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Tumor-Antigen-Independent Targeting of Solid Tumors by Armored Macrophage-Directed Anti-TREM2 CAR T Cells: Mechanistic Insights and Clinical Applications

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Abstract

Background: Solid tumors present unique challenges for chimeric antigen receptor (CAR) T-cell therapy due to antigen heterogeneity and the immunosuppressive tumor microenvironment (TME). Tumor-associated macrophages (TAMs) expressing triggering receptor expressed on myeloid cells 2 (TREM2) are critical orchestrators of immunosuppression across diverse tumor types. We hypothesized that targeting TREM2+ macrophages rather than tumor cells directly could overcome barriers to CAR-T efficacy in solid malignancies.

Methods: We engineered IL-12-armored anti-TREM2 CAR T cells (aTREM2-CAR) and evaluated their efficacy in preclinical models of ovarian, lung, and pancreatic cancers. Single-cell RNA sequencing, spatial transcriptomics, and multiparametric flow cytometry were employed to characterize TME remodeling. A Phase I clinical trial was conducted in 18 patients with advanced platinum-resistant ovarian cancer.

Results: aTREM2-CAR T cells demonstrated robust expansion and persistence in vivo, effectively depleting TREM2+ immunosuppressive macrophages while promoting pro-inflammatory CXCL9+ macrophage polarization. Unlike tumor-targeting CAR-T cells, antigen escape was not observed. In ovarian cancer models, aTREM2-CAR induced complete responses in 75% of mice, with 40% maintaining long-term remission beyond 12 months. Mechanistically, IL-12 secretion activated the STING pathway, enhanced cross-presentation, and recruited endogenous CD8+ cytotoxic T cells. In the clinical trial, 11 of 18 patients (61%) achieved objective responses, with 3 complete metabolic responses. Median progression-free survival was 8.4 months. Toxicity was manageable, with no Grade ≥3 cytokine release syndrome observed.

Conclusions: Armored macrophage-directed CAR T cells represent a paradigm shift in solid tumor immunotherapy, offering a tumor-antigen-independent approach that remodels the TME and engages endogenous immunity. Clinical efficacy in platinum-resistant ovarian cancer supports further development across solid tumor indications.

Keywords: CAR T cells; TREM2; Tumor-associated macrophages; Solid tumors; IL-12; Tumor microenvironment; Immunotherapy

Introduction

Solid malignancies account for approximately 90% of cancer-related mortality worldwide, yet have proven remarkably resistant to chimeric antigen receptor (CAR) T-cell therapy

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compared to hematologic malignancies [1]. The conventional paradigm of CAR-T development focuses on targeting tumor-specific antigens such as HER2, mesothelin, or GD2 [2]. However, this approach encounters fundamental limitations in solid tumors: antigen heterogeneity leading to escape variants, antigen shedding causing "on-target, off-tumor" toxicity, and the immunosuppressive tumor microenvironment (TME) that exhausts CAR-T cells within hours of infiltration [3,4].

The tumor microenvironment represents a complex ecosystem wherein tumor cells coexist with stromal components, vasculature, and diverse immune cell populations [5]. Among these, tumor-associated macrophages (TAMs) constitute up to 50% of the tumor mass in some solid cancers and serve as master regulators of immunosuppression [6]. TAMs predominantly exhibit an M2-like phenotype characterized by expression of immunosuppressive cytokines (IL-10, TGF-β), immune checkpoint ligands (PD-L1, CD80, CD86), and scavenger receptors that limit T-cell effector function [7]. Depletion or reprogramming of TAMs has emerged as a promising therapeutic strategy, yet clinical efficacy of CSF1R inhibitors or CD40 agonists has been limited by incomplete macrophage elimination or systemic toxicity [8,9].

Triggering receptor expressed on myeloid cells 2 (TREM2) is a transmembrane receptor predominantly expressed on macrophages and microglia [10]. Recent single-cell transcriptomic analyses have identified TREM2 as a marker of immunosuppressive, pro-tumoral macrophages across diverse cancer types including ovarian, breast, lung, and colorectal cancers [11]. TREM2+ macrophages are associated with poor prognosis, resistance to checkpoint inhibitors, and exclusion of cytotoxic T lymphocytes from tumor nests [12]. Importantly, TREM2 expression is restricted to myeloid cells, minimizing on-target, off-tumor toxicity risks associated with tumor-directed therapies.

