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CD4⁺ CAR T cells in for the long journey

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Graphical Abstract text (to accompany Figure 1 on the ToC listing):

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In a recently published article, Melenhorst *et al.* performed longitudinal analysis on CAR T cells isolated from patients over 10 years post therapy, revealing expansion of a long-lived $CD4^+$ CAR T cell population with a cytotoxic phenotype.

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Main text

Since the first clinical trials about a decade ago [1, 2], chimeric antigen receptor (CAR) T cell therapy has generated much interest in the clinic due to its remarkable capacity to induce complete responses in a subset of patients. CAR T cell therapy involves genetic modification of autologous T cells to express a synthetic receptor. These target a specific tumour antigen to re-direct T cell killing towards cancer cells. Specifically, CAR T cells targeting the B cell antigen, CD19, have demonstrated the ability to induce durable remissions in the treatment of $CD19^+$ B cell malignancies. Clinical responses vary between cancer types, ranging from as high as 90% complete remission rates in acute lymphoblastic leukaemia (ALL) to 30–50% in chronic lymphoblastic leukaemia (CLL) and non-Hodgkin's lymphoma (NHL) [3,4]. Remarkably, a significant proportion of patients remain in relapse-free remission for years following treatment. Elucidating the characteristics of CAR T cells in these patients may help in further improvement of CAR T cell therapy. Whilst phenotypic markers of CAR T cells that are predictive of persistence during the acute phase of the response have previously been determined [5], a recent study by Melenhorst *et al.* performed the first longitudinal analysis of CTL019 CAR T cells at >10 years clinical remission in 2 CLL patients, revealing fascinating insights into CAR T cell persistence and phenotype over time [6].

Melenhorst *et al.* demonstrated that CTL019 cells were detectable 10 and 9 years post-treatment in patients 1 and 2, respectively, which correlated with sustained B cell aplasia. Through cytometry by time-of-flight (CyTOF) and multiomic single-cell analysis, the CAR T cell phenotypes were assessed over time. Both patients received a 'bulk' CAR T cell product derived from PBMCs, such that it contained a mixture of $CD4^+$, $CD8^+$ and non-conventional

CAR T cells. Whilst $CD4^+$ CAR T cells constituted 70% and 5% of all CAR T cells in the acute phase of the response in patients 1 and 2 respectively, both patients exhibited a marked expansion of $CD4^+$ CAR T cells over time, which constituted >95% of the CAR T cell population from 3.4 years onwards for patient 1 and 7.2 years for patient 2. Characterisation of these $CD4^+CAR^+$ T cells indicates that they exhibited a high proliferative potential with high Ki-67 expression and about 30% of $CD4^+$ CAR T cells in S, G2 or M phase of the cell cycle. Further analysis of these long-lived $CD4^+CAR^+$ T cells demonstrated the expression of PD-1, LAG-3, TIGIT and TIM-3 as well as transcription factors TOX, BATF and EOMES, but low expression of TCF7 and FOS.

Interestingly, $CD4^+$ CAR T cells expressed genes indicative of cytolytic potential including perforin, granzyme A and K (but not granzyme B) and, importantly, had the capacity to degranulate upon co-incubation with antigen-positive tumour cells. In this respect, these cells resemble previously reported $Perforin^+$ $Granzyme\ A^+$ $Granzyme\ B^-$ cytolytic $CD4^+$ T cells found to be important for immune-mediated control of melanoma and bladder cancer [7, 8]. However, as both granzyme A and granzyme K are minimally cytotoxic relative to granzyme B [9, 10], the true cytotoxic potential of the $CD4^+$ CAR T cells described here is unclear. Consistent with previous work indicating a critical role for $CD8^+$ CAR T cells in the acute phase of the response, 25% of the CAR T cells in each patient at the earliest time point of ~2 months were granzyme B^+ $CD8^+$ CAR T cells. Thus, from this data, Melenhorst *et al.* propose that CAR T cell therapy is governed by two major phases, an initial response phase involving $CD8^+$ T cells, followed by long-term remission dominated by $Ki67^{hi}$ $CD4^+$ CAR T cells (**Figure 1**).

