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REVIEW

Current status and regulatory perspective of chimeric antigen receptor-modified T cell therapeutics

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Abstract Chimeric antigen receptor-modified T cells (CAR-T) have emerged as a new modality for cancer immunotherapy due to their potent efficacy against terminal cancers. CAR-Ts are reported to exert higher efficacy than monoclonal antibodies and antibody–drug conjugates, and act via mechanisms distinct from T cell receptor-engineered T cells. These cells are constructed by transducing genes encoding fusion proteins of cancer antigen-recognizing single-chain Fv linked to intracellular signaling domains of T cell receptors. CAR-Ts are classified as first-, second- and third-generation, depending on the intracellular signaling domain number of T cell receptors. This review covers the current status of CAR-T research, including basic proof-of-concept investigations at the cell and animal levels. Currently ongoing clinical trials of CAR-T worldwide are additionally discussed. Owing to the lack of existing approved products, several unresolved concerns remain with regard to safety, efficacy and manufacturing of CAR-T, as well as quality control issues. In particular, the cytokine release syndrome is the major side-effect impeding the successful development of CAR-T in clinical trials. Here, we have addressed the challenges and regulatory perspectives of CAR-T therapy.

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Introduction

In recent years, immunotherapy has attracted considerable research attention as a new modality of cancer treatment. Among the newly developed cancer immunotherapy technologies, those using chimeric antigen receptor-modified T cells (CAR-T) have been of particular interest. Substantial progress has been made in the CAR-T-based cancer immunotherapy field following the initial generation of CAR-T in 1989 (Kershaw et al. 2013; 2014; Wang and Riviere 2015). Currently, dozens of CAR-T clinical trials are ongoing worldwide (Fig. 1). To construct CAR-T, T cells are transduced with genes encoding fusion proteins for cancer antigen-recognizing single chain Fv (scFv) linked to the intracellular signaling domain of T cell receptors.

CAR-Ts are classified as first-, second- and third-generation, depending on the intracellular signaling domain numbers of T cell receptors (Fig. 2). First-generation CAR-T cells were designed to express scFv in the extracellular region and the signaling sequence of the T cell receptor intracellular domain with no co-stimulatory molecules. However, first-generation CAR-Ts were limited in their tumor cell-killing efficacy after specific recognition of tumor cells by antigens. To overcome these limitations, second- and third-generation CAR-Ts were designed to express co-stimulatory molecules in the intracellular domain (Fig. 2, Casucci and Bondanza 2011; Maus et al. 2014). Chimeric antigen receptor (CAR) gene cassettes for second-generation CAR-Ts encompass one co-stimulatory molecule, such as CD28 or 4-1BB. Third-generation CAR-

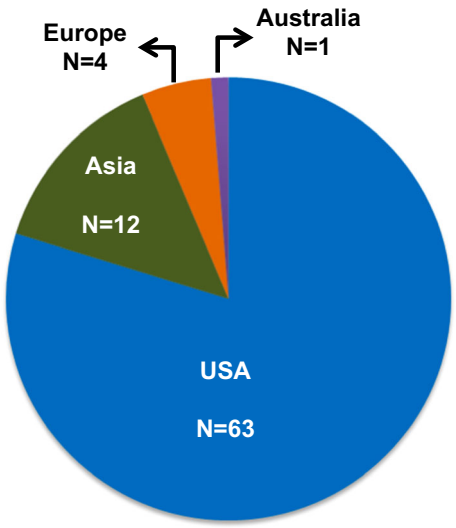

Fig. 1 Clinical trials of CAR-T worldwide

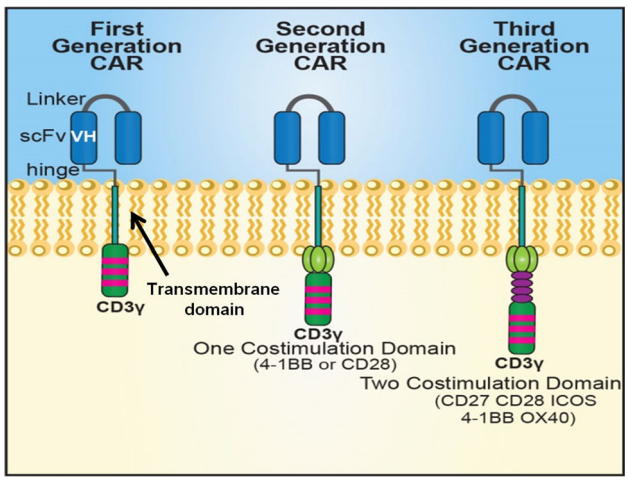

Fig. 2 Construction of CAR in each CAR-T generation

Ts have been further developed to include two co-stimulatory molecules among CD27, CD28, 4-1BBand OX40.

Manufacture and administration of CAR-T

CAR-Ts are manufactured using three consecutive steps (Lee et al. 2012; Wang and Rivière 2015; Levine 2015). The first step is to generate genetic constructs of CAR to encode tumor antigen-specific Fv linked to signaling sequences of T cell receptors. Next, T cells are transduced with CAR using viral, nonviral or physical methods. Retroviral or lentiviral vectors have been successfully employed as viral vectors for transduction of T cells with CAR. In other studies, T cells have been transduced with plasmid DNA (Huang et al. 2012; Kumaresan et al. 2014; Wang et al. 2014a, b) or RNA (Zhao et al. 2010) encoding CAR via electroporation. The third step is cultivation of CAR-T cells.

Several protocols have attempted to activate T cells for transduction, one of which is to use anti-CD3-antibodies and cytokines, such as interleukin-2. Various other T cell activation methods are currently under investigation (Fig. 3). Antigen-presenting cells expressing 4-1BBL and Fc receptor have additionally been employed to activate T cells. Another procedure involves the use of beads modified with anti-CD3 and anti-CD28 antibodies for T cell activation. The resulting CAR-T cells are derived from CD4 or CD8 T cells, expanded using cytokines, and administered to patients via intravenous infusion (Lee et al. 2012).

