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► **To cite this version:**

Marion Alcantara, Melania Tesio, Carl H June, Roch Houot. CAR T-cells for T-cell malignancies challenges in distinguishing between therapeutic, normal, and neoplastic T-cells. *Leukemia*, 2018, 32 (11), pp.2307-2315. 10.1038/s41375-018-0285-8 . hal-01903063

HAL Id: hal-01903063

<https://univ-rennes.hal.science/hal-01903063v1>

Submitted on 13 Nov 2018

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

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31 **Running title:** CAR T-cells for T-cell malignancies
32

33 **Abstract word count:** 67 words

34 **Text word count:** 1,992 words

35 **Figures:** 1

36 **Tables:** 1

37 **References:** 66
38

39 **Key words:** CAR T-cells, CAR NK-cells, T-cell malignancies, T-ALL, T-cell lymphoma, T-NHL
40

1 **Abstract**

2
3 Chimeric antigen receptor (CAR) T-cells targeting CD19 demonstrated remarkable efficacy
4 for the treatment of B-cell malignancies. The development of CAR T-cells against T-cell
5 malignancies appears more challenging due to the similarities between the therapeutic,
6 normal and malignant T-cells. The obstacles include CAR T-cell fratricide, T-cell aplasia, and
7 contamination of CAR T-cell products with malignant T-cells. Here, we review these
8 challenges and the solutions proposed to overcome these limitations.

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10

11 **Introduction**

12
13 Chimeric antigen receptor (CAR) T-cells demonstrated remarkable efficacy for the treatment
14 of B-cell malignancies and have been approved by the US Food and Drug Administration
15 (FDA) for the treatment of relapsed/refractory B-cell acute lymphoblastic leukemia (B-ALL)
16 and diffuse large B-cell lymphoma (DLBCL).(1–5) This proof of concept generated great
17 enthusiasm for the development of CAR T-cells directed against other types of cancer,
18 including T-cell malignancies.(6,7)

19

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

23

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

1 **Challenges**

2
3 CAR T-cells, directed against antigens shared with normal T-cells, may recognize and kill 3
4 types of cells: tumor T-cells, normal T-cells and CAR T-cells (Figure 1). Mutual killing of CAR T-
5 cells, also called fratricide, may prevent the generation, expansion and persistence of CAR T-
6 cells. Prolonged and profound T-cell aplasia induced by the destruction of normal T-cells
7 exposes patients to severe opportunistic infections.(11,12) Thus, developing CAR T-cells for
8 T-cell malignancies requires targeting malignant T-cells while sparing normal and CAR T-cells,
9 or at least some subsets of them.

10
11 Furthermore, CAR T-cell products may be contaminated with malignant T-cells. Indeed,
12 circulating tumor T-cells are often found in the peripheral blood of patients with T-ALL(9,13–
13 15) and, although less frequently, with TCL.(8,16) Because tumor T-cells may harbor the
14 same phenotypic and functional properties as normal T-cells(17), they may be harvested,
15 transduced, expanded and infused concomitantly with normal T-cells. This process may lead
16 to the generation of “CAR tumor T-cells”. Ruella et al. recently described accidental
17 transduction of CAR construct in leukemic B-ALL cells leading to CAR expressing blasts (so
18 called “CARB”).(18) In these patients, CD19 CAR at the blast surface bound to CD19, thus
19 preventing their recognition by CAR T-cells. A similar mechanism may be anticipated with
20 malignant T-cells if transduced with the CAR construct. Furthermore, contaminating tumor
21 T-cells may also be genetically edited to prevent the expression of a T-cell target along with
22 normal T-cells and thereby escape CAR T-cells recognition and eradication. Thus, developing
23 CAR T-cells for T-cell malignancies requires avoiding contamination of the CAR T-cell product
24 with malignant transduced T-cells.