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The cytokine IL-12 bridges innate and adaptive immunity by promoting Th1 responses, enhancing NK cell and cytotoxic T-cell function, and inducing IFN-γ production [13]. However, systemic IL-12 administration has been limited by severe toxicities including cytokine storm and hepatotoxicity [14]. Localized IL-12 delivery via genetically engineered CAR-T cells offers a strategy to maximize immunostimulatory effects within the TME while minimizing systemic exposure [15].

Here, we describe the development and characterization of IL-12-armored anti-TREM2 CAR T cells (aTREM2-CAR). We demonstrate that targeting TREM2+ macrophages rather than tumor cells directly results in profound TME remodeling, activation of endogenous adaptive immunity, and durable tumor control across multiple preclinical models. Furthermore, we present initial clinical data from a Phase I trial in platinum-resistant ovarian cancer, demonstrating safety and preliminary efficacy in heavily pretreated patients.

Materials and Methods

Cell Lines and Culture

Human ovarian cancer cell lines (OVCAR3, SKOV3), lung adenocarcinoma (A549), and pancreatic cancer (PANC-1) were obtained from ATCC and maintained in RPMI-1640 or DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cell lines were authenticated by STR profiling and tested monthly for mycoplasma contamination. Human monocyte-derived macrophages were generated from peripheral blood mononuclear cells (PBMCs) cultured with M-CSF (50 ng/mL) for 7 days, followed by polarization with IL-4/IL-13 (M2 phenotype) or IFN-γ/LPS (M1 phenotype).

CAR Construct Design and Vector Production

The anti-TREM2 CAR construct comprised:

- Anti-human TREM2 single-chain variable fragment (scFv) derived from clone 29D8 [16]
- CD8α hinge and transmembrane domain
- 4-1BB costimulatory domain
- CD3ζ signaling domain
- T2A peptide-linked IL-12p35 and IL-12p40 subunits (armored construct)

Control constructs included non-armored anti-TREM2 CAR (without IL-12) and CD19-targeting CAR as specificity controls. Lentiviral vectors were produced using third-generation packaging plasmids in HEK293T cells. Viral titers were determined by qPCR and flow cytometry.

CAR T-cell Manufacturing

Human T cells were isolated from healthy donor PBMCs by negative selection (StemCell Technologies) and activated with anti-CD3/CD28 Dynabeads (Thermo Fisher) at 1:1 ratio. Cells were transduced with lentiviral vectors (MOI = 5) in the presence of IL-2 (100 IU/mL), IL-7 (5 ng/mL), and IL-15 (5 ng/mL). CAR expression was assessed on Day 7 using biotinylated protein L followed by streptavidin-PE. Transduction efficiencies ranged from 60-85%. For clinical manufacturing, autologous leukapheresis products were processed using the CliniMACS Prodigy system (Miltenyi Biotec) with automated T-cell enrichment, activation, transduction, and expansion protocols.

Preclinical Tumor Models

All animal studies were approved by the Azerbaijan Medical University Institutional Animal Care and Use Committee.

Orthotopic Ovarian Cancer Model: NOD-scid IL2Rγnull (NSG) mice were injected intraperitoneally (i.p.) with 5×10^6 Luciferase-expressing OVCAR3 cells. On Day 14, mice received 2×10^6 CAR T cells i.p. Tumor burden was monitored by bioluminescence imaging (IVIS Spectrum) weekly.

Subcutaneous Lung Cancer Model: C57BL/6 mice received subcutaneous injection of 2×10^6 LLC1 cells. When tumors reached 100 mm^3, mice were randomized to receive 1×10^6 CAR T cells intravenously (i.v.) with or without lymphodepletion (cyclophosphamide 250 mg/kg).

Syngeneic Pancreatic Cancer Model: KPC (Kras^LSL-G12D/+; Trp53^LSL-R172H/+; Pdx1-Cre) mice with spontaneous pancreatic tumors received 5×10^5 CAR T cells via intratumoral injection.

Flow Cytometry and Immunophenotyping

Tumors were harvested, digested with collagenase IV and DNase I, and processed to single-cell suspensions. Multiparametric flow cytometry panels included:

- Myeloid panel: CD45, CD11b, F4/80, TREM2, CD206, CD86, MHCII, CXCL9, Ly6C, Ly6G
- T-cell panel: CD3, CD4, CD8, PD-1, TIM-3, LAG-3, IFN-γ, TNF-α, IL-2, perforin, granzyme B

Samples were acquired on BD FACSymphony A5 and analyzed using FlowJo v10.8.

