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REVIEW

Chimeric antigen receptor T cell therapy: 25 years in the making

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ABSTRACT

Chimeric antigen receptor (CAR) T cell therapy of cancer is generating enormous enthusiasm. Twenty-five years after the concept was first proposed, major advances in molecular biology, virology, and good manufacturing practices (GMP)-grade cell production have transformed antibody-T cell chimeras from a scientific curiosity to a fact of life for academic cellular immunotherapy researchers and, increasingly, for patients. In this review, we explain the preclinical concept, outline how it has been translated to the clinic, and draw lessons from the first years of CAR T cell therapy for the practicing clinician.

Contents

1. Background

In their 1989 paper, Gross, Waks, and Eshhar reported experiments introducing into a cytotoxic T cell the genetic code for an antibody that imparted to the T cell the ability to recognize a hapten, 2,4,6-trinitrophenyl (TNP). They described the results of basic in vitro T cell function studies demonstrating antigen-specific non-MHC-restricted cytotoxicity and interleukin-2 production [1]. The authors concluded that "construction of chimeric T cell receptors with anti-tumor specificity will enable testing of the feasibility of this approach in combating human tumors." These results thus illustrated both the first (production of a chimeric entity) and the final (T cell effector function against tumor cells) steps in successful targeted adoptive cellular immunotherapy for cancer. However, bookended between these two steps lay several chapters that had yet to be completed before CAR T cell therapy could become practical on a clinical level.

Effective and routine clinical use requires the following steps: (1) adequate numbers of T cells must be collected, (2) new genetic material must be introduced efficiently and safely, (3) the genetically modified T cells must be expanded to sufficient numbers for clinical application, (4) once infused the T cells must be able to traffic to the tumor, and (5) cells must expand in vivo, and persist at least long enough to induce a meaningful anti-tumor response.

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Peripheral blood mononuclear cells (PBMC) must first be collected by apheresis or phlebotomy and grown under conditions that will support the expansion and stimulation of T cells.

Genetic material encoding the chimeric antigen receptor is transferred into the patient's T cells using either viral or non-viral approaches, typically at the beginning of the ex vivo expansion stage. Gammaretroviral or lentiviral vectors integrate into the host cell genome and hence lead to permanent transgene expression. In contrast to gammaretroviral vectors, lentiviral vectors can integrate into non-dividing cells, are less susceptible to silencing by host restriction factors, and can deliver larger DNA sequences [2–5]. Long-term follow-up of clinical studies supports the safety of using these vectors in T cells [6], despite early concern about insertional mutagenesis after retroviral transduction of hematopoietic stem cells [4]. Non-viral approaches are typically cheaper and are regarded as potentially safer by regulatory agencies. These include transposon/transposase systems such as Sleeping Beauty, that can deliver a large payload with persistent high-level transgene expression [7–10] or gene transfer using RNA electroporation, the latter as used by our group [11,12].

The CAR construct is modular and consists of an extracellular target-binding domain, a hinge region, a trans-membrane domain that anchors the CAR to the cell membrane, and an intracellular signaling domain (see Fig. 1). The target-binding domain is typically derived from the light and heavy chain portions of a single chain variable fragment (scFv), although other cognate interactions can be used [13]. Notably, CAR-based recognition imparts to the T cell the ability to recognize any cell surface molecule to which an antibody can be made. This has two advantages: (1) proteins, glycoproteins, and glycolipids can all serve as potential targets, and (2) recognition is not MHC-restricted thus there is no requirement for antigen processing and presentation. On the other hand, a major limitation of this approach is that antigens that are solely intracellular cannot be targeted. Cancer-testis and tumor-specific antigens such as the MAGE family and NY-ESO1 are intracellular and hence cannot be targeted by CAR [14].

Engagement of the CAR by its ligand transmits a signal to the intracellular T cell machinery via a signaling domain, typically the CD3 zeta chain. The incorporation of co-stimulatory molecules such as CD27, CD28, CD134 (OX40), or CD137 (4-1BB) can augment the effects of zeta chain signaling and hence enhance T cell proliferation and persistence [15]. CAR constructs with one additional co-stimulatory molecule are known as "second generation" and those with more than one additional co-stimulatory molecule are known as "third generation" CAR.

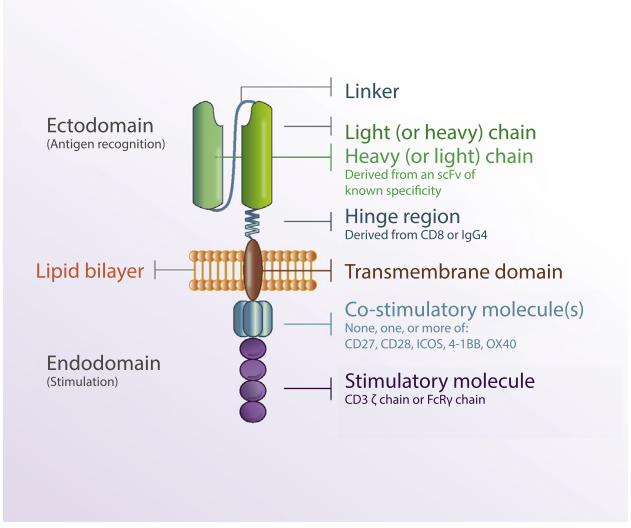

Fig. 1. Anatomy of a CAR. All the indicated components (with the exception of the lipid bilayer, which is an intergral part of the host cell membrane) are typically produced as a single polypeptide encoded by one plasmid.

While second generation CAR are clearly superior to first generation CAR, whether the incorporation of additional co-stimulation (third generation CAR) provides further benefit remains unknown [16]. The optimal design of a given CAR thus remains an area of active investigation and should be empirically evaluated for the treatment of different malignancies. Recent preclinical results indicate that CD137-based costimulatory domains are better at preventing T cell exhaustion than those based on CD28 [17].