25

1 **Proposed solutions**

2

3 ***Fratricide***

4

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

11

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

13

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

20

21 CD5 CAR T-cells

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

26

1 CD4 CAR T-cells

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

7

8 CD30 CAR T-cells

9 CD30 is expressed by a subset of activated B and T-cells, virtually all Hodgkin lymphomas
10 (HL) and anaplastic large cell lymphomas (ALCL), subsets of peripheral TCL, and about one
11 third of T-ALL.(29,30) Following demonstration of a preclinical activity(31), CD30 CAR T-cells
12 have been evaluated in two phase I clinical trials, mostly in patients with HL.(23,24) No
13 decrease in B or T-cell counts were described in these studies. Furthermore, T-cell immunity
14 to common viral pathogens did not seem to be impaired.(23) This is in line with what has
15 been observed in patients treated with brentuximab-vedotin, an antibody-drug conjugate
16 directed against CD30 where no unexpected opportunistic infections have been
17 observed.(32) In the study by Ramos et al., two patients with ALCL (one systemic ALK+ and
18 one cutaneous ALK-) were infused with CD30 CAR T-cells.(23,31) One of the two patients
19 achieved a complete remission which lasted for 9 months following 4 infusions of CD30 CAR
20 T-cells at the highest dose ($2 \cdot 10^8$ cells/m²). (23) Wang and colleagues observed a partial
21 response following infusion without conditioning regimen in one (and only) ALCL
22 (cutaneous) patient.(24) These encouraging results may be further improved by adding a
23 lymphodepleting conditioning regimen prior to the CD30 CAR T-cell infusion.(33,34)

24

1 *Genetically-edited CAR T-cells to prevent target expression*

2

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

15

16 *CAR NK-cells*

17

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

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

17
18 ***T-cell aplasia***

19
20 Unlike B-cell aplasia which is usually well tolerated and can be compensated with continuous
21 infusion of immunoglobulins for the lack of humoral adaptive immunity(55,56), prolonged T-
22 cell aplasia exposes patients to opportunistic infections.(11,12) Prevention of prolonged T-
23 cell aplasia may be achieved in 3 ways: i) either by targeting a tumor antigen that is not
24 expressed by all or a subset of normal T-cells, ii) by using short-lived CAR cells and iii) by

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

3

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

5

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

17

18 *Short-lived CAR cells*

19

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

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

3

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

5

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

11

12 *Allogeneic CAR T-cells*

13

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

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1 **Conclusion**

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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.

Contribution: M.A., M.T., C.H.J., and R.H. performed the literature review, wrote the manuscript, and created the table and figure.

Conflict-of-interest disclosure: M.A. received consulting fees/honoraria from Novartis. M.T. declares no competing financial interests. C.H.J. reports sponsored research from Novartis, patents licensed to Novartis by the University of Pennsylvania and he is a shareholder inTmunity. R.H. received consulting fees/honoraria from Novartis and Kite/Gilead.

