2/ DAP12-	Lymphoma, leukemia	NCT02685670 [26]
CD20	CD3ζ; CD3ζ /CD28	CD20+ malignancies	NCT01735604 [27]
CD19 and CD20	CD3ζ/4-1BB	Leukemia, lymphoma	NCT03097770 [28]
CD22	CD3ζ/CD28	FL, NHL, DLBCL, B-ALL	NCT02315612 [29]
CD30	CD3ζ/CD28	HL, NHL	NCT01316146 [30]
CD33	CD3ζ/CD28	AML	NCT01864902 [31]
CD123	CD3ζ/CD28	AML	NCT02159495 [32]
CD138	CD3ζ/4-1BB	MM	NCT01886976 [33]
ROR1	CD3ζ/4-1BB	CLL, SLL	NCT02194374 [34]
Igκ	CD3ζ/CD28	CLL	NCT00881920 [35]
LeY	CD3ζ/CD28	AML	NCT01716364 [36]
BCMA	CD3ζ/4-1BB	MM	NCT02215967 [37]

AML acute myeloid leukaemia, *B-ALL* B cell-acute lymphoblastic leukaemia, *BCMA* B cell maturation antigen, *CD* cluster of differentiation, *CLL* chronic lymphocytic leukaemia, *DLBCL* diffuse large B-cell lymphoma, *FL* follicular lymphoma, *HL* Hodgkin lymphoma, *Igκ* immunoglobulin kappa cahin, *KIR2D2* simulatory killer immunoglobulin-like receptor 2DS2, *LeY* Lewis Y antigen, *MM* multiple myeloma, *NCI* National Cancer Institute, *ROR1* inactive tyrosine protein kinase transmembrane receptor ROR1, *SLL* small lymphocytic lymphoma

cells/kg. This group reported 93% of CRR, 55% had recurrence free survival (RFS) and 79% overall survival at 1 year. Twenty patients relapsed, 13 with CD19 disease. Patients CTL019 persistence had B-cell aplasia, which continued up last assessment (1–39 months) in 24/34 patients with ongoing CR. Cytokine release was seen in 88% of patients [10–12].

National Cancer Institute (NCI) performed an "intent-to-treat" clinical trial in 21 children and young adults with relapsed or relapsed B-ALL or NHL. They were treated with CD19-CAR-Ts (CD28/CD3ζ), CRR of 60.8% with 90% of responders negative for minimal residual disease (MDR-) was observed. The median leukemia free survival (mLFS) of MDR-CR responders was 18.7 months; the median disease survival of

MDR- CR responders was 49.5%. Severe CRS occurs in 13.5% [13, 14].

The Fred Hutchinson Cancer Research Cancer (FHCRC) group used central memory-enriched CD8 cells for starting material of 29 adults to be treated with CD19-targeted CAR-Ts (CD3ζ/41BB) and defined composition of CD4:CD8 T cells. This treatment approach with a defined subset composition achieved 83% complete response rate. The peak level and duration of persistence of both CD4+ and CD8+ CAR-Ts were associated with clinical response [15, 16]. Posterior update reports 93% of bone marrow remission, these investigators identify as risk factors for severe toxicity CAR-T cell dose and tumor burden [17].

Dr. Porter et al. reported their results of CTL019 in CLL. The overall response rate in heavily pretreated patients CLL was 57% (8/14 patients). The in vivo expansion of CAR-Ts correlated with clinical response. CAR-Ts persisted and remained functional beyond 4 years in the two patients whose achieving CR. All responding patients developed B-cell aplasia and experienced cytokine release syndrome that correspond with T cell proliferation [18].

CD-19 specific CAR-Ts (CD28/CD3ζ) have been studied in DLBCL, indolent lymphoma or CLL. In a multicenter phase 2 trial that enrolled 11 previously treated patients with large B-cell lymphoma, including diffuse large B-cell lymphoma and primary mediastinal B-cell lymphoma. Those patients receiving a conditioning therapy and after CD19-specific CAR-Ts showed 82% objective response, 54% complete response and 28% partial response. With a median follow-up of 15.4 months, 42% of these patients had still response, 40% of them with complete response. The most common adverse events were pyrexia (85%), neutropenia (84%) and anemia 66% [19].

