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RESEARCH LETTER

FAP-Directed CAR-NKT Cells for Multi-Target Suppression of Atherosclerotic Progression

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Metabolically active atherosclerotic lesions remain a major therapeutic challenge in mitigating major adverse cardiovascular events. Despite the use of lipid-lowering and anti-inflammatory therapies, active atherosclerotic lesions harbor cellular heterogeneity and fibrotic barriers that limit therapeutic penetration.

FAP (fibroblast activation protein)-directed CAR (chimeric antigen receptor)-engineered T (FAP.CAR-T) cell therapy has demonstrated disease-modifying effects in fibrotic diseases.[1] Recent advances in FAP-directed immune engineering further support its immunomodulatory capacity in fibrosis-associated inflammation.[2] In human atherosclerotic plaques, FAP is expressed by a subset of modulated vascular smooth muscle cells (VSMCs) enriched within the neo-intima,[3] and genetic deletion of *Fap* attenuates plaque progression.[4]

However, strategies targeting a single cellular component confer limited therapeutic effects in complex inflammatory settings.[2] CAR-T cell therapy yields limited effects in atherosclerosis, largely due to the heterogeneous composition of plaques, including modulated VSMCs, macrophages, and foam cells. Targeting VSMCs alone fails to mitigate plaque-associated inflammatory activity, as macrophage-derived foam cells sustain proinflammatory signaling and promote VSMC activation and migration (Figure [A]).

In addition to cellular heterogeneity, physical barriers within plaques further restrict CAR-T-cell therapeutic activity. Although molecular-scale approaches such as FAP-targeted BiTEs (bispecific T-cell engagers) have demonstrated anti-fibrotic activity in targeting FAP$^+$ VSMCs,[3] cellular therapies remain hindered by steric and structural barriers.

To overcome both cellular heterogeneity and physical barriers, we developed FAP.CAR-NKT (FAP-directed CAR-engineered invariant natural killer T) cells using our previously published protocol,[5] with the CAR construct modified to incorporate a species-crossreactive FAP-specific single-chain variable fragment (MO36). These cells integrate 3 targeting modalities: (1) a FAP-directed CAR targeting modulated VSMCs, (2) an invariant NKT TCR (T-cell receptor) that may recognize CD1d-presented glycolipids on macrophages and foam cells, and (3) natural killer receptors that may recognize stress-induced ligands on foam cells. This multitarget design enables simultaneous elimination of key plaque-resident cell populations (Figure [A]).

We generated syngeneic mouse FAP.CAR-NKT and FAP.CAR-T cells from ApoE$^{-/-}$ mice, achieving high and comparable cell purity and CAR expression (Figure [Bi] through [Bv]). ApoE$^{-/-}$ mice fed a Western diet received a single 5 Gy total-body irradiation as a standard lymphodepleting conditioning regimen 24 hours before administration of the indicated treatments (Figure [Bvi]). Although this conditioning strategy facilitated adoptive cell transfer, future studies should evaluate more clinically relevant conditioning approaches because irradiation may influence immune composition, plaque biology, and therapeutic

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Nonstandard Abbreviations and Acronyms

BiTE	bispecific T-cell engagers
CAR	chimeric antigen receptor
FAP	fibroblast activation protein
FAP.CAR-NKT	FAP-directed CAR-engineered invariant natural killer T
TCR	T-cell receptor
VSMC	vascular smooth muscle cell

cell engraftment. Histological staining revealed reduced numbers of FAP$^+$ and CD1d$^+$ cells within atherosclerotic plaques following FAP.CAR-NKT treatment (Figure [Bvii]). FAP.CAR-T cells showed limited effects on plaque progression, whereas FAP.CAR-NKT cells markedly reduced atherosclerotic burden, as evidenced by decreased lipid-rich plaque area in en face aortas (Figure [Bviii] and [Bix]) and reduced plaque-associated collagen in aortic root sections (Figure [Bx] and [Bxi]). This reduction is consistent with a reduction in FAP$^+$ stromal components within the plaque. The therapeutic effect was attenuated at later treatment time points.