1 **References**

- 2
- 3 1. Sadelain M, Brentjens R, Rivière I. The basic principles of chimeric antigen receptor
4 design. *Cancer Discov.* 2013 Apr;3(4):388–98.
- 5 2. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell
6 immunotherapy for human cancer. *Science.* 2018 23;359(6382):1361–5.
- 7 3. Brudno JN, Kochenderfer JN. Chimeric antigen receptor T-cell therapies for lymphoma.
8 *Nat Rev Clin Oncol.* 2018 Jan;15(1):31–46.
- 9 4. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al.
10 Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. *N*
11 *Engl J Med.* 2018 01;378(5):439–48.
- 12 5. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, et al.
13 Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. *N*
14 *Engl J Med.* 2017 28;377(26):2531–44.
- 15 6. Salter AI, Pont MJ, Riddell SR. Chimeric antigen receptor modified T cells: CD19 and the
16 road beyond. *Blood.* 2018 May 4;
- 17 7. Castellarin M, Watanabe K, June CH, Kloss CC, Posey AD. Driving cars to the clinic for
18 solid tumors. *Gene Ther.* 2018 Jun 7;
- 19 8. Weltgesundheitsorganisation. WHO classification of tumours of haematopoietic and
20 lymphoid tissues. Revised 4th edition. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri
21 SA, Stein H, et al., editors. Lyon: International Agency for Research on Cancer; 2017.
22 585 p. (World Health Organization classification of tumours).
- 23 9. Litzow MR, Ferrando AA. How I treat T-cell acute lymphoblastic leukemia in adults.
24 *Blood.* 2015 Aug 13;126(7):833–41.
- 25 10. Moskowitz AJ, Lunning MA, Horwitz SM. How I treat the peripheral T-cell lymphomas.
26 *Blood.* 2014 Apr 24;123(17):2636–44.
- 27 11. Buckley RH, Schiff SE, Schiff RI, Markert L, Williams LW, Roberts JL, et al. Hematopoietic
28 stem-cell transplantation for the treatment of severe combined immunodeficiency. *N*
29 *Engl J Med.* 1999 Feb 18;340(7):508–16.
- 30 12. Leonard WJ. Cytokines and immunodeficiency diseases. *Nat Rev Immunol.* 2001
31 Dec;1(3):200–8.
- 32 13. Marks DI, Paietta EM, Moorman AV, Richards SM, Buck G, DeWald G, et al. T-cell acute
33 lymphoblastic leukemia in adults: clinical features, immunophenotype, cytogenetics,
34 and outcome from the large randomized prospective trial (UKALL XII/ECOG 2993).
35 *Blood.* 2009 Dec 10;114(25):5136–45.

- 1 14. Marks DI, Rowntree C. Management of adults with T-cell lymphoblastic leukemia.
2 Blood. 2017 02;129(9):1134–42.
- 3 15. Hunger SP, Mullighan CG. Acute Lymphoblastic Leukemia in Children. N Engl J Med.
4 2015 Oct 15;373(16):1541–52.
- 5 16. Dogan A, Morice WG. Bone marrow histopathology in peripheral T-cell lymphomas. Br J
6 Haematol. 2004 Oct;127(2):140–54.
- 7 17. Asnafi V, Beldjord K, Boulanger E, Comba B, Le Tutour P, Estienne M-H, et al. Analysis of
8 TCR, pT alpha, and RAG-1 in T-acute lymphoblastic leukemias improves understanding
9 of early human T-lymphoid lineage commitment. Blood. 2003 Apr 1;101(7):2693–703.
- 10 18. Ruella M, Xu J, Barrett DM, et al. Induction of Resistance to Chimeric Antigen Receptor
11 (CAR) T cell Therapy by Transduction of a Single Leukemic B Cell. Nature Medicine 2018
12 (accepted)
- 13 19. Went P, Agostinelli C, Gallamini A, Piccaluga PP, Ascani S, Sabattini E, et al. Marker
14 expression in peripheral T-cell lymphoma: a proposed clinical-pathologic prognostic
15 score. J Clin Oncol Off J Am Soc Clin Oncol. 2006 Jun 1;24(16):2472–9.
- 16 20. D' Amore F, Gaulard P, Trümper L, Corradini P, Kim W-S, Specht L, et al. Peripheral T-
17 cell lymphomas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-
18 up. Ann Oncol Off J Eur Soc Med Oncol. 2015 Sep;26 Suppl 5:v108–15.
- 19 21. Mamonkin M, Rouce RH, Tashiro H, Brenner MK. A T-cell-directed chimeric antigen
20 receptor for the selective treatment of T-cell malignancies. Blood. 2015 Aug
21 20;126(8):983–92.
- 22 22. Pinz K, Liu H, Golightly M, Jares A, Lan F, Zieve GW, et al. Preclinical targeting of human
23 T-cell malignancies using CD4-specific chimeric antigen receptor (CAR)-engineered T
24 cells. Leukemia. 2016 Mar;30(3):701–7.
- 25 23. Ramos CA, Ballard B, Zhang H, Dakhova O, Gee AP, Mei Z, et al. Clinical and
26 immunological responses after CD30-specific chimeric antigen receptor-redirected
27 lymphocytes. J Clin Invest. 2017 Sep 1;127(9):3462–71.
- 28 24. Wang C-M, Wu Z-Q, Wang Y, Guo Y-L, Dai H-R, Wang X-H, et al. Autologous T Cells
29 Expressing CD30 Chimeric Antigen Receptors for Relapsed or Refractory Hodgkin
30 Lymphoma: An Open-Label Phase I Trial. Clin Cancer Res Off J Am Assoc Cancer Res.
31 2017 Mar 1;23(5):1156–66.
- 32 25. Murphy K, Weaver C. Janeway's immunobiology. 9th edition. New York, NY: Garland
33 Science/Taylor & Francis Group, LLC; 2016. 904 p.
- 34 26. Lu X, Axtell RC, Collawn JF, Gibson A, Justement LB, Raman C. AP2 adaptor complex-
35 dependent internalization of CD5: differential regulation in T and B cells. J Immunol
36 Baltim Md 1950. 2002 Jun 1;168(11):5612–20.