9.3 Multiple Myeloma

CD19-specific CARs-T cells were evaluated in 10 patients with multiple myeloma (MM). Patients received pre-conditioning treatment with autoHSCT and melphalan followed by an infusion of second generation CD19-targeted CARs-T cells (4-1BB/CD3ζ). One patient experienced a complete response for 12 months following treatment and six patients remained progression free [20].

9.3.1 CAR-Ts and Solid Tumors

There are diverse research efforts to evaluate efficacy and safety of CAR-Ts in solid tumors, but results are less exciting than findings in hematological malignances. Prior identification of new possible target antigen and posterior preclinical models of solid tumors it is necessary to evaluate efficacy and animal safety of these therapies based on these antigens. The response to CAR-Ts depends on diverse parameters: (1) A good choice of the target epitope, (2) Specific target antigen, (3) CARs structure, CAR-T cell dose, frequency or administration way, (4) Tumor environment, (5) Patient's lymphodepletion and pre-condition treatment previous to CARs-T administration, (6) CAR-Ts engraftment and trafficking capacity [38].

The ideal target antigen is one that could be found specifically in epithelial cancer cells; however solid tumors have over-expression of proteins that are also expressed in normal cells, making difficult the work of find a specific antigen. Despite of this, there are several efforts to test CAR-T in solid tumors.

9.3.2 EGFR and EGFRvIII

The alternately splice variant of EGFR (EGFRvIII) is commonly associated with glioma cells and is necessary for their survival. A phase 1 trial has been done in 10 recurrent glioblastoma (GBM) patients, whose has been previously treated. After EGFRvIII determination, patients were treated by an infusion of EGFRvIII-CAR-T cells. One patient showed residual stable disease for over 18 months of follow-up. All patients had demonstrable detected transient expansion of EGFRvIII-CAR-T in peripheral blood. Seven patients had posterior surgical intervention, which allowed tissue-specific analysis of

EGFRvIII-CAR-T trafficking into the tumor; patients had a patchy pattern of lymphocyte infiltrate com-posed of CD8 T cells after CARs-T infusion compared with pre-infusion brain tumor specimens from the same patient [39].

9.3.3 HER2

The tyrosine-protein kinase receptor erbB2 (HER2) is commonly expressed in diverse epithelial cells such as gastrointestinal, respiratory, urinary and reproductive systems. However, HER2 overexpression has been detected in tumors cells of gastric, breast, colon and ovarian cancer.

A third generation HER2-CAR-T cell (CD28/4-1BB and CD3ζ) was tested in metastatic cancer (NCT00924287 trial). This trial stopped ahead of time because one colon cancer patient with lung and liver metastases died for acute respiratory failure. Post mortem analysis exhibited signs of systemic ischemia and hemorrhagic microangiopathic injury. The lungs had a diffuse alveolar damage with an immediate accumulation of T lymphocyte demonstrated in the patient's lung. The patient had a marked increase of IFN-ϒ, granulocyte macrophage-colony stimulating factor (GM-CSF), tumor necrosis factor-α (TNF-α), IL and IL-10 after HER-2- CAR-T cell infusion. This trial included in the CAR-T cell structure a scFV based on trastuzumab. One explanation for this severe toxicity could be the recognition and depletion of low levels of ERBB-2 in lung epithelium, triggering pulmonary failure and massive cytokine release [40].

Despite of this severe toxicity there are several ongoing trials with HER2-CAR-T in others tumors such as sarcoma where investigators are using a scFv with lower affinity than trastuzumab-based CAR, therefore with better results in safety setting. Nineteen patients with advanced-stage sarcoma have been treated with these second-generation HER2-specific CAR-T cells (CD28/CD3ζ). HER2-CAR T cells persisted for at least 6 weeks in seven out of nine evaluable patients. HER-CAR-T cells were detected at tumor site in 2 patients. Four patients had stable disease for 12–14 weeks. Three patients with surgery after HER-CAR-T cells infusion had ≥90% necrosis. Median overall survival was 10.3 months [41].