The ex vivo expansion phase is performed by stimulating T cells using anti-CD3 antibodies with or without additional co-stimulating antibodies such as anti-CD28 or with cytokines such as interleukin-2, -7, -12, and/or -15. Alternatively, artificial antigen presenting cells (APC) such as irradiated K562 tumor cells or EBV-transformed cells can be used [18–24]. Approaches are not uniform across centers, and the precise method employed by any given institution typically relates to the preclinical expertise developed at that particular center. However, by making broad comparisons across different centers and under several different settings, it appears that most groups achieve several hundred to several thousand-fold T cell expansion during a culture period ranging from 10 days to 6 weeks [18,19,21–29]. Ex vivo expansion using artificial APC appears to generate higher numbers of T cells at the expense of a longer culture period and hence the preparation time of the final T cell product is prolonged. It is important to generate T cells that are capable of further proliferation after in vivo transfer as this correlates with in vivo persistence. Broadly, this may be done by one of two ways. The first is to modify the manufacturing process either by shortening the ex vivo expansion period such that the T cells that are ultimately derived are less terminally differentiated ("younger"), or by using cytokines such IL-7 and IL-15 [30]. The second approach is to start with T cells of a defined subset composition where the selection of central memory CD8 + T cells prior to ex vivo expansion can lead to infusion products of uniform cellular composition for each patient [31], a concept that is being tested in a clinical trial (NCT01865617).

Upon infusion into patient, CAR T cells must traffic to the tumor site, engage with their cognate antigen, proliferate, avoid inhibitory signals from the tumor microenvironment, kill target cells, and persist long enough to ensure that no residual tumor cells arise. The components of successful adoptive cellular immunotherapy are illustrated in Fig. 2. Preclinical and clinical studies clearly show that T cell homeostatic proliferation and persistence are augmented by lymphodepletion and this is typically achieved using chemotherapy and/or radiation [32]. Upon intravenous infusion, adoptively transferred leukocytes tend to redistribute rapidly from the blood into the tissues and can be seen in the lungs in the first few hours, followed by pooling in the liver and spleen [33,34]. In the setting of CAR T cell therapy, we have noted an initial early peak in CART cell numbers in the peripheral blood, followed by a reduction within the first few days, which is then followed by an increase in CAR T cell numbers in the blood as the cells proliferate [18,35]. CAR T cells clearly traffic to bone marrow and lymph nodes as well as other tissues that contain their target antigen [35]. Local accumulation occurs at the sites of target recognition, whether through local proliferation, trafficking, or both. It is likely that possession of the correct homing molecules plays an important role in this process [36]. We now recognize that the microenvironment of multiple malignancies contains inhibitory and immunosuppressive stimuli [37], and several groups have shown that both cellular (eg regulatory T cells) and molecular (eg immune checkpoints such as PD-1) signals can impede the function of CAR T cells [38–40].

Finally, it is possible that CAR T cells need to persist for at least some time in order to continue to provide immunosurveillance and to prevent relapse. Memory T cells have a lifespan of many years, and as noted, gammaretroviral or lentiviral integration can lead to stable integration of the transgene. Patients with HIV who were infused at our institution with gene-modified T cells over 10 years ago still show evidence of T cell persistence and patients treated with anti-CD19 CAR T cells up to 4 years ago still have circulating CART-19 cells and B cell aplasia [6,18,41].

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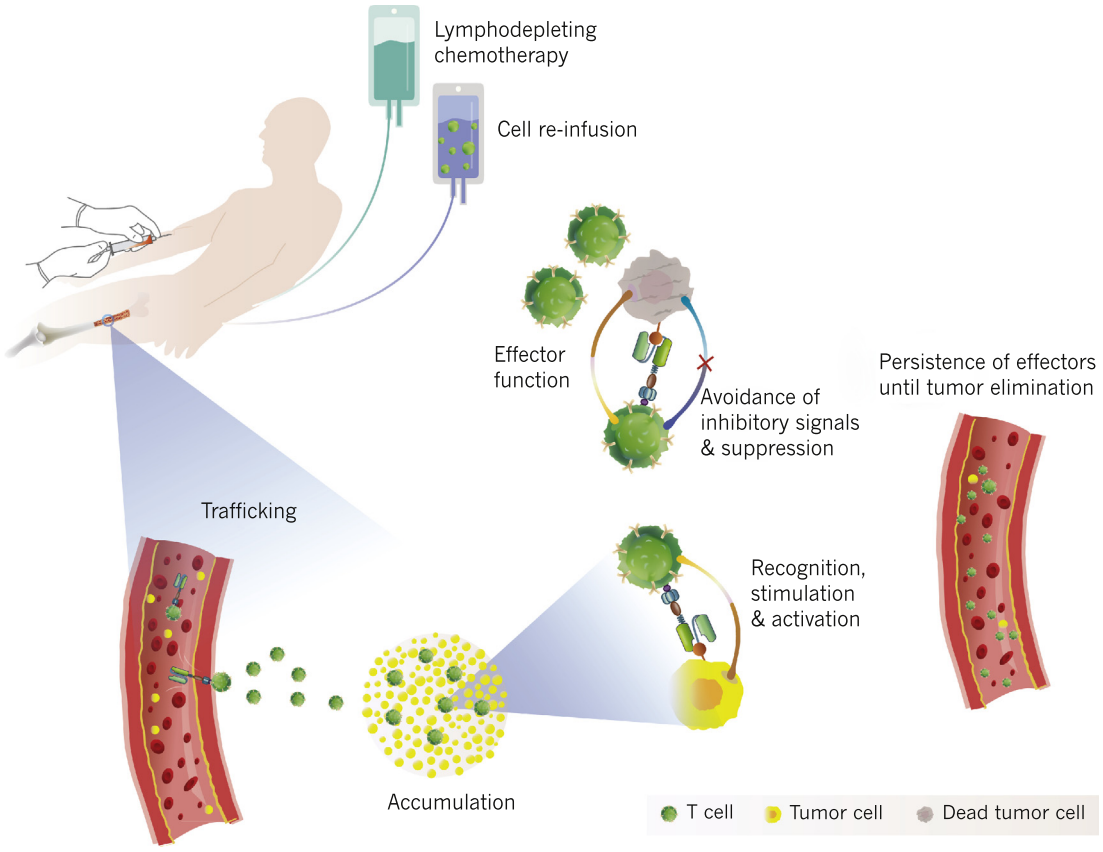

Fig. 2. Components of successful adoptive cellular immunotherapy. The patient undergoes lymphodepleting chemotherapy a variable period of time before the planned infusion. After intravenous infusion, the CAR T cells circulate, traffic out of the circulation into the tumor site, and accumulate locally where they recognize their cognate ligand and are stimulated to proliferate further and produce their effector functions. The T cells must avoid inhibitory signals and suppression from the tumor and the tumor microenvironment and must persist until elimination of all malignant cells.