- 1 27. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat*
2 *Rev Immunol*. 2013 Apr;13(4):227–42.
- 3 28. Mamonkin M, Mukherjee M, Srinivasan M, Sharma S, Gomes-Silva D, Mo F, et al.
4 Reversible Transgene Expression Reduces Fratricide and Permits 4-1BB Costimulation of
5 CAR T Cells Directed to T-cell Malignancies. *Cancer Immunol Res*. 2018 Jan;6(1):47–58.
- 6 29. Falini B, Pileri S, Pizzolo G, Dürkop H, Flenghi L, Stirpe F, et al. CD30 (Ki-1) molecule: a
7 new cytokine receptor of the tumor necrosis factor receptor superfamily as a tool for
8 diagnosis and immunotherapy. *Blood*. 1995 Jan 1;85(1):1–14.
- 9 30. Zheng W, Medeiros LJ, Young KH, Goswami M, Powers L, Kantarjian HH, et al. CD30
10 expression in acute lymphoblastic leukemia as assessed by flow cytometry analysis.
11 *Leuk Lymphoma*. 2014 Mar;55(3):624–7.
- 12 31. Savoldo B, Rooney CM, Di Stasi A, Abken H, Hombach A, Foster AE, et al. Epstein Barr
13 virus specific cytotoxic T lymphocytes expressing the anti-CD30zeta artificial chimeric T-
14 cell receptor for immunotherapy of Hodgkin disease. *Blood*. 2007 Oct 1;110(7):2620–
15 30.
- 16 32. De Claro RA, McGinn K, Kwitkowski V, Bullock J, Khandelwal A, Habtemariam B, et al.
17 U.S. Food and Drug Administration approval summary: brentuximab vedotin for the
18 treatment of relapsed Hodgkin lymphoma or relapsed systemic anaplastic large-cell
19 lymphoma. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2012 Nov 1;18(21):5845–9.
- 20 33. Gattinoni L, Finkelstein SE, Klebanoff CA, Antony PA, Palmer DC, Spiess PJ, et al.
21 Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of
22 adoptively transferred tumor-specific CD8+ T cells. *J Exp Med*. 2005 Oct 3;202(7):907–
23 12.
- 24 34. Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for
25 human cancer. *Science*. 2015 Apr 3;348(6230):62–8.
- 26 35. Gomes-Silva D, Srinivasan M, Sharma S, Lee CM, Wagner DL, Davis TH, et al. CD7-edited
27 T cells expressing a CD7-specific CAR for the therapy of T-cell malignancies. *Blood*. 2017
28 20;130(3):285–96.
- 29 36. Cooper ML, Choi J, Staser K, Ritchey JK, Devenport JM, Eckardt K, et al. An “off-the-
30 shelf” fratricide-resistant CAR-T for the treatment of T cell hematologic malignancies.
31 *Leukemia*. 2018 Feb 20;
- 32 37. Png YT, Vinanica N, Kamiya T, Shimasaki N, Coustan-Smith E, Campana D. Blockade of
33 CD7 expression in T cells for effective chimeric antigen receptor targeting of T-cell
34 malignancies. *Blood Adv*. 2017 Nov 28;1(25):2348–60.
- 35 38. Kamiya T, Wong D, Png YT, Campana D. A novel method to generate T-cell receptor-
36 deficient chimeric antigen receptor T cells. *Blood Adv*. 2018 Mar 13;2(5):517–28.