9.3.4 Mesothelin

Mesothelin (MSN) is a novel attractive target for cancer immunotherapy. This protein is low expressed in normal mesothelial cells, but has a high expression in many solid tumors. Physiologically, MSN is expressed on mesothelial cells of pericardium and peritoneal and pleural cavities.It has been found to be overexpressed in mesothelioma and ovarian cancer, and in other tumors such as lung, pancreatic, gastric, endometrial, colon and breast cancer [42, 43].

A clinical trial tested CAR-T cells with mRNA encoding for second-generation MSN-CAR-T (SS1-4-1BB) in advanced mesothelioma or pancreatic tumors by intravenous or intratumor MSN-CAR-T. Cell infusions were well-tolerated and no off-target toxicities were observed (pleuritis, pericarditis or pericarditis). A severe anaphylaxis and cardiac arrest were reported with the third infusion of MSN-CAR-T, secondary to a high production of IgE antibodies targeted against MSN-CAR-T, probably associated with the murine SS1 scFV. Despite of this severe toxic event, the treatment in general was well- tolerated. Antitumor activity was demonstrated by a decrease in tumor-cell numbers in ascitis and a decrease of peritoneal lesions [44].

9.3.5 Disialogangloside GD2

Disialongangloside GD2 is a glycosphingolipid with low-level expression in neural tissues; however tumors as neuroblastoma overexpress this protein. GD2-CAR-T had been investigated in patients with neroblastoma. They used autologous activated T cells (ATCs) and autologus Epstein barr-virus specific T cells (EV-CTLs), each modified with a distinguishable GD2-specific CAR (GD2-CAR-T). Three patients out of 11 with active disease achieved complete remission and persistence of CAR-ATCs or

CAR-CTL-S beyond 6 weeks associated with superior clinical outcome [45]. Third generation GD2-CAR-T cells (OX40/CD28/CD3ζ) in patients with neuroblastoma, osteosarcoma and melanoma are under investigation. To increase the safety of this CAR-T, the investigators modified the GD2-CAR-T cells to express inducible caspase 9 (icaspas9) suicide gene [46].

9.3.6 Prostate Specific Membrane Antigen

Prostate specific membrane antigen (PSMA) is a type II membrane protein expressed in most of prostate-cancer cells and tumor –associated neovasculature of many solid tumors [47]. A second generation PMSA-CAR-T cell (CD28/CD3ζ) has been tested in prostate cancer patients. No toxicity was reported, two out of three patients included in this study had stable disease at 6 months of follow up [48]. Similar results are reported in other study that treated prostate cancer patients with second generation PMSA-CAR-T cell (CD28/CD3ζ). Patients had decreased PSA levels, and disease progression was delayed in two out of five patients [49].

9.3.7 Other Tumor Antigens

Several tumor antigens in solid tumors are investigated on clinical trial, they are summarized in Table 9.3. Those antigens include glycoproteins as carcinoembryonic antigen (CEA) expressed on many epithelial tumorsfrequently located atthe gastrointestinal tract. Other protein is the neural cell adhesion molecule L1 (CD171) that is expressed in ovarian cancer, neuroblastoma and melanoma. This protein is also expressed on normal tissues as peripheral nerve and kidney, but with a different glycosylation pattern than CD171 expressed in malignant cells, making it a suitable target for CAR T therapy. Other proteins under investigation are the glypican-3, a surface proteoglycan overexpressed on hepatocellular carcinoma, and the IL-13R, a high affinity monomer receptor overexpressed in 50% of glioblastoma, with low expression in normal brain tissues.

9.3.8 Future: Strategies to Improve the Safety and Efficacy of CARs-T

CAR-Ts have impressive results in clinical trials for B-cells malignancies, however, there are still concerns about inability to control CAR-Ts after patient's re-infusion back. CAR-Ts have the capacity to attack normal tissue (off-tumor-cross reaction), being the major limiting factor in the clinical setting.

Future challenges to improve CAR-T cells therapy [67].