Advantages of CAR-T over existing cancer immunotherapy

CAR-Ts have attracted considerable research attention due to their potent efficacy against terminal cancers, relative to monoclonal antibodies and antibody–drug conjugates. Moreover, CAR-Ts act through different mechanisms from T cell receptor-engineered T cells (TCR-T). In TCR-T, TCRs recognize complexes of tumor antigens that are processed in APC cells and presented on APC cell surfaces with MHC class molecules. Unlike TCR-T, CAR-Ts do not require processing and presentation of tumor antigen-

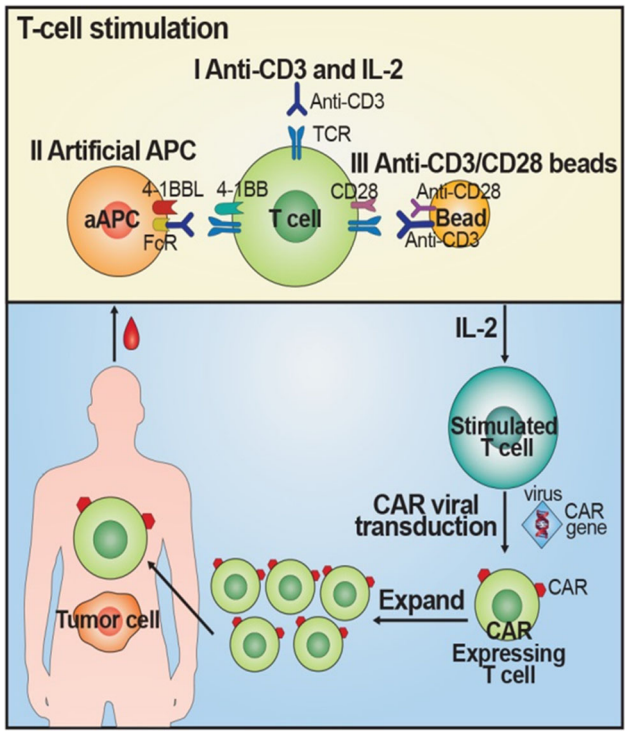

Fig. 3 Activation of T cells and expansion of CAR-T

recognizing moieties with MHC molecules (Kershaw et al. 2014; Fig. 4). The differences between CAR-T and TCR-T are summarized in Table 1 (Gill and June 2015). Notably, the lack of MHC restrictions means that eligible patient groups for CAR-T are wider compared to those for TCR-T that requires the identification of patient MHC types.

Current status of CAR-T studies

The utility of CAR-T for treatment of lymphoma and solid tumors has been examined in several studies, grouped as cell-level (Table 2; Fig. 5), animal-level (Table 2; Fig. 5), preclinical (Table 3; Fig. 5), and clinical trials (Table 4; Fig. 6). Current status summary of CAR-T under basic investigational stages shows that retroviral vectors are most actively used for introducing CAR genes into T cells. Although the use of viral vectors are dominating, nonviral approaches using cationic polymers or electroporations have been attempted (Fig. 5a). Hematological cancers such as lymphoma have been the major target of CAR-T in basic research stage (Fig. 5b). Second-generation CAR-Ts have been most widely investigated in basic cell-level and animal studies, with focus on hematological cancers and solid tumors (Fig. 5c). The application of CAR-T has been extended from cancer immunotherapy to treatment of autoimmune diseases, such as multiple sclerosis in Europe (Fransson et al. 2012). Another recent study reported on the effectiveness of CAR-T in treating fungal infection, suggesting a new field of CAR-T application (Kumaresan et al. 2014). Similar to the investigational stage research, preclinical trial studies used retroviral vectors and second generation CAR constructions in higher frequency than other viral vectors (Fig. 5d), and generations, respectively (Fig. 5f). Notably, the number of preclinical trials for hematological tumors were lower than brain cancers (Fig. 5e).

For introduction of CAR-T genes into T cells, viral vectors have been predominantly used. The duration of CAR-T survival in vivo is reported to be longer than several months. Since month-long sustainable expression of CAR can evoke undesirable side-effects, the optimal length of expression time requires further investigation. To shorten the duration of CAR expression and minimize safety concerns, physical electroporation studies are underway. Introduction of plasmid DNA encoding CAR directly into the cytoplasm of T cells via electroporation may lead to an expected duration of CAR of several days, while avoiding side-effects of delivery vectors.

Currently, more than 80 CAR-T cases are in clinical trials worldwide (www.clinicaltrials.gov). No clinical trials are in phase 3 as yet, but the potential is high, given the number in phase 2 (Table 4). The majority of CAR-T clinical trials is being held in USA, and has also been initiated in Asia, China and Japan (Fig. 1). CD19, widely studied as a tumor antigen target of CAR-T, is overexpressed on the surfaces of leukemia cells of acute lymphocytic leukemia (ALL) patients. CD19 CAR-T therapy is reported to be effective in children with recurrent ALL after bone marrow transplantation (Lee et al. 2015). Among the products in clinical trials, CTL019 (Novartis, USA), CD19-targeted CAR-T (CD19-CAR-T) against ALL has been recognized as a "breakthrough therapy" by the Food and Drug Administration of USA and is in phase 2 development. Several other global pharmaceutical companies are developing CAR-T products in the pipeline. In clinical trials, CD19 has been most extensively used as target tumor antigens of hematological cancers (Fig. 6a). Other antigens in clinical trials include carcinoembryonic antigen (CEA), human epidermal growth factor receptor 2 (HER2), GD2, CD30, and CD20 (Fig. 6a). Hematological cancers have been predominantly studied in clinical phase (Fig. 6b). Until now, phase 2 trial are the most advanced stage for CAR-Ts (Fig. 6c).