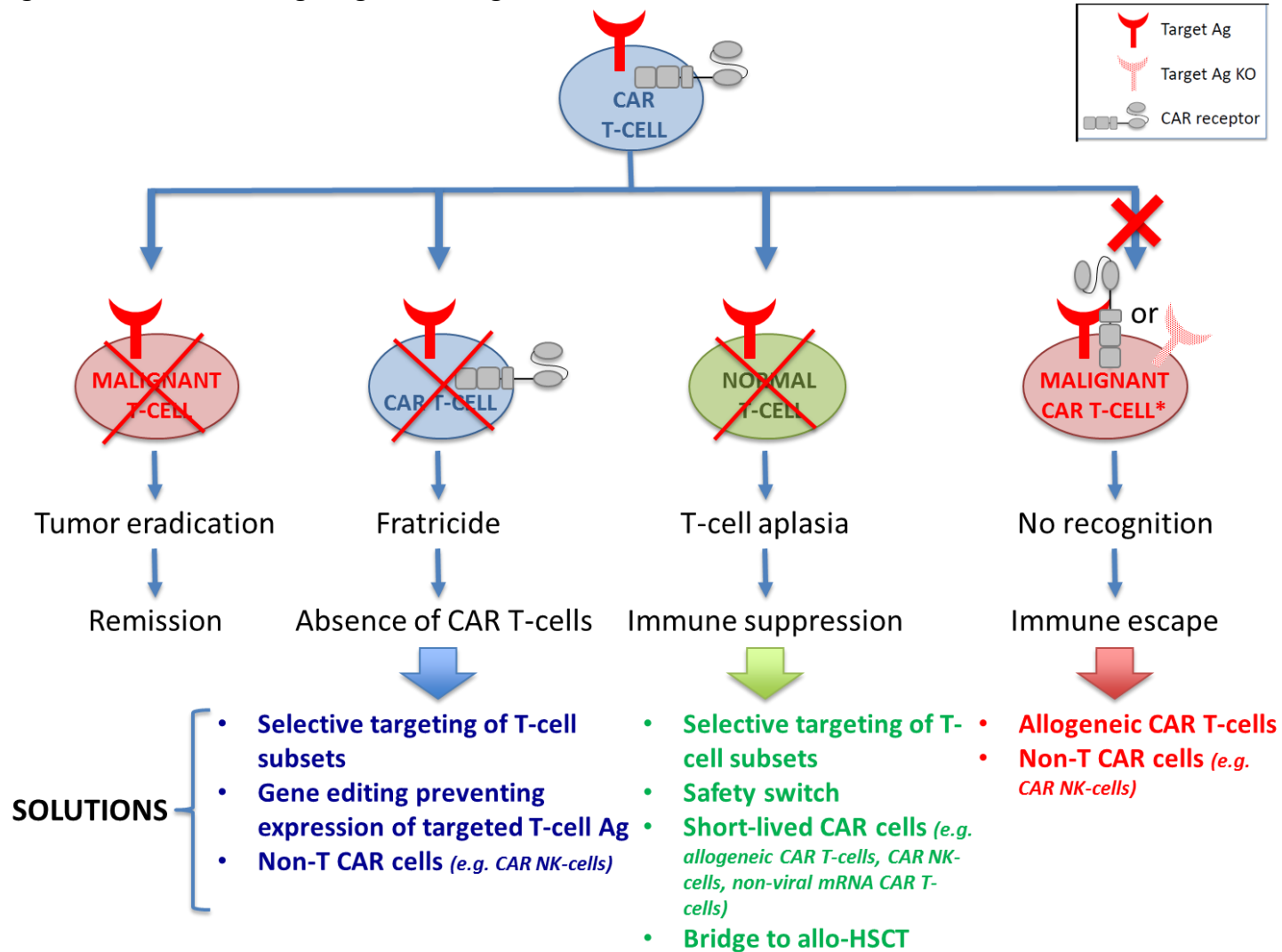
- 1 39. Munro S, Pelham HR. A C-terminal signal prevents secretion of luminal ER proteins. *Cell*.
2 1987 Mar 13;48(5):899–907.
- 3 40. Jackson MR, Nilsson T, Peterson PA. Identification of a consensus motif for retention of
4 transmembrane proteins in the endoplasmic reticulum. *EMBO J*. 1990 Oct;9(10):3153–
5 62.
- 6 41. Daher M, Rezvani K. Next generation natural killer cells for cancer immunotherapy: the
7 promise of genetic engineering. *Curr Opin Immunol*. 2018 Apr;51:146–53.
- 8 42. Caligiuri MA. Human natural killer cells. *Blood*. 2008 Aug 1;112(3):461–9.
- 9 43. Chen KH, Wada M, Pinz KG, Liu H, Lin K-W, Jares A, et al. Preclinical targeting of
10 aggressive T-cell malignancies using anti-CD5 chimeric antigen receptor. *Leukemia*.
11 2017 Oct;31(10):2151–60.
- 12 44. Hauser A, Schratlbauer K, Najdanovic D, Schlossnickel R, Koch A, Hejtman M, et al.
13 Optimized quantification of lymphocyte subsets by use of CD7 and CD33. *Cytom Part J*
14 *Int Soc Anal Cytol*. 2013 Mar;83(3):316–23.
- 15 45. Morvan MG, Lanier LL. NK cells and cancer: you can teach innate cells new tricks. *Nat*
16 *Rev Cancer*. 2016 Jan;16(1):7–19.
- 17 46. Rezvani K, Rouce R, Liu E, Shpall E. Engineering Natural Killer Cells for Cancer
18 Immunotherapy. *Mol Ther J Am Soc Gene Ther*. 2017 Aug 2;25(8):1769–81.