1. Antigen loss
2. On-Target/off tumor toxicity (CAR-T cell recognize normal tissues and can cause severe and life-threatening toxicities, especially in solid tumors).
3. Tumor Microenvironment (Function as a barrier to CAR-T cells penetration).
4. Production difficulties (Autologous T cells manufacturing)

There are several approaches to improve safety and efficacy of CAR-Ts (Fig. 9.3). New strategy to face antigen loss relapse is for example the modification of CAR-Ts with two distinct CAR molecules with two different binding domains called dual-signaling CAR. Tandem CARs (TanCAR) is other approach, with one CAR molecule containing two different binding domains in tandem that simultaneously targets different antigens, for example HER2 and IL13Rα2 to mitigate tumor antigen escape, showing superior antitumor activity compared with pooled CAR-Ts or co-transduced T cells in mouse glioblastoma model [68].

The inhibitory CAR (iCAR) is a fusion of an antigen recognition domain, usually an antigen expressed on normal tissue, with an inhibitory intracellular domain, which could be a programmed cell death protein 1 (PD-1) or a cytotoxic T-lymphocyte-associated protein 4

Table 9.3 Ongoing clinical trials in solid tumors

Target	CAR-T structure	Tumor	Reference
EGFRvIII	CD3ζ and 4-1BB	Glioma	NCT02209376 [50]
	CD3ζ, CD28 and 4-1BB	Glioma	NCT01454596 [51]
HER2	CD3ζ and CD28	Sarcoma	NCT00902044 [52]
	CD3ζ and CD28	Glioblastoma	NCT02442297 [53]
		Glioblastoma multiforme	NCT01109095 [54]
Mesothelin	CD3ζ and 4-1BB	Malignant pleural	NCT01355965 [55]
		Mesothelioma	
		Pancreatic cancer	NCT02465983 [56]
		Pancreatic and ovarian cancer and malignant mesothelioma	NCT01583686 [57]
GD2	CD3ζ, OX40, CD28	Neruoblastoma, osteosarcoma and melanoma	NCT02107963 [46]
		Neuroblastoma	NCT01822652 [58]
PSMA	CD3ζ and CD28	Prostate cancer	NCT01140373 [59]
			NCT00664196 [60]
CEA	CD3ζ and CD28	Liver metastasis	NCT02331693 [61]
FAP	CD3ζ and CD28	Mesothelioma	NCT01722149 [62]
MUC1	CD3ζ and 4-1BB	MUC1 positive solid tumors	NCT02587689 [63]
CD171	CD3ζ and 4-1BB	Neuroblastoma	NCT02311621 [64]
	CD3ζ, CD28 and 4-1BB		
Glypican-3	CD3ζ, CD28 and 4-1BB	Hepatocellular carcinoma	NCT02395250 [65]
IL-13Rα2	CD3ζ and 4-1BB	Glioma	NCT02208362 [66]

CEA carcinoembrionic antigen, *EGFRvIII* epidermal growth factor receptor variant III, *FAP* prolyl endopeptidase FAP/fibroblast activation protein alpha, *HER2* human epidermal growth factor 2 receptor, *IL-13Rα2* interleukin 13 receptor α2, *MUC1* mucin 1, *NCI* National Cancer Insitute (USA), *PSMA* prostate-specific membrane antigen

(CTLA-4). This fusion leads the inhibition of CARs activation and limits its undesired activation [69]. To improve CARs safety there are also another approach such as suicide genes as inducible caspase-9 (iCaspase-9) switch, included in 19-CAR-T cell encoding vector to remove inappropriately active CARs. This "safety switch" approach modulates the effects of CARTs-19 cells and could reduce severe toxicities related to this therapy [70, 71]. Switchable CARs (sCAR-T) are based on engineering bifunctional switched that consists of a tumor antigen-specific Fab molecule engrafted with a peptide neo-epitope, which is bound exclusively by a peptide-specific switchable CAR-T cell. This switch redirects sCAR-T cells activity through the formation of selective immunological synapses. On this way sCAR-T cell switches