Thus, CAR T cells exhibit many of the characteristics that define successful adoptive cellular immunotherapy. Upon infusion into the patient, they are able to traffic to tumor sites, proliferate, release cytokines and lyse tumor cells, and persist long-term as memory cells.

2. CAR T cells for hematologic malignancies

Although the earliest trials of CAR T cell therapy were performed in patients with solid tumors [33,42], it is actually in trials of patients with CD19-expressing B cell malignancies that the most exciting results have recently been obtained [18,26,28,43,44]. B cell malignancies are particularly amenable to targeting using CAR T cell therapy, due to the presence of the CD19 antigen on all B cell malignancies from the most immature B-ALL to the most mature lymphomas [45] and the fact that patients can tolerate prolonged periods of B cell aplasia.

2.1. CAR T cell therapy for B cell malignancies

Indication that patients with B-cell malignancy could be safely treated with genetically engineered B-cell-specific autologous T cells was first provided in 2008 by investigators from the Fred Hutchinson Cancer Research Center and from the City of Hope National Medical Center [46]. Seven patients with indolent or mantle cell lymphoma were treated with anti-CD20-redirected T cells and achieved a partial response (one patient), stable disease (four patients), or maintained a previous complete response (two patients). T cells persisted up to 9 weeks. Gene transfer was achieved by DNA electroporation followed by limiting dilution cloning, a rather laborious and inefficient process. In 2010, the City of Hope group published a follow-up study on patients with relapsed diffuse large B cell lymphoma treated with anti-CD20 (two patients) or anti-CD19 (two patients) CAR T cells, but in this study, T cell persistence was no longer than 7 days, likely related to a cellular anti-transgene immune response in some of the patients [21]. A successful proof of concept report using anti-CD19 CAR T cells was published by the National Cancer Institute group in 2010 [47].

In 2011, our group at the University of Pennsylvania published the outcome of three patients treated with CART19 cells for CLL. We showed dramatic in vivo expansion, cell killing, and delayed tumor lysis syndrome [18,35]. Subsequently, anti-CD19-directed CAR T cell therapy has been shown to be active in NHL [48] and dramatically effective for patients with relapsed and refractory ALL [43,44,49].

Numerous publications describe well over 100 recipients of anti-CD19 CAR T cell therapy to date in various settings (see Table 1). The collective experience from the treatment of these patients across different centers and using somewhat different modalities can be summarized as indicating that patients should receive lymphodepleting chemotherapy, that second generation CAR constructs are superior to first generation constructs, that patients with acute lymphoid leukemia in particular have very high response rates, that patients often develop a severe cytokine release syndrome, and that there is no clear dose–response relationship between the number of CAR T cell infused and the likelihood of response. Furthermore, there appears to be no correlation between initial tumor burden and response, in that patients with marked bone marrow infiltration by leukemia can and do experience complete responses, including the achievement of a "MRD-negative" state (that is absence of disease by high sensitivity testing such as flow cytometry, RTqPCR, or deep sequencing). There may be an indication that bulky lymphadenopathy may be more difficult to eradicate

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Table 1
Clinical trials with anti-CD19 T cell therapy.

Patients	Disease (age)	Disease state	Lymphodepletion	T cell dose	Exogenous cytokines	Toxicities	Best disease response	Time to response	Time to relapse/ progression (months)	CAR persistence (days)
City of Hope, retroviral FMC63 anti-CD19 scFv-CD3ζ with thymidine kinase suicide gene [*Jensen 2010*]										
2	FL (NR)	Refractory	Flu after dose no. 1	$6 \times 10^9/m^2$ total (CAR% NR)	IL-2	Lymphopenia	NE		1–5 months	1
Baylor, retroviral FMC63 anti-CD19 scFv—CD28-CD3ζ [*Savoldo 2011*]										
6	SLL, DLBCL	Relapsed/ refractory	No	2×10^7–$2 \times 10^8/m^2$ total (CAR 20-60%)	No	NR	PD [4], SD [2]		0.5–10 months	6 weeks
University of Pennsylvania, lentiviral FMC63 anti-CD19 scFv-41BB-CD3ζ (Porter 2011 and Kalos 2011)										
3	CLL	Relapsed	B, BR, PC	1.5×10^5–1.6×10^7/kg CAR^+ cells	No	Fever, CRS	CR [2], PR [1]		7–11+	>24 weeks
MSKCC, retroviral SJ25C1 anti-CD19scFv-CD28-CD3ζ, (Brentjens, Blood 2011)										
10	CLL, ALL	Relapsed, refractory	No [3], Cy [7]	1.8×10^8–3.2×10^9 CAR^+ cells	No	Fever, CRS	PD [4], PR [1], SD [2], NE [2]		NA–2+	NR–35
NCI, retroviral FMC63 anti-CD19 scFv-CD28-CD3ζ, [*Kochenderfer 2012*]										
8	FL, CLL, SMZL	Relapsed	Flu Cy	0.5×10^7–5.5×10^7/kg total, 30–71% CAR+	IL-2	Cytopenias, fever, hypotension	CR [1] PR [5] SD [1] NE [1]		6–8+	20–132+
University of Pennsylvania, lentiviral FMC63 anti-CD19 scFv-41BB-CD3ζ (Grupp 2013; Maude, Frey et al. 2014)										
30	ALL	Relapsed/ refractory [24] CR [6]	None-Cy-based	0.76×10^6–20×10^6/kg CAR+	No	CRS (100%), severe CRS (27%)	CR (90%)		67% 6 month EFS	68% 6 month persistence
MSKCC, retroviral SJ25C1 anti-CD19*scFv-CD28-CD3ζ*, (Brentjens 2013 and Davila 2014)										
16	ALL	Relapsed/ refractory [9] CR [7]	Cy	1.4–3.2×10^8 CAR^+	No		CR (88%)		NE (many proceeded to HCT)	Undetectable by 2–3 months
Baylor, *retroviral FMC63 anti-CD19 scFv—CD28-CD3ζ* [*Cruz 2013*], *relapsed post allogeneic HCT*										
8	ALL, CLL	Relapsed, refractory [4] CR [4]	No	1.9×10^7–1.1×10^8	No	No GVHD	CR [3] PR [1] SD [1] PD [3]		NA – 8 months+	1–12 weeks
NCI, retroviral FMC63 anti-CD19 scFv-CD28-CD3ζ, [*Kochenderfer 2015*]										
15	DLBCL, CLL, indolent NHL		Flu Cy	$1\text{-}5 \times 10^6$/kg CAR+	No	Fever, hypotension, delirium	CR [8] PR [4] SD [1] NE [2]		1–23+ months	variable
NCI, retroviral FMC63 anti-CD19 scFv-CD28-CD3ζ, [*Lee 2015*]										
21	ALL [20], DLBCL [1]	Relapsed, refractory	Flu Cy	0.03×10^6–3×10^6/kg CAR+	No	Fever (81%) Severe CRS (14%)	CR (70%)		NE (many proceeded to HCT)	Undetectable by day 68