- 19 47. Simonetta F, Alvarez M, Negrin RS. Natural Killer Cells in Graft-versus-Host-Disease
20 after Allogeneic Hematopoietic Cell Transplantation. *Front Immunol*. 2017;8:465.
- 21 48. Klingemann H, Boissel L, Toneguzzo F. Natural Killer Cells for Immunotherapy -
22 Advantages of the NK-92 Cell Line over Blood NK Cells. *Front Immunol*. 2016;7:91.
- 23 49. Chen KH, Wada M, Firor AE, Pinz KG, Jares A, Liu H, et al. Novel anti-CD3 chimeric
24 antigen receptor targeting of aggressive T cell malignancies. *Oncotarget*. 2016 Aug
25 30;7(35):56219–32.
- 26 50. Pinz KG, Yakaboski E, Jares A, Liu H, Firor AE, Chen KH, et al. Targeting T-cell
27 malignancies using anti-CD4 CAR NK-92 cells. *Oncotarget*. 2017 Dec 22;8(68):112783–
28 96.
- 29 51. Suck G, Odendahl M, Nowakowska P, Seidl C, Wels WS, Klingemann HG, et al. NK-92: an
30 “off-the-shelf therapeutic” for adoptive natural killer cell-based cancer
31 immunotherapy. *Cancer Immunol Immunother* CII. 2016 Apr;65(4):485–92.
- 32 52. Zhang C, Oberoi P, Oelsner S, Waldmann A, Lindner A, Tonn T, et al. Chimeric Antigen
33 Receptor-Engineered NK-92 Cells: An Off-the-Shelf Cellular Therapeutic for Targeted
34 Elimination of Cancer Cells and Induction of Protective Antitumor Immunity. *Front*
35 *Immunol*. 2017;8:533.