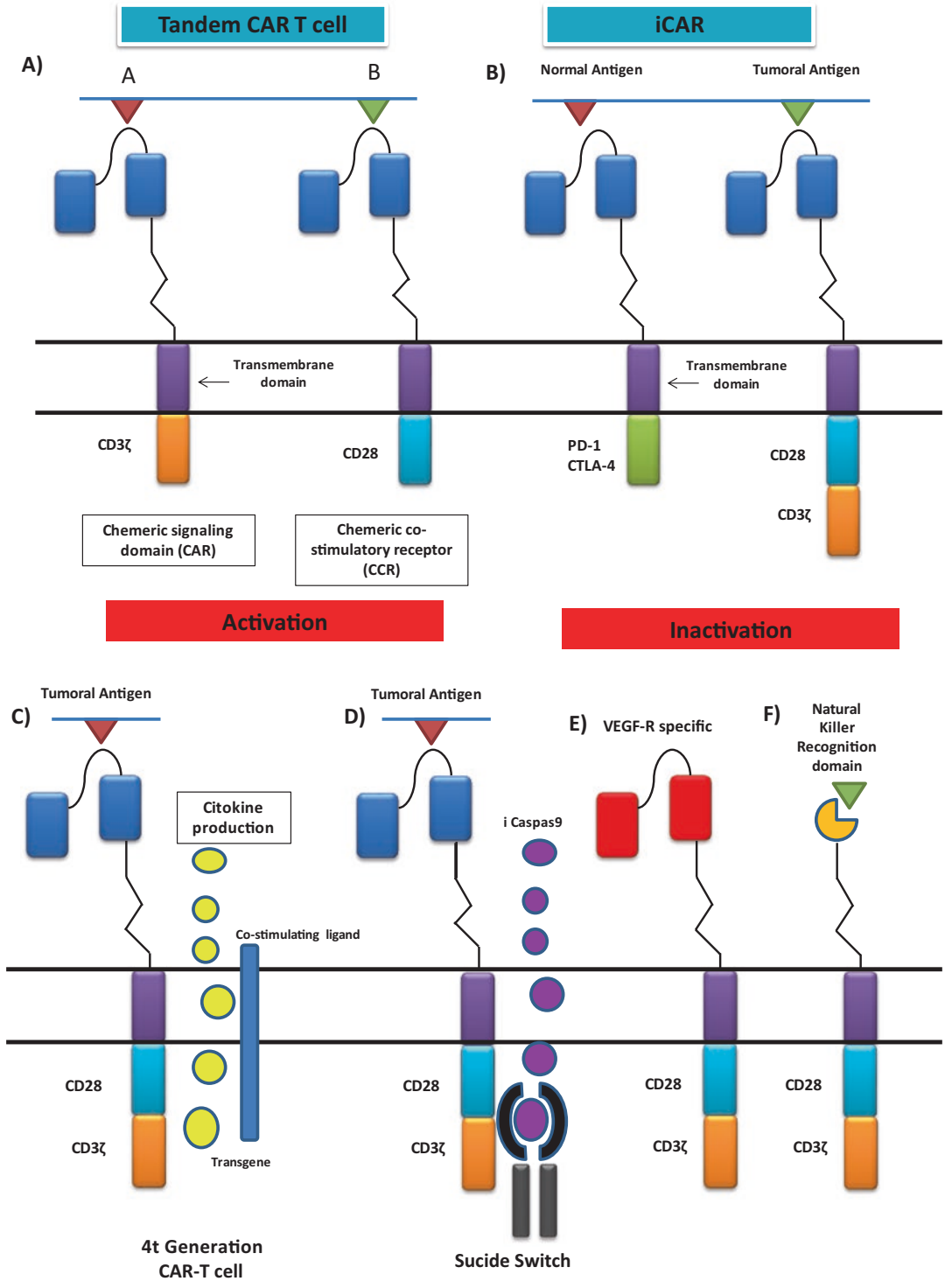

Fig. 9.3 CAR-T: Novel approaches to improve safety and efficacy.
(**a**) Tandem CAR-T cell had two different tumoral antigen (TA) specific scFvs domains to have a synergic activation of both scFV simultaneously. (**b**) iCAR- is a combination of inhibitory receptor specific for the antigen present on normal cell but not on tumor cells, to protect normal cells from a CAR-T cell mediated attract. (**c**) 4th Generation CAR-T cell are associated to a transgene and have the capacity to produce cytokine as IL-12 to increase immune response, (**d**) Switch Suicide CAR-T structure, (**e**) target tumor stroma like VEGFR CAR-T, (**f**) Natural Killer Recognition domain to recognize own antigens and foreign

and targets cells in a structurally defined and temporally control manner [72].