Challenges

Limitations of each generation of CAR-T

Although CAR-T takes advantage of the immune response of T cell killing abnormal cells, tumor cell-killing effects

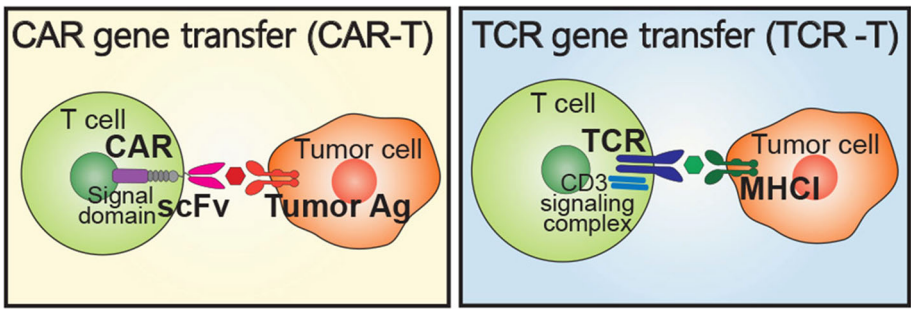

Fig. 4 Different cell surface structures between CAR-T and TCR-T

Table 1 Differences between CAR-T and TCR-T

	CAR-T	TCR-T
Structure of tumor antigen recognition receptor	Expressing tumor antigen-recognizing scFv	Expressing alpha and beta subunits of TCR recognizing MHC-tumor antigen complexes
MHC dependence of tumor antigen recognition	MHC independent	MHC type dependent
Locations of candidate tumor antigens	Antigens on tumor cell surfaces are eligible	Antigens on cell surfaces or inside cells are all eligible as far as they form MHC complexes
Amplification in vivo	The insertion of co-stimulatory molecule in CAR allows the amplification of CAR-T in the body	To promote amplification, additional stimuli using antigen presenting cells are needed

are decreased in solid tumors. This reduced antitumor activity is attributable to the immunosuppressive microenvironment of tumor tissues, resulting in low penetration efficiencies of CAR-T into solid tumor tissues. Moreover, leukocytes in tumor tissues are known to secrete factors that lower T cell activity.

Second-generation CAR-Ts containing a co-stimulatory signaling domain can induce the expression of immunosuppressive receptors, such as T cell membrane protein-3, cytotoxic T lymphocyte associated antigen 4 (CTLA-4), and programmed death-1 (PD-1). To overcome suppression of CAR-T activity by PD-1, the effects of co-administration of anti-PD-1 antibody with CAR-T were recently examined (John et al. 2013). An ongoing clinical trial (NCT00586391) is exploring the effects of co-administration of CAR-T and ipilimumab, an anti-CTLA-4 antibody (Maher 2014).

Third-generation CAR-Ts with two co-stimulatory molecules, such as OX40 and 4-1BB, display enhanced activity in vivo. However, excessive stimulation of T cell activity by two co-stimulatory molecules may induce an abrupt increase in cytokine secretion, known as 'cytokine release syndrome' (CRS). The onset of this severe side-effects one of the biggest safety concerns that require addressing for further successful development of CAR-T.

Side-effects

The biggest hurdle in CAR-T clinical trials is severe side-effects, the most acute being CRS. The mortality list of patients undergoing clinical trials of CAR-T highlights the need for design of improved clinical protocols and regulatory decisions of investigational new drug development applications. Symptoms of CRS include high fever, joint pain, muscle pain, low blood pressure, and dyspnea, with death in a few cases. In 2014, clinical trials were temporarily held in Memorial Sloan Kettering Cancer Center after deaths of two patients within two weeks after infusion with CD19-CAR-T from Juno Therapeutics (USA). CRS has been determined as the main cause of death of patients in clinical trials to date.

Although CRS is the most common side-effect related to CAR-T therapy, the development of CRS has been considered to be correlated to the response to therapy. In previous studies, it has been observed that CAR-T responsive patients developed CRS, whereas non-responsive patients did not develop CRS (Maude et al. 2015). The severity of CRS has been reported to be rather correlated with tumor burden at injection time of CAR-T. Given the importance of CRS in clinical monitoring, the markers which can predict the severity of CRS need to be identified. Peak levels of cytokines such as interferon-γ have been found to be more elevated in severe CRS than mild CRS (Davila et al. 2014). Other studies proposed C-reactive protein as an indicator of severe CRS (Maude et al. 2014). However, the decisive biomarkers for CRS still remains to be studied.

To minimize these side-effects, it is crucial to select the appropriate group among enrolled patients and optimize the CAR-T dose in clinical protocol design. Currently, anti-interleukin 6-antibody or steroid drugs are co-administered with CAR-T to reduce CRS (Davila et al. 2014), although further studies are required in this respect. In addition, safety studies assessing whether CAR-T can induce autoimmunity or graft-versus-host disease are warranted. Several studies have examined the efficacy of co-administration of cytokine inhibitors with CAR-T or a suicide gene, with the aim of reducing CRS. A recent study (Grupp et al. 2013) reported that the co-administration of CAR-T with tocilizumab, an anti-interleukin-6 antibody, alleviates CRS.

