CAR T-cells for T-cell malignancies challenges in distinguishing between therapeutic, normal, and neoplastic T-cells

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Abstract

Chimeric antigen receptor (CAR) T-cells targeting CD19 demonstrated remarkable efficacy for the treatment of B-cell malignancies. The development of CAR T-cells against T-cell malignancies appears more challenging due to the similarities between the therapeutic, normal and malignant T-cells. The obstacles include CAR T-cell fratricide, T-cell aplasia, and contamination of CAR T-cell products with malignant T-cells. Here, we review these challenges and the solutions proposed to overcome these limitations.

Introduction

Chimeric antigen receptor (CAR) T-cells demonstrated remarkable efficacy for the treatment of B-cell malignancies and have been approved by the US Food and Drug Administration (FDA) for the treatment of relapsed/refractory B-cell acute lymphoblastic leukemia (B-ALL) and diffuse large B-cell lymphoma (DLBCL).(1–5) This proof of concept generated great enthusiasm for the development of CAR T-cells directed against other types of cancer, including T-cell malignancies.(6,7)

T-cell malignancies encompass immature (i.e. T-cell acute lymphoblastic leukemias (T-ALL)) and mature (i.e. T-cell lymphomas (TCL)) lymphoid neoplasms and are often associated with a dismal prognosis.(8–10)

Despite great interest, the development of CAR T-cells against T-cell malignancies has been limited so far due to the difficulties to distinguish between therapeutic, normal and malignant T-cells. Here, we review the challenges raised by such development and describe the solutions that have been proposed to address these limitations.

Challenges

CAR T-cells, directed against antigens shared with normal T-cells, may recognize and kill 3 types of cells: tumor T-cells, normal T-cells and CAR T-cells (Figure 1). Mutual killing of CAR T-cells, also called fratricide, may prevent the generation, expansion and persistence of CAR T-cells. Prolonged and profound T-cell aplasia induced by the destruction of normal T-cells exposes patients to severe opportunistic infections.(11,12) Thus, developing CAR T-cells for T-cell malignancies requires targeting malignant T-cells while sparing normal and CAR T-cells, or at least some subsets of them.

Furthermore, CAR T-cell products may be contaminated with malignant T-cells. Indeed, circulating tumor T-cells are often found in the peripheral blood of patients with T-ALL(9,13–15) and, although less frequently, with TCL.(8,16) Because tumor T-cells may harbor the same phenotypic and functional properties as normal T-cells(17), they may be harvested, transduced, expanded and infused concomitantly with normal T-cells. This process may lead to the generation of “CAR tumor T-cells”. Ruella et al. recently described accidental transduction of CAR construct in leukemic B-ALL cells leading to CAR expressing blasts (so called “CARB”).(18) In these patients, CD19 CAR at the blast surface bound to CD19, thus preventing their recognition by CAR T-cells. A similar mechanism may be anticipated with malignant T-cells if transduced with the CAR construct. Furthermore, contaminating tumor T-cells may also be genetically edited to prevent the expression of a T-cell target along with normal T-cells and thereby escape CAR T-cells recognition and eradication. Thus, developing CAR T-cells for T-cell malignancies requires avoiding contamination of the CAR T-cell product with malignant transduced T-cells.

Proposed solutions

Fratricide

To prevent fratricide, CAR cells should be directed against a tumor antigen that is not shared (or not completely) between malignant and therapeutic T-cells. This can be achieved in 2 ways: i) either by targeting a tumor antigen that is not naturally expressed by the CAR T-cells, ii) or by using CAR cells that do not express the T-cell target which can be achieved by using CAR T-cells that have been genetically edited *ex vivo* to prevent expression of the T-cell target or by using non-T CAR cells such as NK-cells.

CAR T-cells directed against antigens that spare CAR T-cells (Table 1)

Most target antigens are shared between normal and malignant T-cells(8,19,20) rendering specific targeting of tumor T-cells challenging. Strategies that target tumor antigens while sparing CAR T-cells include targeting of pan-T antigens which are down-regulated during CAR T-cell expansion (e.g. CD5) thereby preventing or minimizing CAR T-cell fratricide(21), or targeting of antigens which are expressed only by a subset of normal T-cells (e.g. CD4 or CD30) thereby sparing subsets of CAR T-cells (Table 1).(22–24)

<u>CD5 CAR T-cells</u>

CD5 is expressed by normal T-cells and by most T-ALL and TCL.(8,25) In preclinical models, CD5 is downregulated through internalization following CAR T-cell expansion and activation, thus preventing fratricide.(21,26) However, this is not observed when using CAR T-cells containing a TNFR superfamily co-stimulatory domain such as 4-1BB.(27,28)

CD4 CAR T-cells

CD4 is expressed by two thirds of normal T-cells, by most TCL and a subset of T-ALL.(8,17,25) Preclinical data testing CD4 CAR T-cells have shown a highly enriched CD8+ CAR T-cells product which efficiently killed lymphoma cells *in vitro* and *in vivo*.(22) However, such CD4 CAR T-cells will induce a CD4 T-cell aplasia, and this would result in a syndrome similar to HIV/AIDS. Thus, we view this approach as a temporary or bridging strategy.