Notes:
+ indicates ongoing response at time of report. B = bendamustine; BR = bendamustine and rituximab; CLL = chronic lymphoid leukemia; CR = complete response (includes CR with incomplete count recovery), regardless of minimal residual disease status; DLBCL = diffuse large B-cell lymphoma; FL = follicular lymphoma; GVHD = graft versus host disease; HCT = allogeneic hematopoietic cell transplantation; NE = non-evaluable; NR = no response; PC = pentostatin and cyclophosphamide; PD = progressive disease; PR = partial response; SD = stable disease; SMZL = splenic marginal zone lymphoma; FluCy = fludarabine and cyclophosphamide; VP16 = etoposide.

than heavy marrow disease or that lymphadenopathy may take longer to resolve, yet it is also clear that CAR T cells can enter extramedullary sites such as the central nervous system [18,43,44,49–51].

A novel syndrome associated with cytokine release and macrophage activation has been described by several groups [28,41,49]. The cytokine release syndrome (CRS) is characterized by symptoms that can include high persistent fevers, nausea, myalgias, arthralgias, and can evolve to capillary leak, hypoxia, and hypotension that can be life threatening. Standard CTCAE grading for CRS was not designed to describe CRS after cell therapies and novel grading scales have been proposed [52]. CRS is often accompanied by clinical and biochemical changes seen with hemophagocytic lymphohistiocytosis (HLH) or macrophage activation syndrome (MAS); it is almost always accompanied by raised c-reactive protein (CRP) and markedly elevated ferritin. Neurological toxicity has also been described, although the etiology of this is unclear as CAR T cells are not always found in the CNS at the time of CRS [49]. The neurological toxicity consists of confusion, word-finding difficulty, or aphasia, and sometimes leads to seizures; however, neurological toxicity associated with anti-CD19 CAR T cells therapy is generally self-limited [43,49].

A fascinating observation from the treatment of patients with B-ALL has been a handful of CD19-negative relapses that occur while CAR T cells are still detectable [41]. The pathogenesis of this is unclear but is a testament to the power of the immune selection pressure exerted by anti-CD19 CAR T cells. This is an important lesson when treating patients with highly proliferative, immature malignancies such as acute leukemias. Future trials will need to account for the possibility of CD19-negative relapse, perhaps by targeting multiple antigens.

Other B cell antigens have been targeted in preclinical models. These include CD20, CD22, CD23, ROR1, or the kappa light chain. CD22 is highly expressed on mature lymphoid malignancies as well as on B-ALL. This is a rather long molecule and preclinical results have been informative in demonstrating that targeting a proximal epitope on the CD22 molecule leads to superior efficacy [53]. CD23 is expressed on CLL cells but

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not on normal B cells, and preclinical studies have shown that anti-CD23 CAR T cells have some activity against growth of a CLL-like cell line [54]. ROR1 is detected on malignant B cells in CLL and MCL, and at lower levels on normal adipose cells and some B cell precursors and so targeting of this antigen could spare normal B cells [55]. Targeting one of the two light chains would be useful in mature B cell malignancies with surface light chain expression and is another way to spare some of the normal B cell population; preclinical results show that free light chains do not interfere with the function of the CAR T cells and may in fact sustain their proliferation [56]. Preliminary clinical results suggest that this approach is safe and effective [57]. Currently, open clinical trials for the treatment of B cell malignancies are shown in Table 2.

2.2. CAR T cell therapy for acute myeloid leukemia (AML)

Relapsed or refractory AML represents a clear area of need and the encouraging results of the treatment of B-ALL with anti-CD19 CAR T cells suggest that treatment of this aggressive malignancy should be quite feasible, when an appropriate surface antigen is identified. Unfortunately, as AML is a malignancy of the hematopoietic stem cell, it is challenging to find a target that is present on AML blasts and absent from normal hematopoietic cells. A group from the University of Melbourne demonstrated that it is feasible to treat AML patients with an anti-Lewis Y CD28-costimulated CAR T cell product. There was minimal toxicity, and two patients experienced minor responses. Of interest, radiolabelling of the infused CAR T cells clearly demonstrated trafficking to sites of disease [34]. Additional targets are at an early stage of evaluation. The early hematopoietic antigen CD123, the interleukin-3 receptor α chain, has been shown by both the City of Hope and the University of Pennsylvania groups to be expressed on the majority of AML patients. However, CD123 is also present on normal marrow precursors suggesting that CAR T cells targeting this antigen could lead to severe hematopoietic toxicity [58,59]. This observation indicates that clinical trials using anti-CD123 CAR T cells will need to mitigate the possible consequences of myeloablation, possibly by using a transiently expressed CAR or a CAR T cell with limited persistence. It would be logical to incorporate this into a transplant strategy in order to reverse the effects of myeloablation or, at the very least, to have a back-up bone marrow donor in case of irreversible myeloablation. This is the approach taken by the City of Hope in a recently announced clinical trial (NCT02159495). The apparent safety of a CD123-directed diphtheria toxin-conjugated reagent in patients with blastic plasmacytoid dendritic cell neoplasm (BPDCN) is only moderately reassuring in this regard, as the avidity of CAR T cells for their target is likely much higher than that of such cytokine-conjugated therapeutics [60].