To recapitulate the human plaque microenvironment, we employed a multicellular microfluidic vessel-on-a-chip system comprising human umbilical artery endothelial cells, human umbilical artery smooth muscle cells, and macrophages. In this system, oxidized low-density lipoprotein induced foam cell formation and increased FAP expression in VSMCs. Conventional T cells conferred no effects, whereas FAP.CAR-T cells selectively eliminated FAP$^+$ VSMCs but failed to target foam cells, revealing the limitation of single-target strategies in heterogeneous plaques. In contrast, FAP.CAR-NKT cells reduced both FAP$^+$ VSMCs and foam cells (Figure [C]).

We generated human FAP.CAR-T and FAP.CAR-NKT cells, achieving high and comparable cell purity and CAR expression (Figure [Di] through [Div]). Direct in vitro killing assays using oxidized low-density lipoprotein-induced

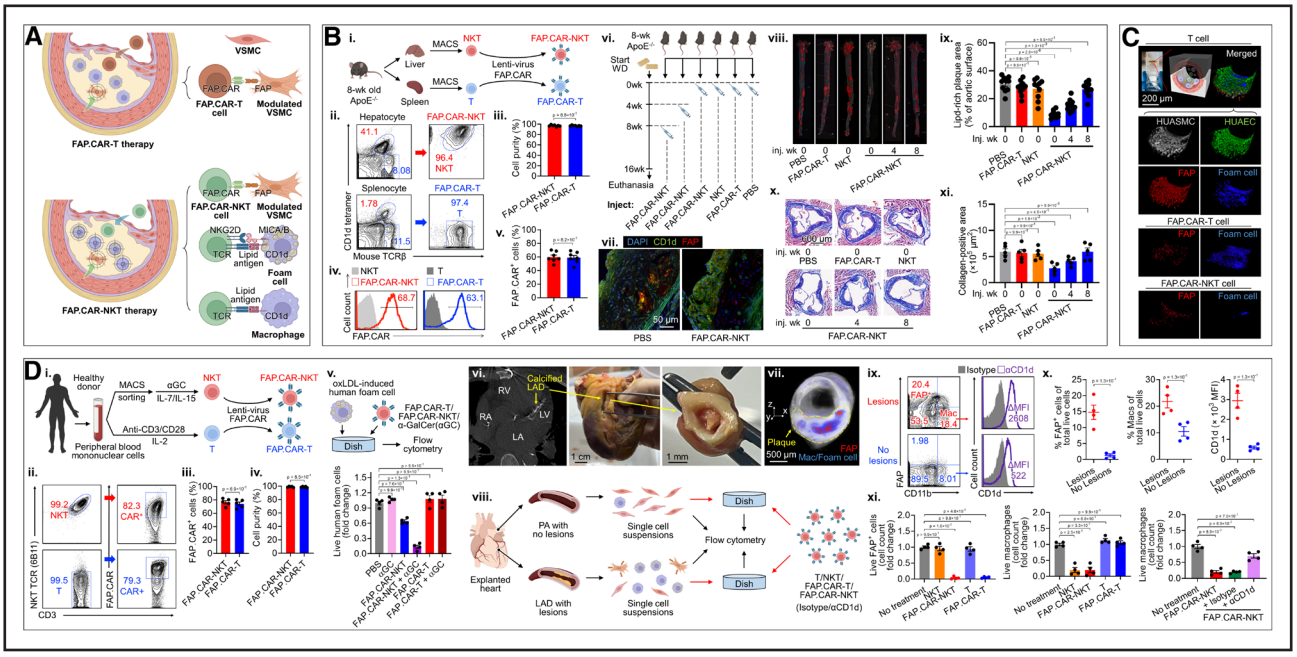

Figure. FAP.CAR-NKT (FAP [fibroblast activation protein]-directed CAR (chimeric antigen receptor)-engineered invariant natural killer T) cells mitigate atherosclerotic progression through multitarget cytotoxicity.