Natural Killer (NK) cells have receptors capable to discriminate between normal and tumor cells. Recently, NK receptors are being used as antigen recognition domains in CAR-T, to improve tumor recognition by T cells. The receptor NKG2-D links to intracellular T-cell signaling domains and enables this receptor to activate T cells. It is under investigation in diseases like AML, MM and myelodysplastic syndrome [73, 74].

The phenotypic heterogeneity of solid tumors hinders CAR-T cell efficacy in these tumors. After initial tumor reduction by CAR-T cell, the antigen-negative tumor cells that are still alive, not recognized by CAR-T, are probably implicated in tumor relapse. TRUCK T cells or fourth Generation CAR-T cells are modified CAR- T cells to secrete pro-inflammatory cytokines (usually cytokine like IL-12). This TRUCK T cell can release this transgenic protein to regulate T-cell response and active innate immune response cells that can kill negative-antigen cancer cells. The transgenic IL-12 is stored into the CAR-T and only is released when is induced [75].

Tumor microenvironment like tumor-vasculature is important for tumor cell survival. VEGF ligand and their receptors are implicated in cancer. VEGFR-2 is overexpressed on tumor stroma cells and some tumor cells. VEGFR-2-specific-CAR-T cells target tumor stroma cells, without harm normal tissue. This is another possible application of CAR-T cells in solid tumors [76].

Genome editing tools as zinc finger nucleases, meganucleases, transcription activator-like effectors nuclease (TALEN), homing endonuceases, and clustered regularly interspaced short palindromic repeats (CRISPR-Cas) system are successfully applied to engineer T cells. Human genome editing led the opportunity generate "universal" CAR-T cells without a functional endogenous TCR or eliminate immunosuppressive signals such as PDL-1 and CTL-4, improving T cells function [77] (Fig. 9.4).

9.4 Toxicities

The most important toxicities related with CAR-T cell therapies are cytokine release syndrome and neurotoxicity and both have very interesting physiopathology. Similar than happens with other immune treatment such as monoclonal antibodies (MoAB), CAR-T cells administration are associated with an immune response mediated by cytokines. Cytokine release syndrome (CRS) has been observed with CD19-specific, CD22-specific and BCMA-specific CAR-T cells therapy, rates of severe CRS are around 25% among various trials. Symptoms occur any time in the first 2 weeks after CAR-T cell infusion and are related with increase cytokine levels. Tumor necrosis factor