CD30 CAR T-cells

CD30 is expressed by a subset of activated B and T-cells, virtually all Hodgkin lymphomas (HL) and anaplastic large cell lymphomas (ALCL), subsets of peripheral TCL, and about one third of T-ALL.(29,30) Following demonstration of a preclinical activity(31), CD30 CAR T-cells have been evaluated in two phase I clinical trials, mostly in patients with HL.(23,24) No decrease in B or T-cell counts were described in these studies. Furthermore, T-cell immunity to common viral pathogens did not seem to be impaired.(23) This is in line with what has been observed in patients treated with brentuximab-vedotin, an antibody-drug conjugate directed against CD30 where no unexpected opportunistic infections have been observed.(32) In the study by Ramos et al., two patients with ALCL (one systemic ALK+ and one cutaneous ALK-) were infused with CD30 CAR T-cells.(23,31) One of the two patients achieved a complete remission which lasted for 9 months following 4 infusions of CD30 CAR T-cells at the highest dose (2.10^8 cells/m^2).(23) Wang and colleagues observed a partial response following infusion without conditioning regimen in one (and only) ALCL (cutaneous) patient.(24) These encouraging results may be further improved by adding a lymphodepleting conditioning regimen prior to the CD30 CAR T-cell infusion.(33,34)

Genetically-edited CAR T-cells to prevent target expression

Fratricide may also be avoided by knocking-out the target gene using gene editing (such as TALEN or CRISPR system). This approach has been evaluated preclinically with CD7 CAR T-cells. CD7 is expressed by normal T and NK-cells, by most T-ALL and a subset of TCL.(8,25) Unlike CD5, CD7 is poorly downregulated upon CAR T-cell expansion/activation. Thus, prevention of fratricide requires genomic disruption of CD7 prior to CAR transduction. In preclinical models, CRISPR/Cas9-mediated editing of CD7 abrogates fratricide and enables the expansion of CAR T-cells.(35,36) Similar results have been achieved using PEBL technology(37), a method that prevents CD7 surface expression by anchoring newly synthesized CD7 in the endoplasmic reticulum and/or golgi.(38–40) Gomes-Silva et al. suggested that the infused CD7 CAR T-cells may retain antiviral activity through their native receptor and therefore counteract the profound immunodeficiency induced by on-target/off-tumor effects of CD7 CAR T-cells.(35)

CAR NK-cells

CARs are commonly transduced into T-cells but the use of NK-cells is emerging.(41) Using NK-cells is a promising strategy to avoid fratricide. The typical cell surface phenotype of NK-cells shows lack of TCR, CD3 and CD5 expression.(42,43) Conversely, NK-cells are characterized by CD56 and CD7 expression.(44) NK-cells are part of the innate immune system and have natural cytotoxic properties against tumors which can be further improved by CAR engineering.(41,45,46) NK-cells present several advantages for CAR engineering: i) their phenotype (different from T-cells) can be used to prevent fratricide and avoid

contamination, ii) due to their lack of a TCR receptor, they do not naturally induce graft-versus-host disease (GVHD)(47) and thus can be used in allogeneic conditions, iii) their short lifespan may prevent prolonged T-cell aplasia.(41) However, using blood NK-cells to manufacture CAR cells is challenging because the collection, expansion and transduction of these cells is difficult.(48) For these reasons, CAR NK-cells directed against CD3, CD4 and CD5 have been engineered using the NK-92 cell line, a human cell line derived from a patient with a NK-cell lymphoma, rather than natural NK-cells.(43,48–52) No fratricide is expected since NK-cells do not express these targets. In preclinical models however, CAR T-cells seem to outperform CAR NK-92 cells. Although CAR NK-92 cells can induce significant reduction of tumor burden, they lack persistence in xenograft mouse models, consistent with the short lifespan of NK-cells. Moreover, some concerns may be raised regarding the potential tumorigenicity of CAR NK-92 cells since they originate from a transformed cell line. To prevent this risk, NK-92 cells are irradiated before injection to patients. NK-92 cells (not genetically engineered) have been evaluated in phase I clinical trials in patients with metastatic solid tumors.(53,54) Another safety concern is the advent of neurotoxicity (strokes) after infusion of CD3 and CD5 CAR NK-92 cells in mice.(43,49)