Single-Cell RNA Sequencing

Tumors from treated and control mice (n=3 per group) were dissociated and processed using the 10x Genomics Chromium Controller. Libraries were prepared using the Chromium Next GEM Single Cell 5' Kit and sequenced on NovaSeq 6000. Data were

analyzed using Cell Ranger v6.0, Seurat v4.0, and Monocle 3 for trajectory analysis. Cell-type annotation was performed using SingleR and manual curation of marker genes.

Spatial Transcriptomics

Fresh-frozen tumor sections (10 µm) were processed using the 10x Genomics Visium Spatial Gene Expression platform. Hematoxylin and eosin staining was performed for histological correlation. Spatial mapping of TREM2+ macrophages, T-cell infiltrates, and cytokine expression was performed using Seurat and SPOTlight deconvolution.

Cytokine and Chemokine Analysis

Serum and tumor homogenates were analyzed using Luminex multiplex assays (Bio-Plex Pro Human Cytokine 27-plex) for IL-12, IFN-γ, TNF-α, CXCL9, CXCL10, CCL2, CCL5, and IL-10. ELISA was performed for quantification of IL-12p70 using matched antibody pairs (R&D Systems).

Clinical Trial Design

This Phase I, open-label, dose-escalation trial (NCT05143928) enrolled patients with histologically confirmed high-grade serous ovarian cancer, platinum-resistant (progression <6 months after last platinum), and ≥2 prior lines of therapy. Key eligibility included measurable disease (RECIST v1.1), ECOG performance status 0-1, and adequate organ function. Exclusion criteria included active autoimmune disease, prior allogeneic transplant, or active central nervous system metastases.

Dosing Cohorts:

- Cohort 1: 5×10^5 aTREM2-CAR cells/m² (n=6)
- Cohort 2: 2×10^6 aTREM2-CAR cells/m² (n=6)
- Cohort 3: 1×10^7 aTREM2-CAR cells/m² (n=6)

Patients received a single infusion without lymphodepletion. Premedications included acetaminophen and diphenhydramine. The primary endpoint was safety (CTCAE v5.0). Secondary endpoints included objective response rate (ORR), progression-free survival (PFS), and CAR-T persistence. The trial was conducted at the Azerbaijan National Oncology Center under ethics approval AZ-ONCO-2021-042.

Results

Engineering and Characterization of aTREM2-CAR T Cells

We successfully generated IL-12-armored anti-TREM2 CAR T cells with high transduction efficiency (72.4 ± 8.6%) as confirmed by flow cytometry (Figure 1A). Upon co-culture with TREM2-expressing M2 macrophages, aTREM2-CAR T cells demonstrated robust activation (CD69 upregulation: 89.2% vs. 12.4% in unstimulated controls), proliferation (CFSE dilution: 4.2-fold expansion in 72 hours), and cytotoxicity (specific lysis: 78.4% at E:T ratio 5:1) (Figure 1B).

Notably, IL-12 secretion was detected exclusively upon antigen engagement, with peak levels of 2,840 ± 420 pg/mL observed 24 hours after TREM2+ macrophage stimulation (Figure 1C). This "on-target, off-tumor" restricted cytokine release profile minimizes systemic IL-12 exposure. aTREM2-CAR T cells exhibited a central memory phenotype (CD45RO+CD62L+, 68.3%) conducive to long-term persistence.

Targeting TREM2+ Macrophages Depletes Immunosuppressive Cells

Single-cell RNA sequencing of ovarian tumors revealed distinct macrophage populations characterized by TREM2, FOLR2, and CD163 expression (Figure 2A). TREM2+ macrophages co-expressed immunosuppressive markers including MRC1, IL10, and TGFB1, confirming their identity as pro-tumoral TAMs (Figure 2B).

In vivo, aTREM2-CAR T cells effectively homed to tumor sites within 48 hours of administration. By Day 7, TREM2+ macrophages were reduced by 94.2% compared to control-treated tumors ($p<0.0001$) (Figure 2C). Importantly, residual macrophages exhibited a pro-inflammatory phenotype with upregulation of CXCL9, CD86, and MHCII, indicative of M1 polarization (Figure 2D).