One of the most obvious targets for the treatment of AML is the myeloid antigen CD33, which is the target of the antibody-drug conjugate gemtuzumab ozogamicin. CD33 is expressed on immature myeloid cells and potent, prolonged targeting with CAR T cells could lead to profound myeloablation. Gemtuzumab led to hepatotoxicity and veno-occlusive disease in a minority of patients, although it is unclear whether that was related to specific targeting of Kupffer cells, or to release of the calicheamycin toxin [61,62]. Preclinical studies indicate that anti-CD33 CAR T cells have equivalent efficacy when compared with anti-CD123 CAR T cells [63,64]. A study in China is currently recruiting patients to an anti-CD33 CAR T cell trial (NCT01864902), and a single patient treated with this modality appears to have had a transient response to anti-CD33 CAR without major hematologic or non-hematologic toxicities [65].

CD44 is an adhesion molecule that is broadly expressed on normal tissues. Its isoform variant 6 (CD44v6) has been shown to be expressed on some AML as well as myeloma cells, and anti-CD44v6 CAR T cells mediated potent anti-tumor effects in mouse models [66]. Despite failing to demonstrate keratinocyte toxicity in vitro after exposure to CAR T cells [66], human trials will likely have to be conducted very cautiously given the report of lethal epithelial toxicity in a patient treated with an anti-CD44v6 monoclonal antibody [67].

NKG2D ligands are found on AML blasts (and indeed on many transformed and virally infected cells). An interesting approach to target NKG2D ligands using an NKG2D CAR is currently being tested clinically (NCT02203825).

The description of these efforts to apply CAR T cell therapy to high-risk relapsed or refractory AML illustrates the scope of the issues associated with targeting a hematopoietic stem cell-derived malignancy. Clearly, clinical development of CAR T cell therapy for AML will have to be undertaken very carefully. The development of approaches to limit the persistence of the infused CAR T cells will be very helpful in this regard. Current approaches include the use of mRNA-electroporated, "biodegradable" CAR T cells, introduction of a suicide gene, or the inclusion of extracellular domain on the T cells to render them potentially susceptible to depletion by clinically available antibodies [58,59,66]. Current AML CAR T cell clinical trials are shown in Table 2.

2.3. CAR T cell therapy for Hodgkin lymphoma

Hodgkin lymphoma does not express B cell-associated surface markers. A characteristic of the malignant cells in Hodgkin lymphoma is bright, uniform expression of CD30. This antigen is the target of the recently approved antibody-drug conjugate brentuximab vedotin. There are preclinical data that CD30+ Hodgkin cell lines can be targeted by CAR T cells [68,69]. Notably, CD30 is present on some activated T cells, thus anti-CD30 CAR T cells could theoretically induce fratricide and impair their own expansion. Two trials targeting CD30 are ongoing at the Baylor College of Medicine (NCT01192464 and NCT01316146).

2.4. CAR T cell therapy for T cell malignancies

Targeting abnormal T cells with antigen-specific CAR T cells represents arguably the most difficult application of the CAR T cell concept. An antigen would have to be found on the malignant cells that is absent from the transgenic cells. Preferably, this antigen would also be absent from residual healthy T cells in order to prevent prolonged T cell lymphopenia. Recent preclinical data suggest that T cells expressing an anti-CD5 CAR escape fratricide by downregulating their own CD5 and may be used for the treatment of CD5-expressing T cell malignancies [70]. As some T cell lymphomas express CD30, theoretically an anti-CD30 CAR T cell could be used for this indication. T cell malignancies could also be treated with CAR-bearing natural killer cells that do not share T cell antigens [71].

2.5. CAR T cell therapy for myeloma

Despite the advent of multiple new agents for the treatment of multiple myeloma (MM), this remains an incurable disease. Several potential targets for CAR T cell therapy have been identified. The Baylor group is targeting the kappa light chain (NCT00881920) and efficacy was shown in pre-clinical studies [56]. A potential shortcoming of this approach is that most myeloma cells do not express kappa chains on their surface [72]. CD138 (also known as syndecan-1) is a cell membrane proteoglycan whose hematopoietic expression is limited to plasma cells, though it is also expressed in various epithelia [73–75]. Clinical trials of an antibody-drug conjugate (ADC) targeting CD138 have reported modest single-agent activity, but with dose-limiting toxicities that included inflammation of the palms and soles (likely an on-target effect) [76]. A phase I/II clinical trial of anti-CD138 CAR T cells is currently recruiting in China (NCT01886976).

Other promising targets for myeloma include the B cell maturation antigen (BCMA), CS-1, CD38, NKG2D ligands, and CD44v6. BCMA is found on mature B cells and plasma cells [77] and is expressed on most, if not all, multiple myeloma cells [78]. Trials of anti-BCMA CAR T cells for MM are currently recruiting patients at the NCI

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Table 2
Currently recruiting CAR T cell therapy trials by antigen.