A, Comparison of FAP.CAR-T and FAP.CAR-NKT therapies. **B**, Mouse FAP.CAR-T and FAP.CAR-NKT generation and therapeutic evaluation in ApoE$^{-/-}$ mice. **i** through **v**, Cell generation, purity, and CAR expression (n=7). **vi**, Experimental design. **vii**, Immunofluorescence of FAP and CD1d within atherosclerotic plaques. **viii** through **ix**, Representative en face Sudan Black-stained aortas and lipid-rich area quantification (n=10; 5 males and 5 females). **x** through **xi**, Representative Masson trichrome staining of aortic root sections and collagen quantification (n=5 males). No animals were excluded. Analyses were blinded. **C**, Vessel-on-a-chip model containing human umbilical artery smooth muscle cells (HUASMCs), human umbilical artery endothelial cells (HUAECs), macrophages, and oxLDL-induced foam cells treated with the indicated cell therapies. Confocal fluorescence microscopy visualized HUASMCs (α-SMA), HUAECs (CD31), FAP$^+$ cells, and foam cells (Dil-oxLDL). **D**, Human FAP.CAR-T and FAP.CAR-NKT characterization and validation. **i** through **iv**, Cell generation, purity, and CAR expression (n=5). **v**, In vitro foam-cell killing assay. **vi**, Calcified LAD lesions identified by computed tomography (CT) and gross imaging. **vii**, Three-dimensional light-sheet imaging of cleared LAD segments showing FAP$^+$ cells and CD68$^+$ macrophages (Mac)/foam cells within the plaque. **viii**, Ex vivo killing assays using cell suspensions from LAD (lesion) and PA (nonlesion). **ix** through **x**, Flow cytometric analysis of FAP$^+$ cells, CD11b$^+$ macrophages (Macs), and CD1d expression (n=4). **xi**, Ex vivo cytotoxicity against FAP$^+$ cells and macrophages/foam cells with or without CD1d blockade (n=4). n denotes biological replicates. Data are mean±SEM. All tests were nonparametric. *P* values: **Bix**, **Bxi**, **Dv**, and **Dxi**: Dunn multiple-comparisons test; **Dx**: Wilcoxon matched-pairs signed-rank test; **Biii**, **Bv**, **Diii**, **Div**: Mann-Whitney *U* test. No experiment-wide multiple testing correction was applied. Analyses used GraphPad Prism 9.

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human foam cells demonstrated superior cytotoxicity of FAP.CAR-NKT cells over FAP.CAR-T cells (Figure [Dv]). Exogenous α-Galactosylceramide enhanced foam-cell killing by FAP.CAR-NKT cells, supporting a role for CD1d-mediated recognition. In explanted hearts from patients with ischemic cardiomyopathy, left anterior descending arteries laden with lesions and pulmonary arteries without lesions were delineated and excised based on imaging and gross pathology (Figure [Dvi]). Multi-view 3D light-sheet fluorescence microscopy of optically cleared left anterior descending artery segments (modified Adipo-Clear) revealed the spatial distribution of FAP$^+$ cells and macrophages/foam cells within plaques (Figure [Dvii]). Flow cytometric analysis further demonstrated enrichment of FAP$^+$ cells and macrophages in lesion versus nonlesion samples, accompanied by increased CD1d expression (Figure [Dviii] through [Dx]). In ex vivo killing assays, FAP.CAR-NKT cells effectively reduced both primary patient sample-derived FAP$^+$ cells and macrophage/foam cells, whereas FAP.CAR-T, NKT, or T cells revealed limited effects. CD1d blockade diminished macrophage/foam cell cytotoxicity, supporting a role for CD1d-mediated recognition, although the relevant endogenous glycolipid antigens remain undefined (Figure [Dviii] and [Dxi]).