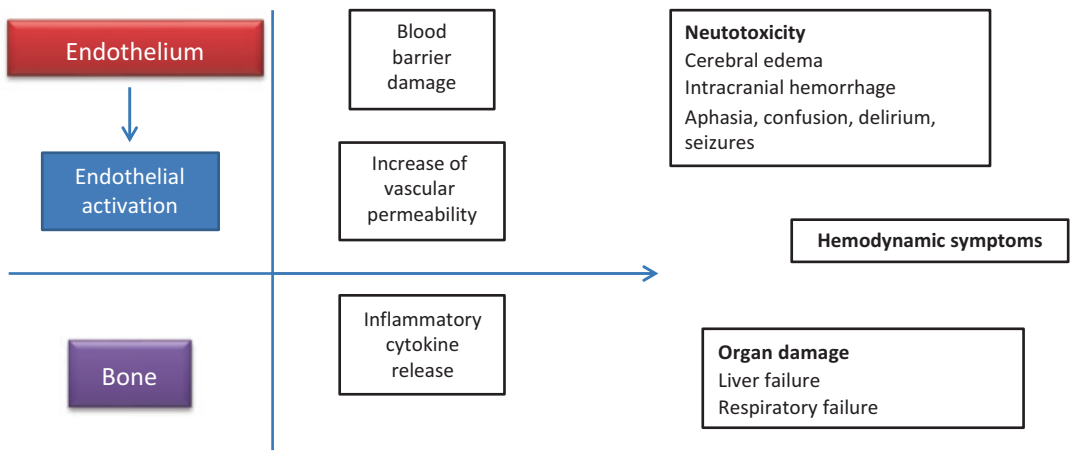

Fig. 9.4 Physiopathology of CAR-T cell toxicity

(TNF) α increases first and is followed by IFNϒ, IL-1ß, IL-1, IL-6; 1L-8 and IL-10 [78]. Others biochemical abnormalities include elevated C-reactive protein and ferritin levels. The CRS severity is related with tumor burden and the anti-tumor responses. The clinical symptoms include fever, hypotension and less common respiratory failure. Consumptive coagulopathy has been also described and is related with severe CRS in children [79]. Lee et al. suggested a CRS grading scale that it is beginning to be used in clinical trials trying to unify outcomes reports (Table 9.4). Most of the CRS related symptoms are manageable with antipyretic, steroid and intravenous fluids. Despite of, some patients will require supportive care as high doses of vasopressors and ventilatory support. Interleukine- 6 (IL-6) is predominantly elevated in these patients and is related with severe CSR. The monoclonal antibody anti-IL6R, tocilizumab, has been used in B-ALL leukemia treated with CD19-CAR-T with good outcomes. Based on these results, tocilizumab is indicated to treat severe CRS [80, 81]. Investigators are working on developing strategies for mitigate CRS occurrence. The group of Seattle Children's Hospital (SCRI) has proposed a strategy which aimed to decrease the rates of severe CRS based on tocilizumab or dexamethasone administration, when patients demonstrate persistent symptoms of mild CRS (Table 9.5). This strategy reduces severe CRS rate in approximately 50%, without impact on efficacy or long-term persistence of the CAR-T cells therapy [49]. When the prevention fails or CRS symptoms remain, MKSCC group have proposed a CRS management algorithm (Fig. 9.5) [82].

Table 9.4 Grading System for Cytokine Release Syndrome (CRS)[a]

Grade	Toxicity related Symptoms[b]	Treatment
1	Fever, nausea, fatigue, headache, myalgias, malaise	Symptomatic treatment only
2	Oxygen requirement of <40%, hypotension responsive to fluids or low dose of one vasopresor. Grade 2 organ toxicity or grade 3 transaminase elevation.	Symptoms require and respond to moderate intervention
3	Oxygen requirement of ≥40%, hypotension high dose or multiple vasopressor. Grade 3 organ toxicity or grade 4 transaminase elevation.	Symptoms require and respond to aggressive intervention
4	Hypotension refractory to high dose vasopressors. Requirement for ventilator support or grade 4 organ toxicity	Life-threatening symptoms
5	Death	

[a]Adapted from Lee et al. [81]
[b]Severe Neurological complications such as dysphasia, confusion, delirium, visual hallucination, seizure-like activity are related with CRS

Table 9.5 CRS management (early intervention)[a]

Symptoms related to CRS	Suggested intervention
Fever ≥38.3 °C	Acetaminophen (12.5 mg/kg) PO/IV up to every 4 h
Persistent fever ≥39 °C for 10 h that is unresponsive to acetaminophen	Tocilizumab (8–12 mg/kg) IV
Persistent fever ≥39 °C after tocilizumab	Dexamethasone 5–10 mg IV/PO up to every 6–12 h with continued fevers
Hypotension	Fluid bolus, target hematocrit >24%
Persistent/recurrent hypotension for longer than 12 h	Tocilizumab (8–12 mg/kg) IV
Use of low-dose vasopressor for hypotension for longer than 12 h	Dexamethasone 5–10 mg IV/PO up to every 6 h with continued use of pressors
Initiation of higher dose vasopressor or second vasopressor	Dexamethasone 5–10 mg IV/PO up to every 6 h with continued use of pressors
Initiation of oxygen supplementation	Tocilizumab (8–12 mg/kg) IV
Increasing of respiratory support	Dexamethasone 5–10 mg IV/PO up to every 6 h with continued use of pressors
Recurrence/persistence of symptoms after ≥48 h of initial dose of tocilizumab	Tocilizumab (8–12 mg/kg) IV