More broadly, these observations further highlight the importance of $CD4^+$ CAR T cells in maintaining durable anti-tumour responses. Previous studies have demonstrated that a defined ratio of $CD8^+$ and $CD4^+$ CAR T cells can lead to greater tumour control [11–13], an observation that has led to one FDA approved product, Breyanzi (lisocabtagene maraleucel), having a pre-defined ratio of $CD8^+$ and $CD4^+$ CAR T cells. Although $CD4^+$ CAR T cells have been shown to elicit cytotoxic function *in vitro* [14], the primary mechanism by which $CD4^+$ CAR T cells enhance therapeutic effects in the early elimination of tumour cells *in vivo* was identified to be through IL-2 mediated support of $CD8^+$ CAR T cell responses, rather than a direct cytotoxic role of $CD4^+$ CAR T cells [12]. While the relative role of $CD4^+$ and $CD8^+$ CAR T cells in the early elimination of tumour cells remains unclear from the present study, these data raise the

possibility that whilst $CD8^+$ CAR T cells play a critical role in early disease elimination, it is cytotoxic $CD4^+$ CAR T cells that are critical for long-term disease control.

In a second aspect of the study, Melenhorst *et al.* performed TCR sequencing and lentiviral vector integration site (LVIS) analysis to investigate the clonal evolution of CAR T cells over time. The lentiviral integration site for the CAR is random for each T cell and so provides a barcode that acts as a proxy measure of CAR T cell clonality. Moreover, theoretically if a CAR inserts into the genome at a point that disrupts the expression of a negative regulator of T cell function this may in itself improve efficacy. Thus, identifying the lentiviral integration site and its impact on clonal evolution is of significant interest to the field, largely sparked by the observation that a TET2 integration event was responsible for the clonal expansion of CAR T cells in one CLL patient [15]. However, in the current study, assessment of CAR integration sites in the dominant clones revealed no common integration sites suggesting this was not a major factor driving CAR T cell persistence. Consistent with this observation, TCR-seq analysis demonstrated little clonality in persisting CAR T cells, suggesting this may be more stochastic than previously anticipated.

Lastly, it is notable that from approximately 5 years post CAR T cell delivery, both patients displayed a near absence of B cells, indicating that anti-CD19 CAR T cells remained active in the depletion of both healthy and malignant B cells populations. This raises the question as to whether the constant antigen stimulation provided by healthy B cells (a form of antigen presenting cell) is critically required for the long-term persistence and maintenance of CD19 targeting T cells. This is of particular importance when considering how to improve CAR T cell persistence in the solid tumours where CAR T cells do not naturally engage with antigen presenting cells. This notion again signifies the importance of strategies that boost CAR T cell persistence and function *in vivo* through vaccine-like approaches [16] and/or utilise the initial CAR T cell response to drive long-lasting endogenous immune responses against the tumour [17].

In summary, these results provide novel insight into the dynamics of long-term CAR T cell persistence, adding further support to the importance of $CD4^+$ CAR T cells in driving clinical efficacy. These data also suggest that lentiviral integration site is not a major determinant of CAR T cell clonality, a finding that corroborates other recent analyses [18]. Further investigations are required to confirm these findings in a larger cohort of patients and determine whether $CD4^+$ CAR T cells play an active role in disease suppression, or are more simply a by-product

of continued targeting of healthy $CD19^+$ B cells. Moreover, this study highlights the probable role that healthy $CD19^+$ cells have in promoting the persistence of anti-CD19 CAR T cells. Given this mechanism is not applicable to CAR T cell therapies targeting solid tumour antigens, where CAR T cell persistence is inferior, an interesting possibility raised by this study is that targeting of endogenous CD19 is actually critical for achieving long-term efficacy. Overall, these findings could help to inform future development of strategies to enhance CAR T cell therapy.

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Conflicts of interest

PAB declares the following conflicts: research funding from AstraZeneca, Bioardis, Bristol-Myers-Squibb and Gilead Sciences.

References

1. Porter DL, Levine BL, Kalos M, *et al.* Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med* 2011; **365**: 725-733.
2. Grupp SA, Kalos M, Barrett D, *et al.* Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med* 2013; **368**: 1509-1518.
3. Maude SL, Frey N, Shaw PA, *et al.* Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med* 2014; **371**: 1507-1517.
4. Park JH, Rivière I, Gonen M, *et al.* Long-Term Follow-up of CD19 CAR Therapy in Acute Lymphoblastic Leukemia. *N Engl J Med* 2018; **378**: 449-459.