Center	Disease	Patients	Co-stimulation	Gene transfer	Notes	Clinicaltrials.gov identifier
BCMA						
NCI	MM	18–73	NA	NA		NCT02215967
CD19						
MSKCC	CLL	>18 yo	*CD28*	RV	Dose-escalation	NCT00466531
BCM	B-cell malignancy	Any	*CD28*	RV	With ipilimumab	NCT00586391
BCM	B-cell malignancy	Any	*CD28*	RV	Dose escalation	NCT00608270
BCM	B-cell malignancy	Any	*CD28*	RV	After AlloHCT, viral co-specificity	NCT00840853
NCI	B-cell malignancy	18–68	*CD28*	RV	With IL2	NCT00924326
MDACC	B-cell lymphoma	18–65			With or without IL2	NCT00968760
MSKCC	B-ALL	>18 yo	*CD28*	RV		NCT01044069
NCI	B-cell malignancy	18–75	*CD28*	RV	Post alloHCT; active GVHD not allowed	NCT01087294
MSKCC	CLL	>18 yo	*CD28*	RV	Upfront therapy	NCT01416974
MSKCC	B-ALL	<19 yo	*CD28*	RV	After AlloHCT, viral co-specificity	NCT01430390
Manchester, UK	B-cell malignancy	>18 yo	*None*	RV		NCT01493453
MDACC	B-cell malignancy	1–65			After AlloHCT	NCT01497184
NCI	B-cell malignancy	1–30 yo	*CD28*	RV		NCT01593696
CHOP	CD19+ leukemia & lymphoma	1–24 yo	*4-1BB*	LV		NCT01623495
Seattle Children's	$CD19^+$ ALL	Age 1–26			$EGFR^+$ construct (may allow deletion)	NCT01683279
Penn	CLL/SLL	>18y	*4-1BB*	LV	2 dose level comparison	NCT01747486
MSKCC	Aggressive B-NHL, relapsed/refractory	18–70	*CD28*	RV	After autologous SCT	NCT01840566
BCM	B-cell malignancy	Up to 75 yo	*CD28+/− 4-1BB*	RV		NCT01853531
MSKCC	B-ALL	<26 yo	*CD28*	RV		NCT01860937
Beijing	B-cell malignancy	5–90 yo	*4-1BB*	RV		NCT01864889
FHCRC	B-cell malignancy	>18y	*4-1BB*	LV		NCT01865617
Penn	B-cell NHL	>18 yo	*4-1BB*	LV		NCT02030834
Seattle Children's	B-ALL	NA	*4-1BB*	LV	$EGFR^+$ construct (may allow deletion)	NCT02028455
Penn	B-ALL	>18 yo	*4-1BB*	LV		NCT02030847
BCM	B-cell malignancy	NA	*CD28*	RV	After AlloHCT	NCT02050347
Beijing	Mantle cell lymphoma	50–80	*4-1BB*	RV		NCT02081937
Sweden	B-cell malignancy	>18 yo	*CD28 and 4-1BB*	RV		NCT02132624
Japan	B cell NHL	20–70	*CD28*	RV		NCT02134262
COH	ALL	>18	*CD28*	LV		NCT02146924
Beijing/Florida	B-cell lymphoma	>18	*CD27*	LV	Caspase 9 suicide gene	NCT02247609
Penn	Hodgkin	>18	*4-1BB*	RNA		NCT02277522
Kite/COH	NHL	>18	*NA*	NA		NCT02348216
Southwest Hospital, China	B-cell malignancy	18–70	*NA*	NA		NCT02349698
Shenzhen	B-cell malignancy	1–85	*CD28*	LV		NCT02456350
CD20						
Beijing	B cell NHL	18–90	*4-1BB*	RV		NCT01735604
CD22						
NCI	B-cell malignancies	1–30	*4-1BB*	LV		NCT02315612
CD30						
BCM	Hodgkin and NHL		*CD28*	RV		NCT01316146
Beijing	CD30+ lymphoma	16–80	*NA*	NA		NCT02259556
CD33						
Beijing	AML	5–90 yo	*4-1BB*	LV		NCT01864902
CD138						
Beijing	Myeloma	18–80 yo	*4-1BB*	LV		NCT01886976
CD171						
Seattle	Neuroblastoma	<18	*CD28 and 4-1BB*	LV		NCT02311621
CEA						
Southwest Hospital, China	CEA+ malignancies	18–70	*NA*	NA		NCT02349724
EGFR						
Beijing	EGFR+ solid tumors	18–80 yo	*4-1BB*	LV		NCT01869166
Renji Hospital	GBM	NA	*NA*	NA		NCT02331693
EGFRvIII						
NCI	GBM	18–66	*CD28*	RV		NCT01454596
Penn/UCSF	GBM	>18	*4-1BB*	LV		NCT02209376
ErbB						
London	Head & Neck cancer	>18 yo	*CD28*	RV	Intratumoral	NCT01818323
FAP						
Zurich	Mesothelioma	18–75	*NA*	RV		NCT01722149
GD2						
Kansas	Neuroblastoma	1.5–17 yo	*None*	RV	Multivirus-specific	NCT01460901
BCM	Neuroblastoma		*CD28 and OX40*		Suicide gene	NCT01822652
BCM	Sarcoma		*CD28 and OX40*	RV	Suicide gene, Co-specificity for VZV	NCT01953900

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Table 2 (*continued*)

Center	Disease	Patients	Co-stimulation	Gene transfer	Notes	Clinicaltrials.gov identifier
NCI	GD2 + solid tumors excluding neuroblastoma	1–35 yo	*CD28 and OX40*		Suicide gene	NCT02107963
Glypican 3						
Renji Hospital	HCC	18–70	*NA*	NA		NCT02395250
Her2						
BCM	Sarcoma		*CD28*	RV		NCT00902044
BCM	GBM		*CD28*	RV	Co-specificity for CMV	NCT01109095
BCM	Lung cancer	>3 yo			TGFbeta resistance, Co-specificity for EBV	NCT01889954
Beijing	Her2 + solid tumors	18–80 yo	*4-1BB*	LV		NCT01935843
IL13Ra2						
BCM	GBM	18–75	*4-1BB*	RV	Intracranial administration	NCT02208362
Kappa light chain						
BCM	B-cell malignancy or myeloma		*CD28*	RV		NCT00881920
Mesothelin						
Penn	Mesothelin-expressing cancer	>18	*4-1BB*	LV		NCT02159716
Penn/UCSF	Pancreatic	>18	*4-1BB*	LV	Combined with anti-CD19 CAR T cells	NCT02465983
NKG2D ligands						
DFCI	AML, MDS, MM	18 yo or older	*NA*	NA		NCT02203825

(NCT02215967) and at the University of Pennsylvania (NCT02546167). The cell surface glycoprotein CS1 is expressed in most multiple myeloma cells and normal plasma cell samples; lower levels of expression have also been noted in other lymphocytes, activated monocytes, and activated dendritic cells, but not in other normal tissues [79]. A soluble antibody targeting CS-1, elotuzumab is in advanced phases of clinical development [80]. A group at Ohio State has published on their preclinical findings of efficacy of a CS1-directed CAR introduced into both NK cells and T cells in vitro and in mouse models [81,82]. Though CS1 is an attractive target, its expression on activated T cells suggests that a potential downside may be fratricide during the manufacturing culture or in vivo, and soluble CS1 may potentially block the CAR-directed cells. Pre-clinical data with CAR T cells directed to CD38 and CD44v6 have shown activity against multiple myeloma and AML cell lines and primary patient samples [66,83]. The main concern with CD38 as a CAR target is its expression on multiple lymphoid and myeloid subsets, and hence the potential for broad myelotoxicity [84], though the soluble anti-CD38 antibody daratumuab does not seem to cause myelosuppression [85]. The main concern with CD44v6 as a CAR target is its expression on keratinocytes; a radioisotope-labeled antibody bivatuzumab caused myelosuppression and a fatality due to skin toxicity, though CAR T cells based on bivatuzumab antibody did not seem to kill keratinocytes in vitro [67]. Our group has published a case report of a patient with multiply relapsed myeloma who achieved a complete response to anti-CD19 CAR T cells after attenuated-dose melphalan conditioning, suggesting that rare CD19-expressing myeloma cells may be responsible for treatment resistance [86].