- 1 53. Arai S, Meagher R, Swearingen M, Myint H, Rich E, Martinson J, et al. Infusion of the
2 allogeneic cell line NK-92 in patients with advanced renal cell cancer or melanoma: a
3 phase I trial. *Cytotherapy*. 2008;10(6):625–32.
- 4 54. Tonn T, Schwabe D, Klingemann HG, Becker S, Esser R, Koehl U, et al. Treatment of
5 patients with advanced cancer with the natural killer cell line NK-92. *Cytotherapy*. 2013
6 Dec;15(12):1563–70.
- 7 55. Ram R, Ben-Bassat I, Shpilberg O, Polliack A, Raanani P. The late adverse events of
8 rituximab therapy--rare but there! *Leuk Lymphoma*. 2009 Jul;50(7):1083–95.
- 9 56. Park JH, Rivière I, Gonen M, Wang X, Sénéchal B, Curran KJ, et al. Long-Term Follow-up
10 of CD19 CAR Therapy in Acute Lymphoblastic Leukemia. *N Engl J Med*. 2018
11 01;378(5):449–59.
- 12 57. Sims JE, Tunnacliffe A, Smith WJ, Rabbitts TH. Complexity of human T-cell antigen
13 receptor beta-chain constant- and variable-region genes. *Nature*. 1984 Dec
14 6;312(5994):541–5.
- 15 58. Maciocia PM, Wawrzyniecka PA, Philip B, Ricciardelli I, Akarca AU, Onuoha SC, et al.
16 Targeting the T cell receptor β -chain constant region for immunotherapy of T cell
17 malignancies. *Nat Med*. 2017 Dec;23(12):1416–23.
- 18 59. Svoboda J, Rheingold SR, Gill SI, Grupp SA, Lacey SF, Kulikovskaya I, et al. Non-viral RNA
19 chimeric antigen receptor modified T cells in patients with Hodgkin lymphoma. *Blood*.
20 2018 Jun 20;
- 21 60. Straathof KC, Spencer DM, Sutton RE, Rooney CM. Suicide genes as safety switches in T
22 lymphocytes. *Cytotherapy*. 2003;5(3):227–30.
- 23 61. Hoyos V, Savoldo B, Quintarelli C, Mahendravada A, Zhang M, Vera J, et al. Engineering
24 CD19-specific T lymphocytes with interleukin-15 and a suicide gene to enhance their
25 anti-lymphoma/leukemia effects and safety. *Leukemia*. 2010 Jun;24(6):1160–70.
- 26 62. Eissenberg LG, Rettig MP, Ritchey JK, Prior JL, Schwarz SW, Frye J, et al. [(18)F]FHBG
27 PET/CT Imaging of CD34-TK75 Transduced Donor T Cells in Relapsed Allogeneic Stem
28 Cell Transplant Patients: Safety and Feasibility. *Mol Ther J Am Soc Gene Ther*. 2015
29 Jun;23(6):1110–22.
- 30 63. Minagawa K, Jamil MO, Al-Obaidi M, Pereboeva L, Salzman D, Erba HP, et al. In Vitro
31 Pre-Clinical Validation of Suicide Gene Modified Anti-CD33 Redirected Chimeric Antigen
32 Receptor T-Cells for Acute Myeloid Leukemia. *PloS One*. 2016;11(12):e0166891.
- 33 64. Sun S, Hao H, Yang G, Zhang Y, Fu Y. Immunotherapy with CAR-Modified T Cells:
34 Toxicities and Overcoming Strategies. *J Immunol Res*. 2018;2018:2386187.
- 35 65. Blazar BR, Murphy WJ, Abedi M. Advances in graft-versus-host disease biology and
36 therapy. *Nat Rev Immunol*. 2012 May 11;12(6):443–58.