To our knowledge, cell-based immunotherapies have demonstrated limited efficacy in atherosclerosis. Our findings demonstrate the translational potential of FAP.CAR-NKT cells to overcome cellular heterogeneity and plaque-associated physical barriers that constrain the efficacy of FAP-directed CAR-T strategies. FAP is minimally expressed in healthy adult tissues,[3] and no overt toxicity was observed following FAP.CAR-NKT treatment in the current study. Although invariant NKT cells are relatively rare, robust ex vivo expansion and CAR engineering have been reported, supporting the translational feasibility of both autologous and allogeneic CAR-NKT therapies.[5] Future studies should define the optimal persistence and dosing strategy for this therapeutic approach. This work establishes a CAR/TCR multi-target cellular immunotherapy framework for atherosclerosis.

ARTICLE INFORMATION

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Author Contributions

E. Zhu conceived the study, designed and performed the experiments, analyzed the data, prepared the figures, and drafted the article. Y.-R. Li and Y.C. Zhu contributed to CAR-T and CAR-engineered invariant natural killer T cell generation and related experiments. J. Cho, S.-K. Park, and P. Zhao contributed to murine atherosclerosis studies. R. Kropp, Y. Sanaiha, O.J. Kwon, M. Justo, and B. Ariannpour contributed to human atherosclerotic tissue procurement and processing. Y. Zhu contributed to vessel-on-a-chip studies. S. Wang contributed to data analysis and figure preparation. M. Gu supervised the vessel-on-a-chip studies, and P. Benharash supervised human tissue procurement. L. Yang and T. Hsiai conceived and supervised the study. All authors reviewed and approved the final article.

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Disclosures

T. Hsiai, L. Yang, E. Zhu, and Y.-R. Li are inventors on intellectual property related to this work. The other authors report no conflicts. The data, analytic methods, and study materials supporting the findings of this study are available from the corresponding authors on reasonable request. The corresponding authors are responsible for maintaining the availability of these materials, subject to institutional policies and ethical regulations governing human tissue specimens. All animal experiments were approved by the University of California, Los Angeles Institutional Animal Care and Use Committee (IACUC; ARC-2014-060). Human tissue collection and use were approved by the University of California, Los Angeles institutional review board (IRB; IRB no. 23-0487). Healthy donor peripheral blood mononuclear cells were obtained from the UCLA/CFAR Virology Core Laboratory without identification information under federal and state regulations.

REFERENCES

1. Rurik JG, Tombácz I, Yadegari A, Méndez Fernández PO, Shewale SV, Li L, Kimura T, Soliman OY, Papp TE, Tam YK, et al. CAR T cells produced in vivo to treat cardiac injury. *Science*. 2022;375:91–96. doi: 10.1126/science.abm0594
2. Li X, Li J, Li G, Zhu L, Cheng G, Li H, Lin H, Jia N, Hong X, Liu Y, et al. Engineered immunosuppressive dendritic cells protect against cardiac remodelling. *Nature*. 2026;653:1205–1215. doi: 10.1038/s41586-026-10346-5
3. Amrute JM, Jung IH, Yamawaki T, Lin WL, Bredemeyer A, Diekmann J, Hayat S, Zhang X, Wakefield DL, Luo X, et al. Targeting modulated vascular smooth muscle cells in atherosclerosis via FAP-directed immunotherapy. *Science*. 2026;392:eadx1736. doi: 10.1126/science.adx1736
4. Stein S, Weber J, Nusser-Stein S, Pahla J, Zhang HE, Mohammed SA, Oppi S, Gaul DS, Paneni F, Tailleux A, et al. Deletion of fibroblast activation protein provides atheroprotection. *Cardiovasc Res*. 2021;117:1060–1069. doi: 10.1093/cvr/cvaa142
5. Li YR, Zhou Y, Yu J, Kim YJ, Li M, Lee D, Zhou K, Chen Y, Zhu Y, Wang Y-C, et al. Generation of allogeneic CAR-NKT cells from hematopoietic stem and progenitor cells using a clinically guided culture method. *Nat Biotechnol*. 2025;43:329–344. doi: 10.1038/s41587-024-02226-y

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