The majority of CAR-Ts for clinical trials have been constructed using viral vectors for CAR gene transfection. Although these vectors are designed to be non-replicating, a long-term study (over a number of years) should be performed to monitor potential replicative ability. Profiling and standardization of cytokines after CAR-T administration is necessary. The deaths of two patients in 2014 have highlighted CRS as the most severe limitation in clinical

Table 2 In vitro and in vivo CAR-T studies

Generation	Disease	Antigen	Delivery	Co-stimulatory molecules	In vitro, or in vivo	Ref.
3	Lymphoma	TRAIL-receptor1	Retro	CD28, 4-1BB	In vitro	Kobayashi et al. (2014)
3	Lymphoma	CD19	Lenti	–	In vitro	Wang et al. 2012)
2	B-cell lymphoma	CD20	Retro	CD28	In vitro	Watanabe et al. 2015)
2	Osteosarcoma	HER2	Lenti	CD28	In vitro	Mata et al. 2014)
2	Leukemia	CD19	Lenti	CD28	In vitro	Saito et al. 2014)
2	Leukemia	HA-1 H/HLA-A2	Retro	CD28	In vitro	Inaguma et al. 2014)
2	Breast cancer	ERBB2	Retro	CD28	In vitro	Hu et al. 2012)
2	Glioblastoma	EGFRvIII	Retro	CD28	In vitro	Morgan et al. 2012)
2	B-cell lymphoma	CD19	Electro-poration	CD28	In vitro	Torikai et al. (2012)
1	Multiple myeloma	CD138	Lenti	–	In vitro	Jiang et al. 2014)
3	Neuroblastoma	GD2	Retro	CD28, 4-1BB	In vivo	Heczey et al. 2014)
2	B-cell lymphoma	CD19	Retro	CD28	In vivo	Tsukahara et al. 2015)
2	Breast cancer	ERB2	Electro-poration	CD28	In vivo	Wang et al. 2014a, b)
2	Aspergillus infection	Dectin1	Electro-poration	CD28	In vivo	Kumaresan et al. (2014)
2	Breast cancer	HER2	Lenti	CD28	In vivo	Sun et al. (2014)
2	Colorectal cancer	CEA	JetPEI	CD28	In vivo	Blat et al. (2014)
2	Melanoma, breast carcinoma	CSPG4	Retro	CD28	In vivo	Geldres et al. (2014)
2	Prostate cancer	PSMA	Retro	CD28	In vivo	Ma et al. (2014)
2	Multiple myeloma	CS1	Retro	CD28	In vivo	Chu et al. (2014)
2	Glioblastoma	EphA2	Retro	CD28	In vivo	Chow et al. (2012)
2	Multiple myeloma	NY-ESO-1	Retro	CD28	In vivo	Schuberth et al. (2013)
2	Multiple sclerosis	MOG	Lenti	CD28	In vivo	Fransson et al. (2012)
2	Melanoma	B7	Retro	CD28	In vivo	Shin et al. (2012)
2	Head/neck cancer	ERBB receptors	Retro	CD28	In vivo	Davies et al. (2012)
2	Osteosarcoma	HER2	Retro	CD28	In vivo	Rainusso et al. (2012)
2	Ovarian cancer	Mesothelin	Lenti	CD28	In vivo	Lanitis et al. (2012)
2	Osteosarcoma	IL-11Rα	Electro-poration	CD28	In vivo	Huang et al. (2012)

CEA carcinoembryonic antigen, *PSCA* prostate stem cell antigen, *CD138* syndecan-1, *CSPG-4* chondroitin sulfate proteoglycan-4, *PSMA* prostate specific membrane antigen, *MOG* myelin oligodendrocyte glycoprotein

trials of CAR-T. This excessive increase in cytokine secretion results from amplification of CAR-T cells in vivo and may be sufficiently fatal to cause death. To monitor and control CRS, identification of the specific roles of individual cytokines and types of relevant cytokines is required to predict the risk of CRS in clinical trials. Moreover, profiling of cytokines and CRS marker cytokines during clinical trials should be standardized.

In the case of CD19-CAR-T, anticancer effects are accompanied by a certain level of side effects. The possibility of segregating efficacy from side-effects of CAR-T products should be explored, with the aim of establishing an optimal regimen with minimization of CRS. Autoimmunity of CAR-T is another potential concern. Low doses of CAR-T and serial tumor cell-killing effects of single CAR-T cells may lower the possibility of autoimmunity. However, the artificial nature of CAR-T may increase the risk of autoimmunity. Thus, to guarantee safety, regulatory concerns regarding the autoimmunity issue should not be overlooked.

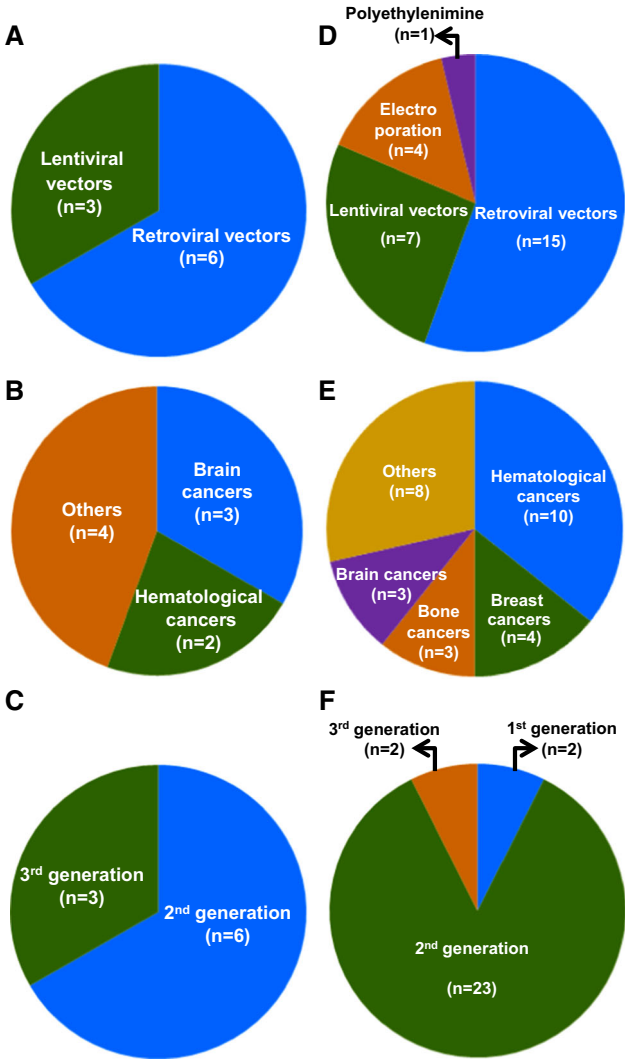

Fig. 5 Current status of CAR-T therapeutics under investigational stages and preclinical trials. CAR-T therapeutics under investigational stages were analyzed by delivery vectors (**a**), target diseases (**b**), and generations (**c**). CAR-T therapeutics in preclinical trials were analyzed by delivery vectors (**d**), target diseases (**e**), and generations (**f**)

