

Chimeric antigen receptor T-cell therapy for atherosclerosis

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Dear Editor,

Atherosclerosis is a chronic disease of the arterial wall initiated by excessive accumulation of low-density lipoprotein (LDL) particles in the arterial intima. Macrophage uptake of oxidized LDL (oxLDL) generates lipid-laden foamy macrophages, a defining feature of atherosclerotic plaques¹. These cells participate in inflammatory signaling, lipid handling, and necrotic core formation, thereby shaping plaque progression and stability^{2,3}. Despite their central roles in initiation and progression of atherosclerosis, therapies that directly target pathogenic foamy macrophages are still lacking. Current preventive strategies remain dominated by systemic risk factor control, particularly lipid lowering, yet substantial residual cardiovascular risk persists¹, underscoring the need for cell-directed approaches that remove disease-promoting plaque cell populations.

Recent advances in cellular immunotherapy directed against cell-surface antigens have shown remarkable efficacy in multiple cancers⁴. More recently, this approach has been extended beyond oncology. Chimeric antigen receptor (CAR) T cells targeting cardiac fibroblasts in cardiac fibrosis^{5,6}, senescent cells in aging^{7,8}, and B cells in autoimmune disease⁹, have shown therapeutic potential through selective depletion of disease-driving cell populations in preclinical or clinical settings. Given the pathogenic role of foamy macrophages in atherosclerosis, we therefore hypothesized that a CAR T-cell approach directed against an antigen enriched in plaque foamy macrophages could attenuate established atherosclerosis.

To identify a suitable cell-surface marker enriched in foamy macrophages, we integrated multiple publicly available single-cell RNA-sequencing (scRNA-seq) datasets from human arteries and mouse aortas. Macrophages constituted one of the major cellular components in these datasets (Supplementary Fig. S1a, c). Subclustering of the macrophages revealed multiple subpopulations, consistent with the current view of macrophage heterogeneity¹⁰, with foamy macrophages representing the predominant subset in both human and mouse arteries (Fig. 1a; Supplementary Fig. S1b, d, e). Among genes enriched in foamy macrophages, triggering receptor expressed on myeloid cells 2 (*Trem2*) emerged as one of the top three cell-surface markers in both species (Fig. 1b, c; Supplementary Fig. S1f), consistent with recent studies^{11,12}. Together, these findings identify *Trem2* as a promising antigen for a *Trem2*-enriched plaque macrophage population.

As epitope choice can substantially affect CAR activity, we evaluated three distinct single-chain variable fragments (scFvs) from independent sources, each previously reported to recognize both human and mouse *Trem2* (see Supplementary Information). For each scFv, we generated species-matched CARs containing human or mouse CD28 and CD3 ζ signaling domains, yielding hCAR1-3 and mCAR1-3 (Supplementary Fig. S2a, b). Human and mouse primary T cells were activated for 48 h and transduced with lentiviral or retroviral vectors, respectively; all constructs produced robust CAR expression by day 4 (Fig. 1d; Supplementary Fig. S2c). When co-cultured with *Trem2*⁺ THP1 cells, all three types of CAR T cells exhibited potent cytotoxicity, whereas cytotoxicity against *Trem2*⁻ NALM6 cells was negligible, indicating *Trem2*-dependent target cell killing (Fig. 1e; Supplementary Fig. S3). Likewise, mCAR1-3 T cells efficiently killed *Trem2*⁺ RAW264.7 cells, although activated mouse primary T cells showed low levels of nonspecific cytotoxicity, which was independent of the expression of *Trem2* (Supplementary Fig. S4a–d). Target cell killing was accompanied by IFN- γ and TNF- α release, with hCAR1 and mCAR1-2 among the strongest cytokine-producing constructs (Fig. 1f; Supplementary Fig. S4e).

We next assessed human CAR T-cell activity in a vascular context using a co-culture system comprising human induced pluripotent stem cell (hiPSC)-derived vessel organoids (VOs), THP1 cells, and CAR T cells. THP1 and CAR T cells remained largely at the organoid surface rather than infiltrating the VOs (Supplementary Fig. S5). Nevertheless, hCAR T cells efficiently eliminated THP1 cells without a detectable reduction in vessel area or vascular junction number (Fig. 1g–j). As an orthogonal *in vivo* test of target cell clearance, luciferase-expressing THP1 cells were implanted subcutaneously in immunodeficient mice, followed by intravenous CAR T-cell administration and longitudinal imaging for 45 days. THP1 cell mass and bioluminescent signal progressively increased in mock-treated mice, whereas CAR T-cell treatment suppressed both readouts; hCAR1 produced the most sustained response without affecting body weight (Fig. 1k, l). Taken together, these studies demonstrate that CAR T cells can efficiently eliminate *Trem2*⁺ target cells in these more complex models.