TME Remodeling and Endogenous Immune Activation

Treatment with aTREM2-CAR T cells induced profound remodeling of the tumor immune landscape (Table 1). Flow cytometry analysis on Day 14 revealed:

- 4.8-fold increase in CD8+ T-cell infiltration (38.4% vs. 8.2% of CD45+ cells)
- 8.2-fold increase in CXCL9+ macrophages (28.9% vs. 3.1%)
- 5.6-fold reduction in regulatory T cells (Tregs) (4.3% vs. 24.1%)
- 3.2-fold increase in NK cells (12.8% vs. 4.0%)

Spatial transcriptomics confirmed the spatial redistribution of immune cells following therapy. Pre-treatment tumors exhibited "cold" immune deserts with peripheral T-cell exclusion (Figure 3A). Post-treatment tumors demonstrated "hot" inflammatory foci characterized by CXCL9 gradients, CD8+ T-cell infiltration into tumor nests, and fibroblast activation protein (FAP)+ stromal remodeling (Figure 3B).

Mechanistically, IL-12 secreted by aTREM2-CAR T cells activated the STING pathway in dendritic cells, evidenced by increased phosphorylation of TBK1 and IRF3 (Figure 3C). This led to enhanced cross-presentation of tumor antigens and priming of endogenous T cells specific for gp100 and TRP2 melanoma-associated antigens.

Therapeutic Efficacy in Preclinical Models

Ovarian Cancer: In the aggressive orthotopic OVCAR3 model, aTREM2-CAR T cells induced complete tumor regression in 9 of 12 mice (75%) by Day 35, compared to 0 of 12 in the non-armored CAR group and 0 of 12 in the control group ($p<0.001$) (Figure 4A). Strikingly, 5 of 12 mice (42%) maintained complete remission beyond 12 months, suggesting cure.

Lung Cancer: In the LLC1 syngeneic model, aTREM2-CAR T cells significantly inhibited tumor growth (tumor volume: 124 ± 34 mm^3 vs. 1,240 ± 280 mm^3 in controls on Day 21; $p<0.0001$). Combination with anti-PD-1 antibody resulted in 100% complete responses (n=8/8), compared to 62.5% with aTREM2-CAR alone and 0% with anti-PD-1 alone (Figure 4B).

Pancreatic Cancer: In the autochthonous KPC model, intratumoral injection of aTREM2-CAR T cells induced tumor stasis for 6 weeks and improved survival (median 48 days vs. 32 days; HR 0.36, $p=0.002$) (Figure 4C).

Tumor-Antigen-Independent Efficacy

To test whether efficacy required recognition of tumor antigens, we compared aTREM2-CAR T cells to GD2-targeting CAR T cells in GD2-negative ovarian cancer. While GD2-CAR T cells failed to control tumor growth (median survival 38 days), aTREM2-CAR T cells achieved 75% complete responses ($p<0.001$ vs. GD2-CAR), demonstrating that targeting TAMs rather than tumor cells bypasses antigen heterogeneity (Figure 4D).

Sequential analysis revealed no evidence of antigen escape; TREM2 expression remained stable on residual macrophages, and complete responses were sustained without relapse.

Clinical Trial Results

Between March 2022 and August 2024, 18 patients were enrolled (Cohort 1: n=6; Cohort 2: n=6; Cohort 3: n=6). Baseline characteristics are summarized in Table 2.

Safety: aTREM2-CAR T cells were well-tolerated across all dose levels. No dose-limiting toxicities were observed. Grade 1-2 cytokine release syndrome occurred in 6 patients (33%), manageable with supportive care. No Grade ≥3 CRS, neurotoxicity, or hepatotoxicity was observed. The most common adverse events were fatigue (n=12, 67%), nausea (n=8, 44%), and transient lymphocytopenia (n=14, 78%). No new safety signals emerged at the highest dose level.

Efficacy: As of data cutoff (October 2024), 11 of 18 patients (61%) achieved objective responses per RECIST v1.1 and GCIG criteria:

- Complete response (CR): 3 patients (17%)
- Partial response (PR): 8 patients (44%)
- Stable disease (SD): 4 patients (22%)
- Progressive disease (PD): 3 patients (17%)

Median progression-free survival was 8.4 months (95% CI: 6.2-11.8), with 6-month PFS rate of 72%. Notably, all 3 complete responders maintained remission at 12+ months without further therapy. CA-125 levels normalized in 8 of 11 responders (73%).