In addition to CRS, another concern is "on-target/off-tumor" side effects. The side effect is due to that majority of target antigens for CAR-Ts are existing on both tumor and normal tissues, showing overexpression on tumor cells (Kakarla and Gottschalk 2014). To minimize the "on-target/off-tumor" side effects, the discovery of new target molecule exclusively expressing on tumor tissues would be necessary. Another approach is to remove inappropriately activated CAR-Ts. A recent study reported that small molecule drug AP1903 could induce caspase 9 and apoptosis in transduced cells, killing only activated cells expressing high levels of CAR (Gargett and Brown 2014).

Regulatory perspective

CTL019, a CD19-CAR-T designed by Novartis (USA), is the first known compound in the CAR-T class that has entered phase 2 clinical trials. From the regulatory perspective, manufacturing and clinical trials are major concerns (Puri 2014). Production issues include consistency of CAR-T products, patient-dependent variations in T cell transfection efficiency, optimal T cell types for CAR transfection, and labeling of CAR-T. The major clinical trial concerns include potency and safety.

Pharmacokinetics and biodistribution of CAR-T

Pharmacokinetics and biodistribution experiments provide essential information for predicting the possible side-effects of CAR-T. A recent study reported that CAR-T is distributed to the bone marrow after intravenous administration and circulates in the blood up to 10 months post-injection (Ritchie et al. 2013). Further quantitative analyses of pharmacokinetics, tissue distribution and retention in the body are essential. The tumor antigen, HER-2, has been shown to be expressed in brain tissues and the mammary gland (Wang et al. 2010). Carcinoembryonic antigen,

Table 3 CAR-T in preclinical trials

Generation	Disease	Antigen	Delivery	Co-stimulatory molecule	Ref.
3	Neuroblastoma	GD2	Retro	CD28,OX40	Nishio et al. 2014)
3	Various cancers	FITC	Retro	CD28, 4-1BB	Tamada et al. 2012)
3	B-cell NHL	CD20	Retro	CD28, 4-1BB	Budde et al. (2013)
2	Glioblastoma	IL13Rα2	Lenti	CD28	Krebs et al. 2014)
2	Glioma	IL13R	Retro	CD28	Kong et al. 2012)
2	Various cancers	HER-2, CD19	Retro	CD28	Grada et al. 2013)
2	Ovarian cancer	Mesothelin. a-folate receptor (FRa)	Lenti	CD28	Lanitis et al. 2013)
2	Various cancers	ROR-1	Lenti	4-1BB	Hudecek et al. (2013)
2	Leukemia	CD19	Retro	CD28	Barrett et al. (2013)

Table 4 CAR-T in clinical trials

Antigen	Disease	Organization	Country	Phase	NCT ID	Dose (cells)	Enrolled patients
CD19	B-cell leukemia	Children's Hospital of Philadelphia	USA	I, II	NCT01626495	NA	20
	B-cell leukemia; lymphoma	Abramson Cancer Center of the University of Pennsylvania	USA	I	NCT01029366	NA	14
	B-cell NHL, ALL, CLL	Baylor College of Medicine	USA	I	NCT00586391	$2 \times 10^7/m^2$, $1 \times 10^8/m^2$, $2 \times 10^8/m^2$	54
	NHL, CLL	Baylor College of Medicine	USA	I	NCT00709033	$2 \times 10^7/m^2$, $1 \times 10^8/m^2$, $2 \times 10^8/m^2$	3
	B-cell leukemia	Abramson Cancer Center of the University of Pennsylvania	USA	I	NCT01029366	NA	14
	CLL	Abramson Cancer Center of the University of Pennsylvania	USA	II	NCT01747486	$1–5 \times 10^8$, $1–5 \times 10^7$	34
	Mantle cell lymphoma	Chinese PLA General Hospital	China	I, II	NCT02081937	NA	2
	Mediastinal B-cell lymphoma	National Cancer Institute	USA	I, II	NCT00924326	NA	56
	NHL	Memorial Sloan Kettering Cancer Center	USA	I	NCT01840566	5×10^6/kg 1×10^7/kg 2×10^7/kg	18
	B-cell NHL	Jichi Medical University	Japan	I, II	NCT02134262	1×10^5/kg, 2×10^5/kg, 3×10^5/kg, 6.6×10^6/kg 1×10^6/kg, 2×10^6/kg, 5×10^6/kg, 6.6×10^7/kg	18
	B-cell NHL, ALL, CLL	Fred Hutchinson Cancer Research Center	USA	I, II	NCT01865617	NA	104
	Leukemia, lymphoma	M.D. Anderson Cancer Center	USA	I	NCT01497184	Not to exceed $10^6/m^2$	96
	B cell leukemia	Seattle Children's Hospital	USA	I	NCT01683279	NA	18
	ALL, NHL	National Cancer Institute	USA	I	NCT01593696	NA	90
CD19	B-cell ALL	Memorial Sloan Kettering Cancer Center	USA	I	NCT01860937	5×10^6/kg	55
	NHL, ALL, CLL	Baylor College of Medicine	USA	I	NCT02050347	5×10^5/kg, 1×10^6/kg, 5×10^6/kg	56