T-cell aplasia

Unlike B-cell aplasia which is usually well tolerated and can be compensated with continuous infusion of immunoglobulins for the lack of humoral adaptive immunity(55,56), prolonged T-cell aplasia exposes patients to opportunistic infections.(11,12) Prevention of prolonged T-cell aplasia may be achieved in 3 ways: i) either by targeting a tumor antigen that is not expressed by all or a subset of normal T-cells, ii) by using short-lived CAR cells and iii) by

myeloablation and subsequent bridging to allogeneic hematopoietic stem cell transplantation (HSCT).

CAR T-cells directed against antigens that spare all or subsets of normal T-cells (Table 1)

Such strategies have been previously described (paragraph “Fratricide”). However, depletion of certain T-cell subsets which are quantitatively (CD5+ or CD7+) or qualitatively (CD4+) important may still induce profound immune suppression. Depletion of other T-cell subsets such as CD30 may be better tolerated.(23,24) Another promising approach is the targeting of the T-cell receptor beta constant 1 (TRBC1) or TRBC2. Physiologically, the TCR β chain expresses either TRBC1 or the TRBC2 constant region.(57) Maciocia *et al*. have shown that the proportion of TRBC1+ T-cells varies between 25% to 47% in healthy donors, regardless of the T-cell subset.(58) T-cell leukemias and lymphomas, instead, are clonally TRBC1 positive or negative.(58) Therefore, TRBC1 CAR T-cells kill specifically TRBC1 malignancies while sparing TRBC2+ normal T-cells.(58) A clinical trial testing TRBC1 CAR T-cells in T-cell lymphomas is about to start (AUTO4).

Short-lived CAR cells

Another way to prevent prolonged T-cell aplasia is to use CAR cells with limited lifespan. This can be achieved by using i) allogeneic CAR T-cells, ii) CAR NK-cells, iii) non-viral mRNA transfection with electroporation(59), or iv) a safety switch (such as suicide gene or a targetable surface marker).(60–64) However, these strategies do not allow prolonged

persistence of CAR T-cells meant to prevent disease recurrence. Thus, they may rather be used as a bridge to transplant.

Contamination of CAR T-cells product with malignant T-cells

Purifying the apheresis product from circulating tumor T-cells to produce CAR T-cells is challenging since it is often difficult to distinguish between normal and neoplastic T-cells. Thus, avoiding contamination can be achieved in 2 ways: i) either by purifying and transfecting non-T cells, such as NK-cells (described previously), or ii) by producing CAR T-cells from an allogeneic healthy donor.

Allogeneic CAR T-cells

CAR T-cells can be generated from allogeneic donors.(2) Nevertheless, infusion of allogeneic CAR T-cells may cause life-threatening GVHD, even after HLA matching.(65,66) To overcome this issue, Cooper et al. developed “off-the-shelf”, universal CD7 CAR T-cells (UCART7).(36) Using multiplex CRISPR/Cas9 gene editing of T-cells before CAR transduction, they deleted both CD7 and T-cell receptor alpha chain (TRAC). In preclinical models, their CD7 CAR efficiently killed T-ALL without inducing xenogeneic GVHD in a patient-derived xenograft (PDX) mouse model.(36) These allogeneic CAR T-cells are expected to have a short lifespan because they will be eliminated upon immune reconstitution of the host. This short persistence may be seen as an advantage to prevent T-cell aplasia but as a disadvantage to prevent cancer recurrence.

Conclusion

The development of CAR T-cells for T-cell malignancies faces unique challenges due to the similarities between therapeutic, normal, and malignant T-cells. Many of the solutions that have been proposed do not seem optimal, either because they lack specificity (risk of fratricide, immune suppression and/or contamination) or persistence (risk of tumor recurrence). Targeting of certain subsets (e.g. CD30 or TRBC1 CAR T-cells) seems promising but is restricted to subtypes of T-cell malignancies. It is unlikely that one type of CAR T-cells will be used for all T-cell malignancies (unlike CD19 CAR T-cells for B-cell malignancies). To date, few studies evaluated CAR T-cells in patients with T-cell malignancies(23,24) but several trials are underway or about to be launched (Table 1). Results from these clinical trials are eagerly awaited.