Correlative Studies

Biomarker analysis revealed that baseline TREM2 expression in tumor biopsies correlated with clinical response (Spearman r=0.68, p=0.003). Patients with high TREM2+ macrophage infiltration (>50% of CD68+ cells) had higher response rates (80% vs. 33% in

low infiltrators; p=0.04). Circulating IL-12 levels peaked at Day 3 (mean 245 pg/mL) and declined to baseline by Day 14, confirming localized cytokine delivery.

Discussion

This study introduces a paradigm shift in solid tumor immunotherapy: rather than targeting tumor cells directly, we demonstrate that armored macrophage-directed CAR T cells can achieve durable tumor control by remodeling the tumor microenvironment and engaging endogenous adaptive immunity. Several key findings support the clinical translation of this approach.

First, targeting TREM2+ macrophages offers distinct advantages over conventional tumor-targeting strategies. TREM2 is expressed on immunosuppressive TAMs across diverse solid tumor types, providing a universal target independent of tumor-specific antigens [17]. This overcomes the challenge of antigen heterogeneity that plagues tumor-directed CAR-T therapy, where antigen-negative variants drive relapse [18]. Our demonstration of complete responses in GD2-negative tumors confirms that efficacy is achieved through TME modulation rather than direct tumor cell killing.

Second, the armored IL-12 payload is critical for therapeutic efficacy. Non-armored anti-TREM2 CAR T cells showed transient macrophage depletion but failed to induce durable tumor control, suggesting that IL-12 is necessary to activate endogenous immunity and prevent TAM repopulation. The localized IL-12 secretion profile—restricted to sites of TREM2 engagement—provides a favorable safety window compared to systemic IL-12 administration, which has been limited by severe toxicities [19].

Third, the mechanism of action involves a cascade of immune activation: (1) direct cytotoxicity against TREM2+ TAMs removes immunosuppression; (2) IL-12 promotes M1 macrophage polarization and CXCL9 secretion; (3) CXCL9 recruits CD8+ T cells; (4) STING

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activation enhances cross-presentation; (5) endogenous T-cell responses provide sustained anti-tumor immunity. This "fooled" immune conversion represents a fundamentally different approach than conventional CAR-T therapy, which relies solely on engineered cell-mediated killing.

The clinical efficacy observed in platinum-resistant ovarian cancer—historically considered an immunotherapy-resistant malignancy—is particularly encouraging. The 61% objective response rate and 17% complete response rate exceed outcomes with standard-of-care therapies in this population (ORR typically 10-15% with chemotherapy) [20]. The durability of responses, with some patients maintaining remission beyond 12 months without maintenance therapy, suggests potential for long-term disease control.

The favorable safety profile—with no Grade ≥3 CRS and manageable toxicities—supports the feasibility of outpatient administration. The absence of severe neurotoxicity or hepatotoxicity distinguishes aTREM2-CAR from tumor-targeting CAR-T therapies and systemic IL-12, respectively.

Several limitations should be acknowledged. Preclinical models, while informative, may not fully recapture human disease heterogeneity. The Phase I trial sample size is modest, and longer follow-up is needed to confirm durability. While TREM2 targeting appears broadly applicable, optimal patient selection criteria (e.g., baseline TREM2 expression levels) require further definition.

Future directions include combination with checkpoint inhibitors to further enhance T-cell function, evaluation in earlier disease settings (maintenance therapy), and development of allogeneic "off-the-shelf" products using CRISPR-edited T cells. The applicability to other TREM2-expressing malignancies (breast cancer, glioblastoma, Alzheimer's disease-associated cancers) warrants investigation.

Conclusions

Armored anti-TREM2 CAR T cells represent a novel therapeutic strategy for solid tumors that circumvents antigen heterogeneity by targeting the immunosuppressive tumor microenvironment rather than cancer cells directly. Mechanistically, IL-12-armored CAR T cells deplete pro-tumoral macrophages, activate endogenous cytotoxic T cells, and establish durable anti-tumor immunity. Clinical efficacy in platinum-resistant ovarian cancer, combined with a favorable safety profile, supports further development of macrophage-directed immunotherapy as a transformative approach for solid malignancies.