Table 4 continued

Antigen	Disease	Organization	Country	Phase	NCT ID	Dose (cells)	Enrolled patients
	B-cell ALL	Abramson Cancer Center of the University of Pennsylvania	USA	II	NCT02030847	1 to 5 × 10^8	24
	NHL	Abramson Cancer Center of the University of Pennsylvania	USA	II	NCT02030834	1 to 5 × 10^8	55
	B-cell leukemia	Children's Hospital of Philadelphia	USA	I	NCT01626495	NA	20
	ALL	Seattle Children's Hospital	USA	I, II	NCT02028455	NA	80
	ALL	Memorial Sloan Kettering Cancer Center	USA	I	NCT01044069	10^6/kg	40
	Leukemia	Memorial Sloan Kettering Cancer Center	USA	I, II	NCT00466531	NA	30
	NHL, ALL, CLL	Baylor College of Medicine	USA	I	NCT01853631	1 × 10^6/m^2, 5 × 10^6/m^2, 2× 10^7/m^2	14
	B-cell lymphoma, leukemia	Uppsala University	Sweden	I, II	NCT02132624	NA	15
	ALL	Affiliated Hospital to Academy of Military Medical Sciences	China	I	NCT02186860	NA	5
	Mediastinal B-cell lymphoma	National Cancer Institute	USA	I, II	NCT00924326	NA	56
	NHL	Professor Robert Hawkins	USA	I	NCT01493453	10^9	24
	B-cell lymphoma	M.D. Anderson Cancer Center	USA	I	NCT00968760	NA	60
CD19	NHL, leukemia	National Cancer Institute	USA	I	NCT01087294	NA	48
	NHL, CLL	Baylor College of Medicine	USA	I	NCT00709033	2 × 10^7/m^2 1 × 10^8/m^2 2 × 10^8/m^2	3
	ALL	Memorial Sloan Kettering Cancer Center	USA	I	NCT01430390	NA	26
	ALL	University College, London	England	I, II	NCT01195480	2 × 10^8/m^2	30
	NHL, ALL, CLL	Baylor College of Medicine	USA	I, II	NCT00840853	1.5 × 10^7/m^2 4.5 × 10^7/m^2 1.2 × 10^8/m^2	68
	Leukemia	Fred Hutchinson Cancer Research Center	USA	I, II	NCT01475058	NA	30
	Lymphoma	City of Hope Medical Center	USA	I, II	NCT01318317	NA	57
	Lymphoma	City of Hope Medical Center	USA	I	NCT00182650	NA	5
	B-cell lymphoma	Peking University	China	I, II	NCT02247609	NA	20
	ALL	City of Hope Medical Center	USA	I	NCT02146924	NA	24
CD20	Leukemia, lymphoma	Fred Hutchinson Cancer Research Center	USA	I	NCT00012207	NA	12
	B-cell CLL	National Cancer Institute	USA	I	NCT00621452	NA	12
	Lymphoma	PLA General Hospital	China	I	NCT01735604	NA	10

Table 4 continued

Antigen	Disease	Organization	Country	Phase	NCT ID	Dose (cells)	Enrolled patients
CD22	Follicular lymphoma, ALL, NHL	National Cancer Institute	USA	I	NCT02315612	3×10^5/kg, 1×10^6/kg, 3×10^6/kg, 1×10^7/kg	57
CD30	NHL	Chinese PLA General Hospital	China	I, II	NCT02259556	NA	30
	NHL	Baylor College of Medicine	USA	I	NCT01316146	2×10^7/m^2, 1×10^8/m^2, 2×10^8/m^2	18
	NHL	Baylor College of Medicine	USA	I	NCT01192464	2×10^7/m^2, 5×10^7/m^2, 1×10^8/m^2	18
	Lymphoma	Peking University	China	I, II	NCT02274584	NA	20
CD33	Myeloid leukemia	Chinese PLA General Hospital	China	I, II	NCT01864902	NA	10
CD123	Leukemia	City of Hope Medical Center	USA	I	NCT02159495	NA	24
CD138	Multiple myeloma	Chinese PLA General Hospital	China	I, II	NCT01886976	NA	10
LewisY	Multiple myeloma	Peter MacCallum Cancer Centre	Australia	I	NCT01716364	NA	6
Kappa	Lymphoma	Baylor College of Medicine	USA	I	NCT00881920	2×10^7/m^2, 1×10^8/m^2, 2×10^8/m^2	54
NKG2D	Leukemia	Celdara Medical	USA	I	NCT02203825	1×10^6, 3×10^6, 1×10^7, 3×10^7	24
HER2	Sarcoma	Baylor College of Medicine	USA	I	NCT00902044	1×10^8/m^2	36
	Solid tumors	Chinese PLA General Hospital	China	I, II	NCT01935843	NA	10
	Metastatic cancer	National Cancer Institute	USA	I	NCT00924287	NA	1
	Glioblastoma	Baylor College of Medicine	USA	I, II	NCT01109095	1×10^6/m^2 3×10^6/m^2 1×10^7/m^2 3×10^7/m^2 1×10^8/m^2	16