We then tested therapeutic efficacy in *Apoe*^{-/-} mice with established atherosclerosis. After 12 weeks of high-fat diet (HFD) treatment, mice received 5×10^6 mCAR T cells intravenously and were analyzed 4 weeks later. En face Oil Red O (ORO) staining revealed that mCAR1 reduced aortic plaque

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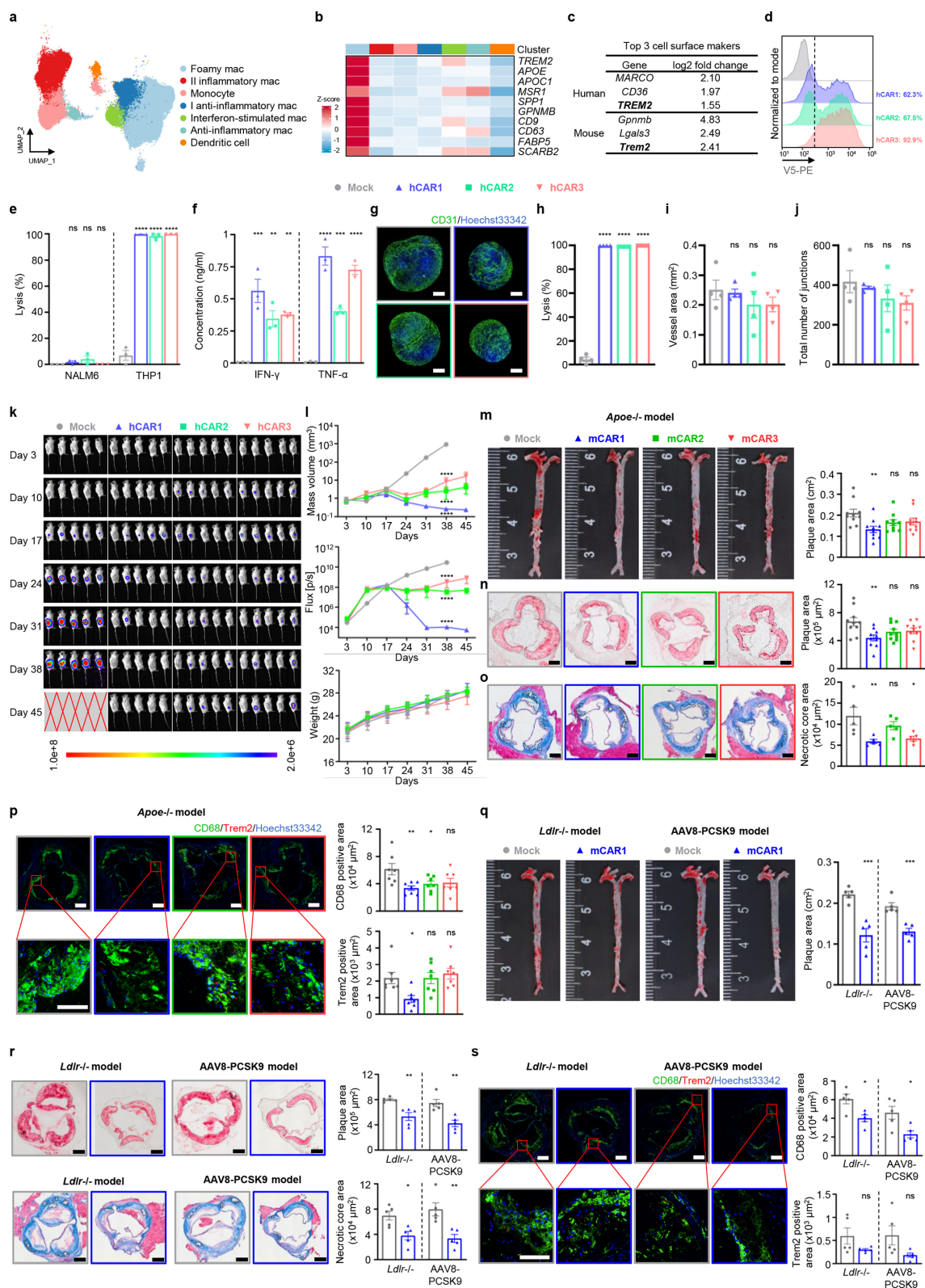