[a]Adapted from Annesley et al. [84]
Tocilizumab is a humanized, immunoglobulin $G1_K$ ($IgG1_k$) anti-human interleukina 6 (IL-6) receptor monoclonal antibody. Suggested doses is 8–12 mg/kg) IV

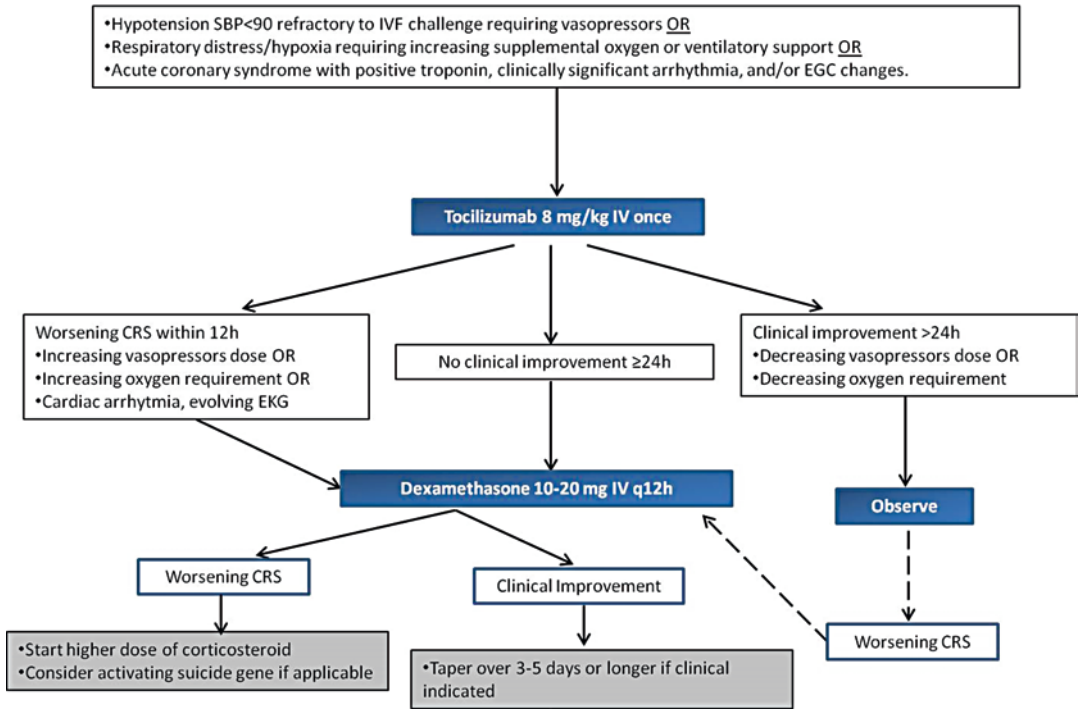

Fig. 9.5 MSKCC for CRS Management Algorithm (for Grade≥2)

Neurotoxicity has been observed associated with CAR-T cell therapy, with a wide spectrum of symptoms since mild confusion and aphasia to life-threatening encephalopathy and intractable seizures. Apparently these symptoms are related with cerebral edema secondary to immune activation. It is not known if cerebral edema is a consequence of CAR-T cell therapy as an extreme manifestation of CRS or it is an independent symptom. The major explanation for this pathology is endothelial injury related to cytokine release, contributing to the onset of neurotoxicity, but the exact mechanism of action is still poorly understood [83]. The Fig. 9.4 summarizes the physiopathology of CRS and Neurotoxicity.

Grades 2–4 refer to CTCAE v.4.0 grading.

9.5 Take Home Messages

1. CAR-Ts are a novel precision immunotherapy strategy specifically designed to attack a tumor antigen, using patient's T cells engineered modification.
2. It is important to select the best target antigen to generate CAR-T cell effective against a specific tumor.
3. Next generation CAR-T cells will be available to improve immune response, decrease off target/on tumor risk, to be more capable to penetrate tumor microenvironment, and to program death or apoptosis.
4. Cytokine release syndrome management is a new challenge in the clinical oncology practice.

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