Table 4 continued

Antigen	Disease	Organization	Country	Phase	NCT ID	Dose (cells)	Enrolled patients
	HER2 positive malignancy	Baylor College of Medicine	USA	I	NCT00889954	$1 \times 10^4/m^2$, $3 \times 10^4/m^2$, $1 \times 10^6/m^2$, $3 \times 10^6/m^2$, $1 \times 10^7/m^2$, $3 \times 10^7/m^2$, $1 \times 10^8/m^2$	18
GD2	Neuroblastoma	Baylor College of Medicine	USA	I	NCT00085930	$2 \times 10^7/m^2$	19
	Neuroblastoma	Children's Mercy Hospital Kansas City	USA	I	NCT01460901	NA	5
	Neuroblastoma	Baylor College of Medicine	USA	I	NCT01822652	1.5×10^8, 2×10^8	38
	Sarcomas	Baylor College of Medicine	USA	I	NCT01953900	$1 \times 10^6/m^2$ $1 \times 10^7/m^2$ $1 \times 10^7/m^2$ $1 \times 10^8/m^2$	26
	Various cancers	National Cancer Institute	USA	I	NCT02107963	1×10^5/kg, 1×10^6/kg, 3×10^6/kg, 1×10^7/kg	72
CD171	Neuroblastoma	Seattle Children's Hospital	USA	I	NCT02311621	5×10^5/kg. 1×10^6/kg, 5×10^6/kg, 1×10^7/kg	80
CEA	Cancer	Roger Williams Medical Center	USA	I	NCT00004178	NA	–
	Various cancers	Cancer Research UK	England	I	NCT01212887	NA	14
	Colorectal cancer	Roger Williams Medical Center	USA	I	NCT00673322	10^9, 10^{10}, 10^{11}	1
	Liver metastases	Roger Williams Medical Center	USA	I	NCT01373047	10^8, 10^9, 10^{10}	8
	Breast cancer	Roger Williams Medical Center	USA	I	NCT00673829	Phase Ia: 10^9, 10^{10} Phase Ib: 10^{11}	26
CEA	Metastatic cancers	Roger Williams Medical Center	USA	II	NCT01723306	NA	48

Table 4 continued

Antigen	Disease	Organization	Country	Phase	NCT ID	Dose (cells)	Enrolled patients
EGFR	Advanced EGFR-positive solid tumors	Chinese PLA General Hospital	China	I, II	NCT01869166	NA	10
	Advanced glioma	RenJi Hospital	China	I	NCT02331693	NA	10
EGFR vIII	Glioma	Abramson Cancer Center of the University of Pennsylvania	USA	I	NCT02209376	NA	12
	Glioblastoma	National Cancer Institute	USA	I, II	NCT01454596	NA	160
PSMA	Prostate cancer	Memorial Sloan Kettering Cancer Center	USA	I	NCT01140373	1×10^7/kg 3×10^7/kg 1×10^8/kg	–
Folate receptor	Ovarian cancer	National Cancer Institute	USA	I	NCT00019136	NA	–
IL-13 zetakin	Brain tumor	City of Hope Medical Center	USA	I	NCT00730613	10^8	3
IL13 receptor a2	Adult anaplastic astrocytoma	City of Hope Medical Center	USA	I	NCT02208362	NA	44
ErbB T4+	Head and Neck cancer	King's College London	England	I	NCT01818323	10^7–10^9	30
FAP	B-cell CLL	Fred Hutchinson Cancer Research Center	USA	I	NCT01722149	10^6	12
ROR1	CLL	M.D. Anderson Cancer Center	USA	I	NCT02194374	10^5/kg	48

ALL acute lymphoblastic leukemia, *CLL* chronic lymphocytic leukemia, *NHL* non-Hodgkin's lymphoma, *NA* not available

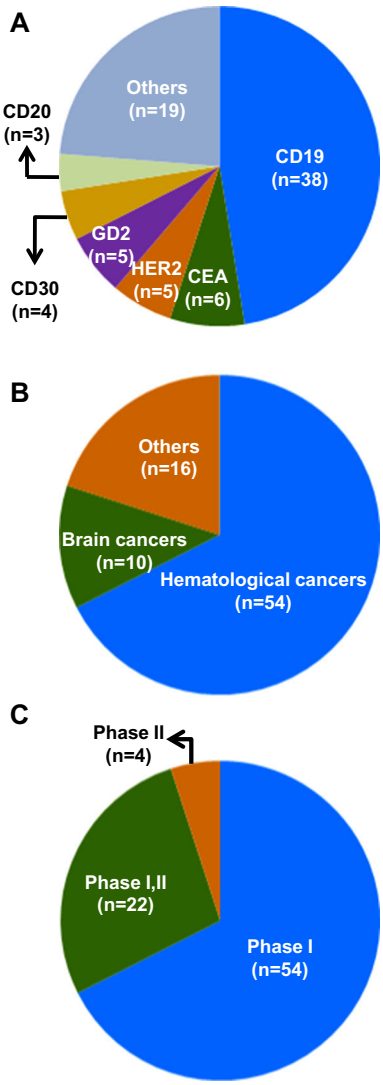

Fig. 6 Current status of CAR-T therapeutics in clinical trials. CAR-T therapeutics in preclinical stages were analyzed by tumor antigens (**a**), target diseases (**b**), and generations (**c**)

another tumor antigen, has been identified in colon or normal tissues (Schölzel et al. 2000).The nonexclusive expression patterns of tumor antigens in normal tissues increase the possibility of normal tissue damage upon administration of CAR-T targeting these tumor antigens. From this viewpoint, regulatory considerations associated with the distribution and pharmacokinetics of CAR-T should be addressed.

Efficacy of CAR-T

One concern with regard to the efficacy of CAR-T is the consideration of optimal T cell subsets. The majority of CAR-Ts in clinical trials introduce CAR genes after isolation of total T cells from patients. The issue of whether the T cell subtype affects the transfection efficiency and potency of CAR-Ts should be examined.

Efficacy evaluation methods for CAR-T should be further explored. Currently, tumor cell lysis induction and cytokine secretion capabilities of CAR-Ts are under examination. Establishment of a relevant method reflecting the efficacy of CAR-T may facilitate standardized evaluation of CAR-T products. Moreover, owing to in vivo amplification, it is necessary to assess the relationships among the ratio of CAR-T within total administered T cells, CAR copy numbers and efficacy.

Unlike chemical or protein drugs, CAR-T is a living drug that amplifies in the body after administration. This in vivo amplification property leads to an increase in the actual effective dose of CAR-T. The severity of symptoms and ages of patients may serve as factors affecting the in vivo amplification efficiency of CAR-T. The discrepancy between administration and working doses is a unique feature of CAR-T. Further analysis of the relationships between administered and working doses and therapeutic effects is warranted.