Fig. 1 Trem2-targeted CAR T cells attenuate atherosclerosis. **a** UMAP visualization of monocyte/macrophage subpopulations identified by integration of scRNA-seq datasets from human arteries. **b** Heat maps showing genes upregulated in the human foamy macrophage cluster relative to the other monocyte/macrophage clusters. **c** Top three enriched cell-surface marker genes in human and mouse foamy macrophages. **d** Flow cytometric analysis of the expression of CARs in human primary T cells 4 days after transduction. **e** Trem2-dependent *in vitro* cytotoxicity of human CAR T cells against Trem2⁺ NALM6 and Trem2⁺ THP1 cells. Cells were co-cultured at an effector-to-target (E:T) ratio of 1:20 for 4 days; $n = 3$ independent experiments. **f** IFN- γ and TNF- α concentrations in supernatants from the THP1-CAR T co-cultures; $n = 3$ independent experiments. **g** Representative immunofluorescence images of human hiPSC-derived VOs stained for CD31 and Hoechst 33342 after co-culture with THP1 cells and human CAR T cells. Untransduced human primary T cells were used as a mock control. Scale bars, 200 μ m. **h** THP1-cell lysis in the VO co-culture system; $n = 4$ organoids. **i, j** Quantification of vessel area (i) and total junction number (j) using

AngioTool; $n = 4$ organoids. **k** Longitudinal bioluminescence imaging of mice bearing luciferase-expressing THP1 xenografts after treatment with mock or hCAR1-3 T cells. Red crosses denote that mock group mice were euthanized before imaging on day 45. **l** Quantification of THP1 mass volume (Top), bioluminescent flux (Middle), and body weight (Bottom) over 45 days. $n = 5$ mice per group. **m** En face ORO staining of whole aortas and quantification of plaque area in $Apoe^{-/-}$ mice; $n = 10$ mice. Untransduced mouse primary T cells were used as a mock control. **n** ORO staining of aortic roots and quantification of plaque area in $Apoe^{-/-}$ mice; $n = 10$ mice. **o** Masson's trichrome staining of aortic roots and quantification of necrotic core area; $n = 5$ mice. **p** Immunofluorescence staining of $Apoe^{-/-}$ aortic roots for CD68 and Trem2, with quantification of CD68⁺ and Trem2⁺ areas; $n = 7$ mice. **q** En face ORO staining and quantification of whole aorta plaque area in $Ldlr^{-/-}$ and AAV8-PCSK9 atherosclerosis models treated with mCAR1; $n = 5$ mice. **r** En face ORO and Masson's trichrome staining of aortic roots, with quantification of plaque and necrotic core areas in $Ldlr^{-/-}$ and AAV8-PCSK9 models; $n = 5$ mice. **s** Immunofluorescence staining of aortic roots for CD68 and Trem2, with quantification of CD68⁺ and Trem2⁺ areas in $Ldlr^{-/-}$ and AAV8-PCSK9 models; $n = 5$ mice. One-way ANOVA with Dunnett's multiple comparisons test was conducted for *in vitro* and $Apoe^{-/-}$ mouse studies. Two-way ANOVA with Tukey's multiple comparisons test was conducted for panel **l**. Student's *t*-test was conducted for $Ldlr^{-/-}$ and AAV8-PCSK9 mouse studies. ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. Scale bars: 300 μ m in panels **n**, **o**, **p**, **r**, and **s**; 50 μ m in magnified images in panels **p** and **s**.

area by approximately 30%, with a particularly pronounced effect in the descending aorta (Fig. 1m; Supplementary Fig. S6a). mCAR2 and mCAR3 showed a trend toward reduced plaque size, although this did not reach statistical significance (Fig. 1m; Supplementary Fig. S6a). Consistently, aortic root analysis confirmed a reduction in plaque area with mCAR1 (Fig. 1n; Supplementary Fig. S6b, c). mCAR1 and mCAR3 also reduced necrotic core area (Fig. 1o), while mCAR1 produced the most consistent reduction in plaque macrophage burden, including both CD68⁺ and Trem2⁺ areas (Fig. 1p). Notably, the anti-atherosclerotic effect was not accompanied by changes in circulating LDL-C, total cholesterol, or triglycerides (Supplementary Fig. S7a–c), indicating that plaque reduction was independent of systemic lipid lowering. No significant increases in serum IFN- γ or TNF- α were detected at 72 h or 7 days after treatment (Supplementary Fig. S7d, e).