5. Fraietta JA, Lacey SF, Orlando EJ, *et al.* Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. *Nat Med* 2018; **24**: 563-571.
6. Melenhorst JJ, Chen GM, Wang M, *et al.* Decade-long leukaemia remissions with persistence of CD4$^+$ CAR T cells. *Nature* 2022; **602**(7897): 503-509.
7. Cachot A, Bilous M, Liu Y-C, *et al.* Tumor-specific cytolytic CD4 T cells mediate immunity against human cancer. *Sci Adv* 2021; **7**: eabe3348.
8. Oh DY, Kwek SS, Raju SS, *et al.* Intratumoral CD4$^+$ T Cells Mediate Anti-tumor Cytotoxicity in Human Bladder Cancer. *Cell* 2020; **181**: 612-1625 e13.
9. Kaiserman D, Bird CH, Sun J, *et al.* The major human and mouse granzymes are structurally and functionally divergent. *J Cell Biol* 2006; **175**: 619-630.
10. Susanto O, Stewart SE, Voskoboinik I, *et al.* Mouse granzyme A induces a novel death with writhing morphology that is mechanistically distinct from granzyme B-induced apoptosis. *Cell Death Differ* 2013; **20**: 1183-1193.
11. Turtle CJ, Hanafi L-A, Berger C, *et al.* CD19 CAR-T cells of defined CD4$^+$:CD8$^+$ composition in adult B cell ALL patients. *J Clin Invest* 2016; **126**: 2123-2138.
12. Moeller M, Haynes NM, Kershaw MH, *et al.* Sustained antigen-specific antitumor recall response mediated by gene-modified CD4$^+$ T helper-1 and CD8$^+$ T cell*s. Cancer Res* 2007; **67**: 11428-11437.
13. Moeller M, Haynes NM, Kershaw MH, *et al.* Adoptive transfer of gene-engineered CD4$^+$ helper T cells induces potent primary and secondary tumor rejection*. Blood* 2005; **106**: 2995-3003.
14. Yang Y, Kohler ME, Chien CD, *et al.* TCR engagement negatively affects CD8 but not CD4 CAR T cell expansion and leukemic clearance. *Sci Transl Med* 2017; **9**: eaag1209.
15. Fraietta JA, Nobles CL, Sammons MA, *et al.* Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells. *Nature* 2018; **558**(7709): 307-312.
16. Slaney CY, von Scheidt B, Davenport AJ, *et al.* Dual-specific Chimeric Antigen Receptor T Cells and an Indirect Vaccine Eradicate a Variety of Large Solid Tumors in an Immunocompetent, Self-antigen Setting*. Clin Cancer Res* 2017; **23**: 2478-2490.
17. Lai JY, Mardiana S, House IG, *et al.* Adoptive cellular therapy with T cells expressing the dendritic cell growth factor Flt3L drives epitope spreading and antitumor immunity*. Nat Immunol* 2020; **21**: 914-926.
18. Sheih A, Voillet V, Hanafi L-A, *et al.* Clonal kinetics and single-cell transcriptional profiling of CAR-T cells in patients undergoing CD19 CAR-T immunothera*py. Nat Commun* 2020; **11**: 219.

Figure 1. Dynamics of CAR T cell response over 10 years. **(a)** The anti-CD19 CAR T cell response can be sub-divided into the acute phase (Phase I) where CAR T cells initially expand and subsequently contract in line with reduced numbers of $CD19^+$ cells (either tumour cells or healthy B cells). This is followed by a longer phase where CAR T cells persist at low levels and the numbers of $CD19^+$ cells remain low. **(b, c)** In the initial stages, the CAR T cell population is constituted of a mixture of $CD8^+$ T cells with a classical cytotoxic phenotype and $CD4^+$ CAR T cells that provide IL-2 support to this response. In the long-term remission phase, $CD4^+$ T cells make up the majority of CAR T cells and their persistence may in part be driven by healthy B cells.