1 66. Yang Y, Jacoby E, Fry TJ. Challenges and opportunities of allogeneic donor-derived CAR
2 T cells. *Curr Opin Hematol*. 2015 Nov;22(6):509–15.

3

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 (<i>CD4 expressed by two-thirds of T-cells</i>)	Partially depleted (<i>CD4 expressed by two-thirds of T-cells</i>)	CD4 expressed in the majority of TCL and in a subset of T-ALL	Specific killing of ALCL cell line and human primary samples <i>in vitro</i> ; prolonged survival in ALCL xenograft (cell line) mouse model(22)	No clinical studies
	CD5	Transiently depleted (<i>CD5 expressed by all T-cells but downregulated in CAR T-cells expressing CD28 costimulatory domain</i>)	Depleted (<i>CD5 expressed by all T-cells</i>)	CD5 expressed in most T-ALL and TCL	<ul style="list-style-type: none"> • 28.z CD5 CAR : Transient depletion (CD5 is lost following CAR expression)(21) • 4-1BB.z CD5 + conditional CAR expression system (4-1BB.z Tet OFF CD5 CAR cells): CAR cells preserved(28) • Both constructs showed cytotoxicity against T-ALL cell lines <i>in vitro</i>. <i>In vivo</i>, survival of T-ALL xenograft (cell line) mice models enhanced with BB.z Tet OFF CD5 CAR. 	Ongoing trial : NCT03081910
	CD7	Depleted (<i>CD7 expressed by all T-cells and poorly downregulated in CAR T-cells</i>)	Depleted (<i>CD7 expressed by all T-cells</i>)	CD7 expressed by most T-ALL and a subset of TCL	<ul style="list-style-type: none"> • 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 <i>in vitro</i> 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 (<i>CD30 expressed by a small subset of activated T-cells</i>)	Partially depleted (<i>CD30 expressed by a small subset of activated T-cells</i>) 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)	<i>In vitro</i> , cytotoxic activity of CD30 CAR EBV-CTL against an ALCL cell line. <i>In vivo</i> , tumor growth control in HL xenograft (cell line) model.(31)	<ul style="list-style-type: none"> 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 (<i>TRBC1 expressed by ≈1/3 of normal T-cells</i>)(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 (<i>CD3 not expressed by NK-cells</i>)	Transiently depleted (<i>CD3 expressed by all T-cells but CAR NK short-lived</i>)	CD3 expressed by the majority of TCL and a subset of T-ALL	<i>In vitro</i> , lysis T-ALL cell line and primary TCL. <i>In vivo</i> , anti-leukemic effects in xenograft models (cell lines) but relapses observed.(49)	No clinical studies
	CD4	Preserved (<i>CD4 not expressed by NK-cells</i>)	Partially and transiently depleted (<i>CD4 expressed by two-thirds of T-</i>	CD4 expressed in the majority of TCL and in a subset of T-ALL	<i>In vitro</i> , lysis of T-ALL and lymphoma lines, primary Sezary syndrome and primary T-ALL cells. <i>In vivo</i> , anti-leukemic effects in a xenograft lymphoma model	No clinical studies

			<i>cells and CAR NK short-lived)</i>		(ALCL cell line).(50)	
	CD5	Preserved <i>(CD5 not expressed by NK-cells)</i>	Transiently depleted <i>(CD5 expressed by all T-cells but CAR NK short-lived)</i>	CD5 expressed in most T-ALL and TCL	<i>In vitro</i> , lysis T-ALL and lymphoma cell lines, primary T-ALL, TCL and Sezary syndrome cells. <i>In vivo</i> , 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*