3. CAR T cells for solid malignancies

Although hematologic malignancies represent a minority of human cancer, they have played an outsized role in advancing cancer treatment as the proving grounds for novel therapies such as multi-agent chemotherapy, monoclonal antibodies, and tyrosine kinase inhibitors, to name but a few. In contrast, in the setting of CAR T cell therapy, some of the earliest trials and the most important lessons were drawn from the field of solid tumors [33,42]. The greatest challenge in developing CAR T cell therapy for solid tumors is the identification of suitable target antigens. Although hematologic malignancies are routinely classified based on the expression of cell surface markers, solid tumors are more often characterized based on combinations of anatomic location, histology, immunohistochemical stains which do not always distinguish between surface and intracellular expression, and specific mutations of signaling molecules at the molecular level; none of these methods directly yields a CAR target. In cases where therapeutic antibodies have been developed for a solid tumor, translation to a CAR construct may be straightforward, but the risk of on-target off-tumor toxicity appears to be greater with CAR-modified T cells than with the equivalent naked soluble antibody. For example, an anti-HER2/neu CAR based on the clinically well-tolerated monoclonal antibody trastuzumab led to fatal pulmonary toxicity in a colon cancer patient [87]. This was attributed to low levels of target expression in pulmonary vascular endothelium even though no such toxicity is observed when trastuzumab is administered to breast cancer patients. However, a recent publication described 19 patients treated with a lower dose of anti-Her2 CAR T cells with no dose-limiting toxicity [88]. Similarly, in a clinical trial of CAR T cells directed to carbonic anhydrase IX, which is overexpressed in renal cell cancer, there was toxicity to the biliary tract due to low-level expression of the antigen [42]. Novel methods to mitigate toxicity could include pre-treatment with antibody to block antigen expression on normal tissues before CAR T cell infusion [89] or the use of combinatorial CAR T cells that are activated only in the presence of multiple antigens [90,91].

There are currently several open trials of CAR T cells for solid tumors, including glioblastoma multiforme (GBM), ovarian cancer, pancreatic cancer, and mesothelioma. Two trials using CAR T cells redirected to the Epidermal Growth Factor Receptor variant III (EGFRvIII) antigen are presently open (NCT02209376 and NCT01454596) and are particularly interesting because EGFRvIII is a tumor-specific antigen that is not thought to be expressed on normal tissues. Mesothelin is being targeted for multiple solid tumors at three different centers; the anti-mesothelin antibody conjugated to pseudomonas toxin caused pleuritis and pericarditis and was short lived, but the toxicity seems to be related to the toxin. The persistence and efficacy of the drug could be increased by the addition of lymphodepletion [92,93]. PSMA is being targeted in prostate cancer in combination with a suicide gene (HSV thymidine kinase) at MSKCC (NCT01140373). Results have not yet been published. The ganglioside GD-2 is being targeted with CAR T cells at Baylor for the treatment of neuroblastoma (NCT 01822652); because the antibody to GD-2 causes a significant pain syndrome due to target expression on peripheral nerves, the investigators have included a fast-acting suicide gene into the CAR-modified T cells. Alternatively, it is possible that CAR T cells will function as a vaccine in solid tumors. Multiple injections of CAR T cells electroporated with mRNA encoding an anti-mesothelin CAR led to a case of anaphylaxis, but also led to a vaccine effect with

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epitope spreading and transient responses [12,94]. Using CAR T cells as a vaccine approach is being tested with a cMet-directed CAR in melanoma (NCT 01837602). Given the relatively localized nature of some solid tumors, local delivery of genetically modified T cells may be an attractive way to limit systemic toxicity. Several trials have been reported, including one that targets the IL-13 receptor alpha chain in GBM [95], another where anti-CEA CAR T cells are injected intra-arterially for liver metastases [96] and more are planned such as an intraperitoneal infusion of anti-MUC16^{ecto} T cells that secrete IL-12 in ovarian cancer [97].

Aside from selection of a suitable target antigen, the big question in solid tumors will be if CAR T cells can home to non-lymphoid organs and penetrate tumor stroma. Pre-clinical data suggest that homing to tumor is feasible, as xenografted human tumors in mouse models can be controlled with CAR T cells [36]. However, xenograft models do not typically have the tumor stroma that is characteristic of many solid tumors such as pancreatic adenocarcinoma. The immune microenvironment of solid tumors is not adequately replicated in xenografted mice and can only be studied adequately in immune competent mice; however, these types of models require the development of entirely syngeneic models with mouse T cells, which do not have the same costimulatory requirements as human T cells. Notably, checkpoint blockade is likely to relieve some of the immunosuppressive effects of the tumor microenvironment and may enhance the function of CAR T cells. Some investigators are targeting tumor stroma directly with CAR T cells targeting fibroblast activation protein (FAP). FAP is an integral membrane proteinase selectively expressed in reactive stromal fibroblasts of epithelial cancers and in several types of malignancies and tumor stroma. Preclinical data suggest that this approach can deplete tumor stroma but at the cost of significant toxicity from cachexia and myelosuppression, an undesirable outcome in patients with advanced cancer [98,99]. If a safe dose window were identified, it would be interesting to combine stroma and tumor-directed CAR T cells to achieve synergy in solid tumors. Current clinical trials using CAR T cells against solid tumors are shown in Table 2.