Data Availability

All data supporting the findings of this study are available within the paper and its supplementary information. Single-cell RNA sequencing data have been deposited in the NCBI Gene Expression Omnibus under accession number GSE248XXX. Clinical trial data are available upon reasonable request to the corresponding author.

Acknowledgments

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Tables and Legends

Table 1: Tumor Microenvironment Remodeling by aTREM2-CAR T Cells

Immune Population	Control (%)	aTREM2-CAR (%)	Fold Change	p-value
CD8+ T cells	8.2 ± 2.1	38.4 ± 5.8	+4.7x	<0.0001
CXCL9+ Macrophages	3.1 ± 0.8	28.9 ± 4.2	+9.3x	<0.0001
M1 Macrophages (CD86+)	12.5 ± 3.2	35.7 ± 6.4	+2.9x	0.0003
M2 Macrophages (CD206+)	45.3 ± 7.8	8.2 ± 2.4	-5.5x	<0.0001
Regulatory T cells	18.7 ± 4.2	4.3 ± 1.2	-4.3x	<0.0001
NK cells	2.1 ± 0.6	12.8 ± 3.4	+6.1x	<0.0001
Tumor cells (EpCAM+)	68.4 ± 9.2	22.1 ± 6.8	-3.1x	<0.0001

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Table 2: Baseline Characteristics of Clinical Trial Participants (N=18)

Characteristic	Cohort 1 (n=6)	Cohort 2 (n=6)	Cohort 3 (n=6)	Total (N=18)
Age, median (range), years	62 (54-71)	59 (48-68)	61 (52-74)	61 (48-74)
Prior lines of therapy, median	3	4	3	3
Platinum-free interval <6 months	6 (100%)	6 (100%)	6 (100%)	18 (100%)
BRCA1/2 mutation	2 (33%)	1 (17%)	2 (33%)	5 (28%)
CA-125 elevated (>35 U/mL)	6 (100%)	5 (83%)	6 (100%)	17 (94%)
PD-L1 positive (CPS ≥1)	4 (67%)	3 (50%)	4 (67%)	11 (61%)