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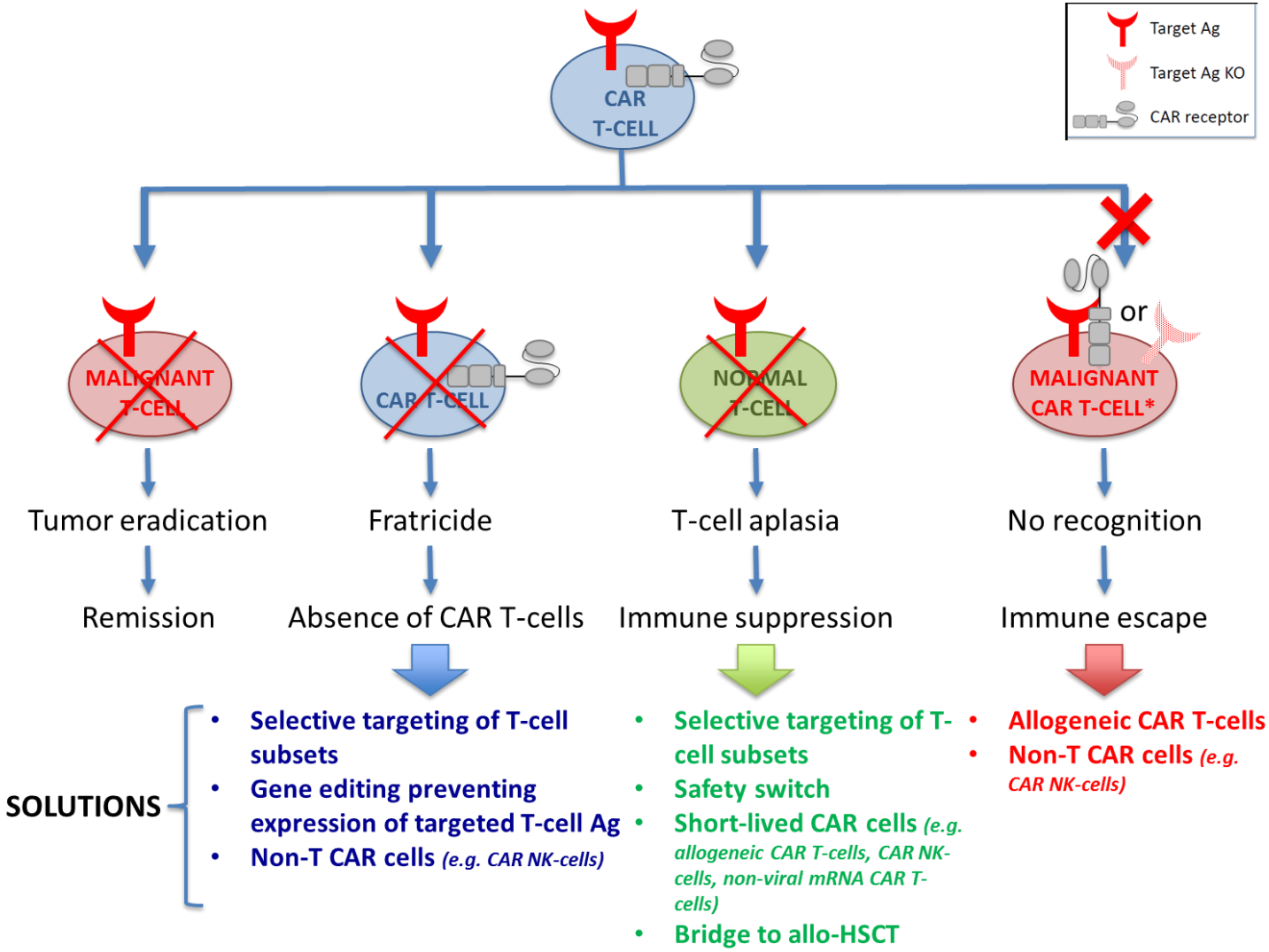
Figure 1. Challenges and solutions in targeting T-cell antigens with CAR T-cells



**Contaminating malignant T-cells transduced with CAR may escape recognition by normal CAR T-cells if the chimeric receptor binds to its target at the cell surface or if it has been genetically edited to prevent target expression.*

Table 1. Effects of different CAR cells constructs on the three T-cell compartments (therapeutic, normal and malignant T-cells)