Since mCAR1 T cells showed the strongest therapeutic efficacy in $Apoe^{-/-}$ mice, we further evaluated this construct in two additional models of atherosclerosis: $Ldlr^{-/-}$ mice and AAV8-PCSK9-treated mice. In both models, mice were fed an HFD for 16 weeks before CAR T-cell treatment and analyzed 4 weeks later. En face Oil Red O (ORO) staining revealed that mCAR1 T cells significantly reduced total plaque area by approximately 50% in $Ldlr^{-/-}$ mice and 30% in the AAV8-PCSK9 model (Fig. 1q; Supplementary Fig. S8a, b). Aortic root analyses similarly showed smaller lesions and necrotic cores in both models after treatment (Fig. 1r; Supplementary Fig. S8c, d). CD68⁺ macrophage area was also reduced, whereas the decrease in Trem2⁺ area did not reach statistical significance in these two models (Fig. 1s). Thus, the plaque-reducing activity of mCAR1 was reproducible across three independent preclinical models of atherosclerosis. To further investigate the long-term therapeutic effect of mCAR1, we extended the $Apoe^{-/-}$ mouse experiment to 8 weeks after a single mCAR1 T cell injection, corresponding to a total of 20 weeks of HFD feeding. At this later time point, the reduction in plaque burden observed at 4 weeks was no longer detectable. No significant differences in en face plaque area, aortic root lesion area, necrotic core area, and CD68⁺ and Trem2⁺ areas were observed between mCAR1 T-cell treated and control mice (Supplementary Fig. S9). Thus, under continued hyperlipidemic stress, the benefit observed at 4 weeks was not sustained at 8 weeks after a single injection.

Given that CAR T-cell kinetics and tissue distribution are important determinants of therapeutic efficacy and safety, we next examined the number of circulating CAR T cells in both $Apoe^{-/-}$ and $Ldlr^{-/-}$ mice. CAR⁺ T cells increased rapidly

after injection, reached their highest measured level on day 1, declined substantially by day 3, and approached background levels by days 7–14 (Supplementary Fig. S10), indicating rapid systemic exposure followed by contraction rather than prolonged high-level persistence in the circulation. Tissue distribution was further assessed by V5 tag immunostaining. At day 7, sparse V5⁺ cells were detected in aortic root plaques, whereas V5 signal was not readily detected in the examined brain, intestine, kidney, liver, lung, or spleen sections (Supplementary Fig. S11). Since tissue distribution was assessed at a single time point, these findings do not exclude earlier trafficking, transient tissue accumulation, or low-level engagement below the detection limit. Collectively, this kinetic profile is compatible with an early, transient exposure and a time-limited therapeutic response. In parallel, we examined the short-term safety of the treatment. mCAR1 T cell treatment was not associated with significant changes in serum ALT and AST levels (Supplementary Fig. S12a, b) or serum renal function markers (Supplementary Fig. S12c, d), behavioral performance (Supplementary Fig. S12e–j), bone turnover biomarkers (Supplementary Fig. S12k–m), major organ histology (Supplementary Fig. S13), or Evans blue vascular permeability (Supplementary Fig. S14). Therefore, no overt short-term toxicity was detected in the assays or at the time points examined.