4. New concepts in CAR T cell therapy

4.1. Expansion and persistence

Observations from recent clinical trials indicate a remarkable capacity for in vivo expansion and long-term persistence in some patients treated with CAR T cells. It is notable that the initial clinical studies that were associated with disappointing clinical outcomes were also associated with poor T cell persistence [21,100,101]. In contrast, the current generation of clinical trials, employing second generation CAR T cells based on CD28 or CD137-costimulation, are associated with approximately 3 log-folds expansion and with persistence of months to years. In some patients, B cell aplasia continues beyond the last point at which CART19 cells can be detected by flow cytometry while the transgene is still detectable by quantitative PCR, suggesting that B cell aplasia may be a suitable functional readout of persistent CART19 activity [43].

4.2. Toxicity

Specific on-target toxicity in CART19 trials relates to depletion of normal B cells. To date, no unusual infectious or autoimmune complications have been reported, and patients receive repletion with pooled immunoglobulins for hypogammaglobulinemia. A cytokine release syndrome that overlaps with macrophage activation syndrome was described by our group and others after CAR T cell therapy of CD19+ malignancies [41,43,49]. We have found that the use of the anti-IL6 receptor antibody tocilizumab is very effective at aborting this syndrome, although we do not know whether premature intervention impairs the anti-tumor effect [52]. The etiology of the neurotoxicity that has been reported after anti-CD19 CAR T cell therapy remains unclear [49]. Distinct from CRS, some patients experience tumor lysis syndrome, which may be delayed and appears temporally related to the peak of T cell activity [18]. Tumor lysis syndrome is a testament to the potency of this therapy. Despite theoretical concerns about integrating viruses leading to insertional mutagenesis, there have been no reports of transformation during hundreds of patient-years of follow-up using several different T cell therapies [6].

5. Conclusions

This is a genuinely exciting time in the development of CAR T cells as a novel therapeutic modality. Years of basic research in the fields of virology, molecular biology, and T cell expansion have coalesced in an explosion of preclinical research that continues to deliver new insights on a regular basis and that has been translated into a burgeoning array of clinical trials in both liquid and solid malignancies. CAR T cell treatment of B-cell malignancies in particular has demonstrated that this approach is feasible and can engender impressive and profound responses in patients with otherwise treatment-refractory cancer. CAR T cell therapy has many advantages over monoclonal antibody therapy such as the ability for dramatic in vivo expansion magnifying any potential response, and long-term persistence providing on-going vaccine-like activity; therefore, in our opinion, they will prove superior to bi-specific antibody or antibody-drug conjugate technology.

Important lessons can be drawn from the past 8 years of clinical trials using CAR T cells in both hematologic and solid malignancies. Where CAR constructs are based on existing monoclonal antibodies, it is crucial to realize that apparent safety of the antibody may underestimate the toxicity of CAR T cells, as T cells are subject to dramatic in vivo proliferation that amplifies their activity, and also due to the superior avidity of CAR T cells [42,87]. Hence, careful studies of antigen expression on normal tissues must be performed, and ideally new CAR T cell constructs should be tested in clinically relevant models [58]. The power of this modality is truly unprecedented and should not be underestimated.

Published results describing over 100 patients treated with anti-CD19 CAR T cells to date, and preliminary results presented in abstract form covering many more, indicate that responses are heterogeneous. For example, ALL may be a better CAR T cell target than CLL. The reasons for this are at present unclear, although preclinical data show that CLL cells inhibit T cell function [102]. Future work should define the susceptibility of particular tumor types to CAR T cells. Reports that several patients have relapsed with a CD19-negative ALL after CAR T cell therapy highlight both the strength of this approach and one of its potential weaknesses—concentrating on a single antigen in a highly proliferative immature malignancy such as ALL could induce antigen-loss variants [41]. In future, this issue could be addressed by infusing pooled CAR T cell products where each product is specific for one antigen, covering a range of possible antigen-escape mutants.

Different centers currently have diverse approaches to selecting the optimal CAR construct to take forward into clinical trials, and employ different cell production methods. The myriad ways that anti-CD19 CAR T cells are now produced may impact the differing response rates at the various centers. This issue in particular cannot be resolved without careful comparative trials. In our opinion, this issue is as important as the discovery of additional targets for CAR T cell therapy as it will impact the field as a whole.

CD19 represents one of the low-hanging fruit given its ubiquitous expression among B-cell malignancies and the relative benign course of patients with prolonged B cell aplasia. Nonetheless, whether effective CAR T cell therapy can be applied universally to other hematologic and solid malignancies remains to be seen. However, the design and implementation of novel CAR T cell products are accelerating and the time from antigen discovery to clinical trial is likely to get shorter and shorter. We hope to see the explosion of CAR T cell-based clinical trials translated into standard of care for patients with cancer in the near future.

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Practice points

These are the most important points of relevance in current clinical practice:

1. CAR T cell therapy can induce profound and durable remissions in patients with multiply relapsed and refractory leukemia.
2. T cell proliferation and in vivo activity can lead to a severe cytokine-release syndrome that must be managed carefully by experienced physicians.
3. Some degree of T cell persistence is likely important for prevention of relapse after initial response to therapy.
4. Selection of antigens that are suitable for CAR T cell-based therapy must be conducted very carefully and modeled using relevant pre-clinical models. Every caution should be exercised in the implementation of novel CAR T cell reagents in order to mitigate severe toxicity.

Research agenda

These are some questions will likely repay further research:

1. What is the optimal CAR construct, with regard to issues of co-stimulatory domain, vector, and T cell production?
2. What novel cell-surface tumor-specific antigens are available for each malignancy to be targeted?
3. Are there any universal tumor antigens that can be employed to facilitate translation of CAR T cell therapy to more patients?
4. Given that CRS is the major toxicity from CART19 therapy, are there any approaches that will ameliorate CRS without compromising efficacy?
5. What features of the tumor microenvironment play a role in facilitating or impeding CAR T cell attack?

Conflict of interest

SG: Research support: Novartis; Intellectual property interests and potential royalty payments: Novartis.

MVM: Research Support: Novartis; Intellectual property interests and potential royalty payments: Novartis.

DLP: Research support: Novartis; Intellectual property interests and potential royalty payments: Novartis. Employment: Genentech (spouse).

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