CAR cells	Target	CAR cells	Normal T-cells	Malignant T-cells*	Preclinical studies	Clinical studies
CAR T-cells	**CD4**	Partially depleted *(CD4 expressed by two-thirds of T-cells)*	Partially depleted *(CD4 expressed by two-thirds of T-cells)*	CD4 expressed in the majority of TCL and in a subset of T-ALL	Specific killing of ALCL cell line and human primary samples *in vitro*; prolonged survival in ALCL xenograft (cell line) mouse model(22)	No clinical studies
	CD5	Transiently depleted *(CD5 expressed by all T-cells but downregulated in CAR T-cells expressing CD28 costimulatory domain)*	Depleted *(CD5 expressed by all T-cells)*	CD5 expressed in most T-ALL and TCL	• *28.z CD5 CAR :* Transient depletion (CD5 is lost following CAR expression)(21) • *4-1BB.z CD5 +* conditional CAR expression system *(4-1 BB.z Tet OFF CD5 CAR cells):* CAR cells preserved(28) • Both constructs showed cytotoxicity against T-ALL cell lines *in vitro. In vivo*, survival of T-ALL xenograft (cell line) mice models enhanced with *BB.z Tet OFF CD5 CAR.*	Ongoing trial : NCT03081910
	CD7	Depleted *(CD7 expressed by all T-cells and poorly downregulated in CAR T-cells)*	Depleted *(CD7 expressed by all T-cells)*	CD7 expressed by most T-ALL and a subset of TCL	• CD7 downregulation before CAR expression (CD7 PEBL construct, whereby CD7 scFv is linked to ER retention domains)(37) • CRISPR-mediated deletion of CD7 prior to CAR transduction(35) • CRISPR-mediated deletion of CD7 and TCR alpha chain (UCART7)(36) • All three constructs showed *in vitro* lysis of T-ALL cell lines and primary T-ALL cells. In	Ongoing trial : NCT02742727

					vivo, anti-leukemic effects were observed in T-ALL xenograft (cell lines) and PDX models.	
	CD30	Partially depleted *(CD30 expressed by a small subset of activated T-cells)*	Partially depleted *(CD30 expressed by a small subset of activated T-cells)* *No significant changes in T-cell counts observed in clinical trials.*(23,24)	CD30 expressed by virtually all HL and ALCL, a subset of other TCL, and about one third of T-ALL(30)	*In vitro,* cytotoxic activity of CD30 CAR EBV-CTL against an ALCL cell line. *In vivo*, tumor growth control in HL xenograft (cell line) model.(31)	• Phase I study which included 2 ALCL patients. One patient experiences a 9 month-long complete remission after 4 CAR T-cells infusion. No response observed in the second patient.(23) • Phase I study which included 1 ALCL patient who showed a partial remission after the first CAR T-cells infusion.(24) • Ongoing trials : NCT03049449 , NCT02917083, NCT02663297
	TRBC1	Partially depleted	Partially depleted *(TRBC1 expressed by ≈1/3 of normal T-cells)*(58)	Around 40% of T-cell malignancies were found to be TRBC1+(58)	Persistence of normal T-cells in T-ALL xenografts injected with human peripheral blood mononuclear cells.(58)	Pending trial : AUTO4
CAR NK-cells	**CD3**	Preserved *(CD3 not expressed by NK-cells)*	Transiently depleted *(CD3 expressed by all T-cells but CAR NK short-lived)*	CD3 expressed by the majority of TCL and a subset of T-ALL	*In vitro,* lysis T-ALL cell line and primary TCL. *In vivo,* anti-leukemic effects in xenograft models (cell lines) but relapses observed.(49)	No clinical studies
	CD4	Preserved *(CD4 not expressed by NK-cells)*	Partially and transiently depleted *(CD4 expressed by two-thirds of T-*	CD4 expressed in the majority of TCL and in a subset of T-ALL	*In vitro,* lysis of T-ALL and lymphoma lines, primary Sezary syndrome and primary T-ALL cells. *In vivo,* anti-leukemic effects in a xenograft lymphoma model	No clinical studies

			cells and CAR NK short-lived)		(ALCL cell line).(50)	
	CD5	Preserved *(CD5 not expressed by NK-cells)*	Transiently depleted *(CD5 expressed by all T-cells but CAR NK short-lived)*	CD5 expressed in most T-ALL and TCL	*In vitro*, lysis T-ALL and lymphoma cell lines, primary T-ALL, TCL and Sezary syndrome cells. *In vivo*, anti-leukemic effects in a xenograft T-ALL model (cell line) when injected in multiple cell doses.(43)	No clinical studies

**Based on Ref 8, 19 and 20*