Together, these data provide proof of concept that Trem2-targeted CAR T cells can attenuate established atherosclerosis by targeting a Trem2-enriched plaque macrophage subpopulation. The recent demonstration that anti-oxLDL CAR regulatory T cells reduce atherosclerotic plaque further supports the broader potential of engineered cellular immunotherapy in vascular disease¹³. Genetic and pharmacological studies have reported both pro-atherogenic and protective effects of Trem2, reflecting its roles in lipid uptake, macrophage survival, efferocytosis, and necrotic core formation^{11,12,14}. Using Trem2 agonistic antibody (4D9), Piollet *et al.* showed reduced necrotic core size and macrophage content¹², whereas another Trem2 agonistic antibody (AL002a) was reported to increase lesion size and expand macrophage area, an effect attributed to enhanced macrophage survival¹⁵. Ablation of Trem2 in $Apoe^{-/-}$ mice led to reduced lesion size and foam cell accumulation¹⁴. These apparently divergent findings likely reflect differences in disease stages and experimental models. Our strategy addresses a different question from direct modulation of Trem2 signaling. Here, Trem2 serves primarily as a recognition antigen that enables CAR T cells to eliminate Trem2-

expressing cells. These strategies are therefore not mechanistically equivalent, and our findings should not be interpreted to suggest that the depletion of Trem2⁺ macrophages will be beneficial across all stages of the disease. Importantly, CAR T cells were administered after prolonged HFD feeding, when plaques and foamy macrophages were already present. The observed response therefore shows that a disease-associated macrophage population remains therapeutically targetable after lesion establishment rather than only during disease initiation. This may also help reconcile our findings with previous studies reporting either protective or detrimental consequences of altering Trem2 activity.

The reproducibility of the mCAR1 activity across *Apoe*^{-/-}, *Ldlr*^{-/-}, and AAV8-PCSK9 models strengthens this conclusion since these models establish hypercholesterolemia and plaque formation through distinct mechanisms. In all three settings, mCAR1 reduced plaque burden, and the accompanying reduction in necrotic core area indicates an effect on lesion composition in addition to total lesion size. In *Apoe*^{-/-} mice, reduced CD68⁺ and Trem2⁺ areas further linked therapeutic efficacy to a lower plaque macrophage burden. Trem2⁺ area did not reach statistical significance in the *Ldlr*^{-/-} and AAV8-PCSK9 models despite reductions in plaque burden and CD68⁺ macrophage area, which may reflect differences in plaque macrophage composition between models as well as the smaller sample sizes. Likewise, although a reduction in necrotic core area points toward a more favorable phenotype, we did not directly measure fibrous cap thickness or collagen content, and thus cannot confirm definitive plaque stabilization.

An important feature of the treatment response is the plaque reduction without changes in circulating lipids. This separates the observed effect from conventional lipid lowering and supports a direct cell-targeted mechanism. Persistently high circulating lipid levels remain the upstream driver of atherosclerosis, and targeting a Trem2-enriched plaque macrophage population would not be expected to prevent continued lipid deposition or the subsequent recruitment of new monocytes. Thus, Trem2-directed CAR T-cell therapy should be viewed as a potential complement rather than an alternative to lipid-lowering treatment. Consistent with this interpretation, the plaque-reducing effect observed at 4 weeks was no longer detectable at 8 weeks after a single injection under continued HFD feeding, indicating a time-limited therapeutic response rather than durable disease modification. A clinically relevant population, if this strategy can be translated, may therefore be patients who remain at high plaque-related or inflammatory risk despite effective control of circulating lipids.

Target selectivity remains an important consideration as Trem2 is not restricted to plaque macrophages and can be expressed in other macrophage populations. Within the experimental window examined here, mCAR1 treatment was not associated with detectable abnormalities in major organs. Circulating CAR T cells contracted rapidly, and V5⁺ cells were not readily detected in the examined organs at day 7. These findings are consistent with an initial safety window but do not establish long-term safety. For a chronic non-malignant disease, a controllable CAR response may be preferable to

prolonged systemic persistence, and transient or tunable CAR platforms may therefore offer a practical route for future translation. Together, our work provides a framework for developing cellular immunotherapy for atherosclerosis by targeting a Trem2-enriched plaque macrophage population.

DATA AVAILABILITY

This study did not generate new sequencing data.

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AUTHOR CONTRIBUTIONS

Y.L., X.M., and K.F. conducted the *in vitro* experiments and analyzed the data. L.K. and Y.Y. generated VOs. Y.S. and L.W. contributed to T cell culture. Y.L. conducted the *in vivo* experiments and analyzed the data. P.W. analyzed the scRNA-seq data. Y.L., X.K., Y.H.T., and J.Z.Z. wrote and revised the manuscript. Y.H.T. and J.Z.Z. conceived and supervised the study and acquired funding.

COMPETING INTERESTS

The authors declare no competing interests.

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ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at <https://doi.org/10.15302/vita.2026.10.0080>.

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