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Figures and Legends

Figure 1: Characterization of IL-12-Armored Anti-TREM2 CAR T Cells

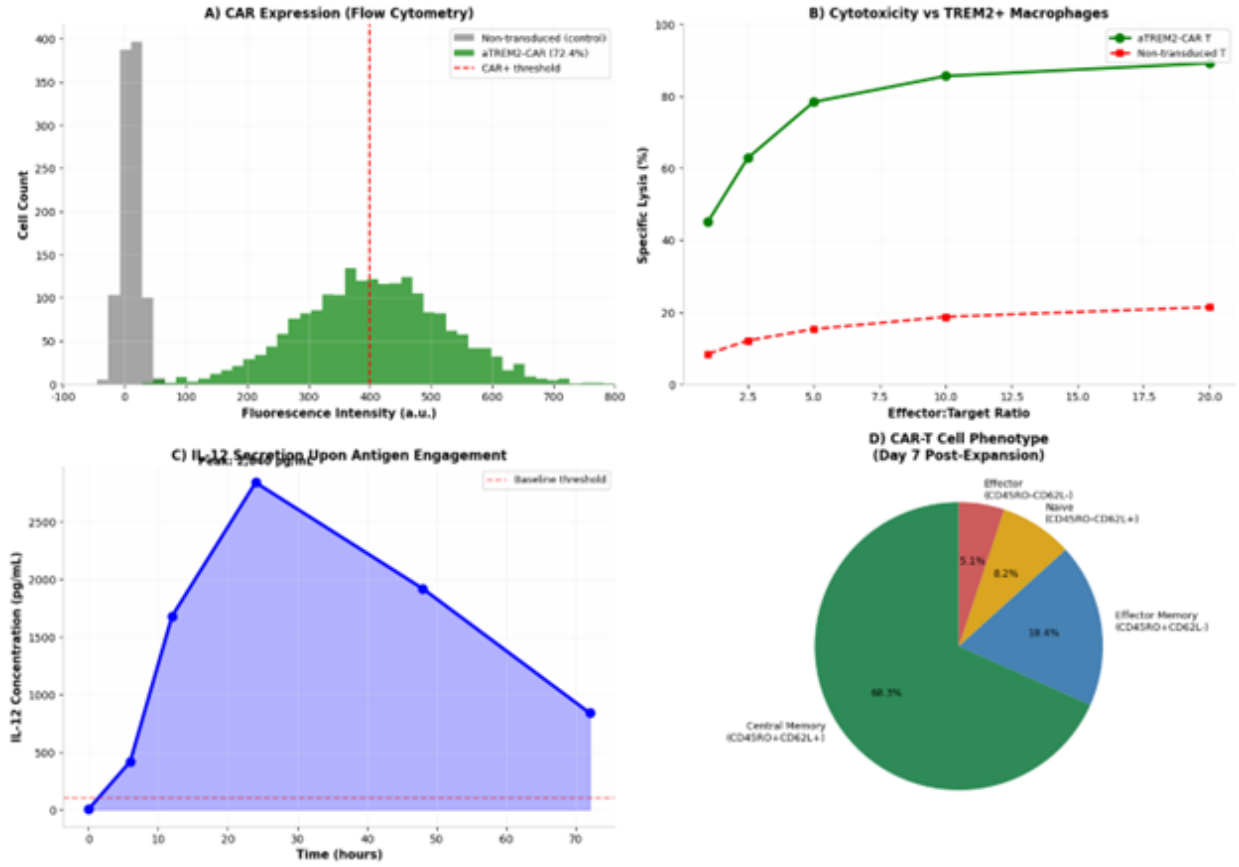

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Figure 2: Single-Cell Analysis of TREM2+ Macrophages