The specific T cell subtypes contributing to anticancer activity need to be identified. Currently, CAR-T is administered as mixtures of various T cell subsets. A recent study reported that increasing the frequency of CD8(+) CD45RA(+)CCR7(+) CAR-T cells, a subset closest to T cell memory stem cells, within total CAR-T enhances anticancer activity in an animal model (Xu et al. 2014).

Clinical trials of CAR-T

Several issues require clarification with regard to the optimal dose of CAR-T. As a result of in vivo amplification of intravenously infused CAR-T, the initial doses infused are not the same as the actual working cell number. Moreover, adjustment criteria of doses should be fixed between the weight and body surface of patients. Considering the amplification of CAR-Ts in bone marrow after infusion, we need to ascertain whether measurement of CAR-T numbers in the blood reflects the amplification extent in bone marrow.

Manufacture and quality control of CAR-T products

To validate consistency in CAR-T quality among batches, regulatory studies on chemistry, manufacturing and control (CMC) are essential. Analysis of quality control for each step of CAR-T production is important (Fig. 7). Quality control should be performed to maintain transfection efficiency of CAR among different batches, with parameters including the acceptable ranges of gene-modified CAR-T ratios among total T cells and quantification of copy

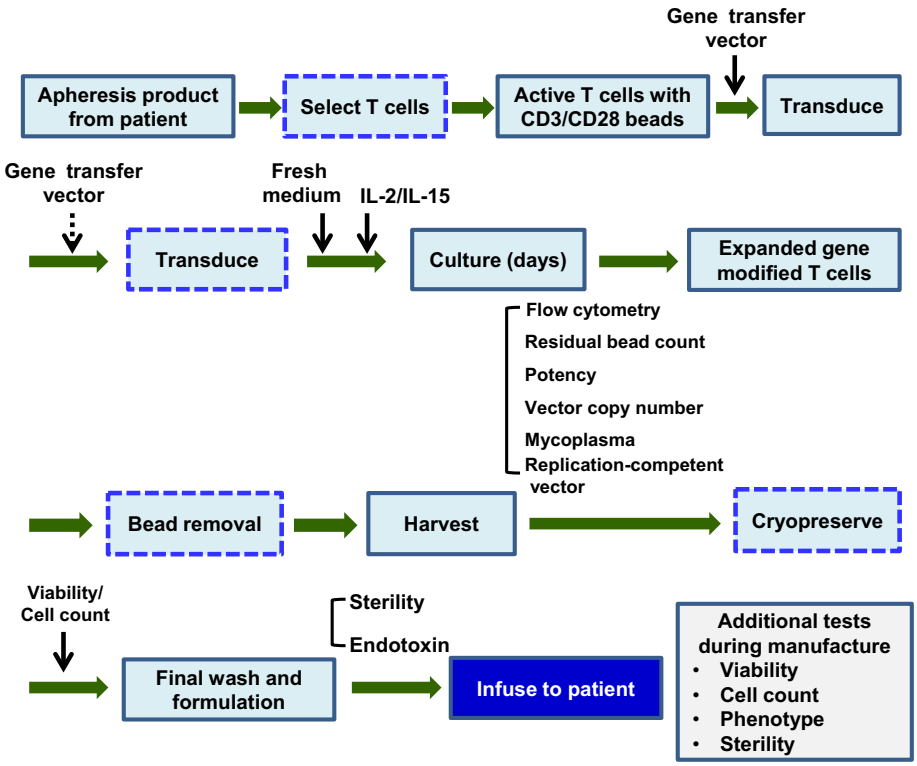

Fig. 7 Quality control flow chart for production of CAR-T

numbers of the CAR gene per cell. For consistent production of CAR-T among batches, standardization of the stock of viral vectors is required to provide constant multiplicity of infection. Moreover, effects of patient age and medical treatment history on transfection of T cells with CAR-encoding vectors should be assessed.

Viral vectors, such as retroviral or lentiviral vectors, are frequently used for introduction of CAR into T cells (Wang and Riviere 2015). Quality control of viral vectors for CAR gene delivery should be performed in terms of purity, safety, T cell transfection efficiency, and physicochemical characterization.

Moreover, for quality control, validation of CAR-T sterility is essential. Since CAR-T is manufactured ex vivo by isolation of T cells, introduction of CAR genes, amplification, microbial assays and the scope of microorganisms for CAR-T need to be established.

Production and distribution of CAR-T need to be standardized. After conceptual design and proof-of-concept studies, production techniques have mostly been transferred from the laboratory benches of academia to industry. The manufacturing processes of CAR-carrying viral vectors and CAR-T are complex and differ among developers. CAR-T products under clinical trials are generated and distributed to patients using different protocols. The production and distribution processes of gene-modified cells are sufficiently crucial to affect quality. Quality control and standardization of manufacturing and distribution processes should thus be performed under good practice principles.

Labeling of CAR-T products should be carefully assessed. Given the autologous nature of CAR-T in which patient T cells are transfected with CAR genes and infused back into the same patient, it is important to clarify labeling of CAR-T to minimize the fatal risk of potential administration to the wrong patients.

Conclusions

CAR-Ts have attracted considerable research attention as a novel and potent modality of cancer immunotherapy. Global pharmaceutical companies have started investing in CAR-Ts, with several products in the pipeline for approval. However, substantial regulatory issues for CAR-T need to be addressed. The living nature of CAR-T necessitates careful assessment of safety and efficacy issues. The ex vivo manufacturing process of CAR-T highlights the significance of validating sterility and extensive product quality control. Further focus on regulatory studies and establishment of regulatory science-based guidelines may expedite the development of effective CAR-T products for patient use.

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