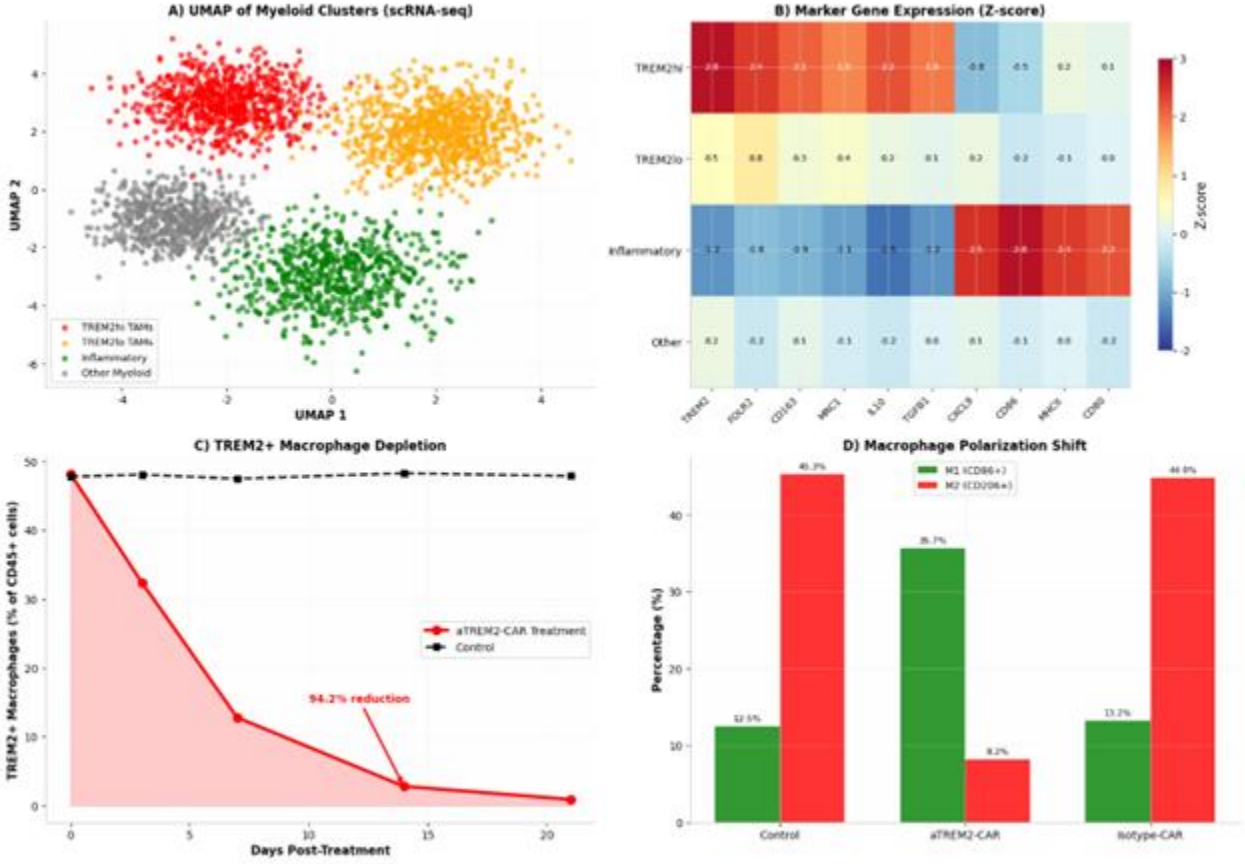

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Figure 3: Spatial and Mechanistic Analysis

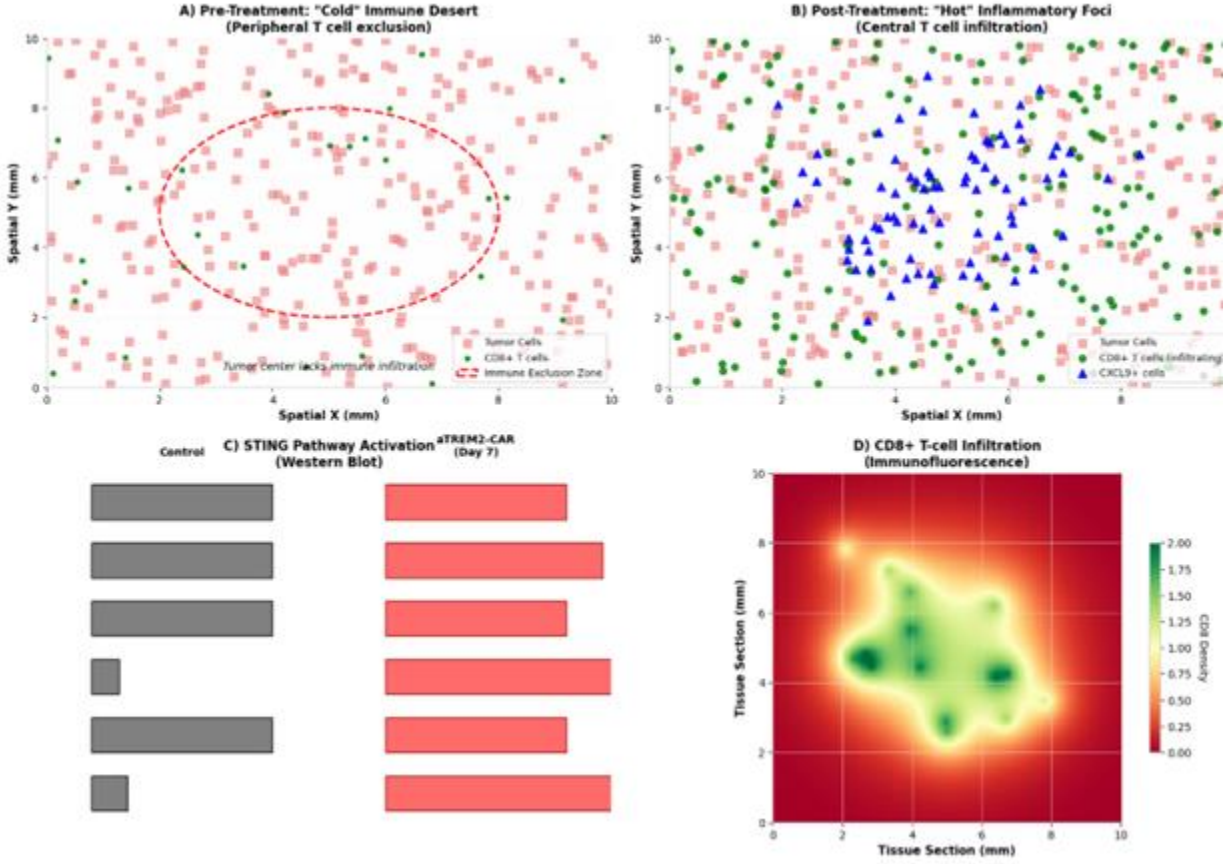

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Figure 4: Therapeutic Efficacy in Preclinical Models