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Armored Macrophage-Targeted CAR-T Cells Reset and Reprogram the Tumor Microenvironment and Control Metastatic Cancer Growth: A Phase I/II Clinical Trial

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Abstract

Background: Tumor-associated macrophages (TAMs), which commonly express triggering receptor expressed on myeloid cells 2 (TREM2) or folate receptor beta (FOLR2), are enriched in solid tumors and maintain the tumor microenvironment (TME) in an immunosuppressive state. Chimeric antigen receptor T-cell (CAR-T) therapies targeting tumor cells directly have shown limited efficacy in solid malignancies due to antigen heterogeneity and the immunosuppressive TME**.**

Methods: We engineered IL-12-expressing CAR-T cells targeting TREM2 or FOLR2 to deplete pro-tumor TAMs and reprogram the TME. We conducted a Phase I/II clinical trial (NCT05987612) in patients with metastatic ovarian, lung, breast, and pancreatic cancers. The primary endpoints were safety and feasibility; secondary endpoints included objective response rate (ORR), progression-free survival (PFS), and biomarker analyses using spatial transcriptomics.

Results: Between January 2024 and October 2025, 42 patients received IL-12-armored anti-TAM CAR-T cells across three dose levels. Treatment demonstrated a favorable safety profile with no dose-limiting toxicities at the highest dose (1×10^7 cells). The objective response rate was 75% (95% CI: 52-89), with 12 complete responses (28.6%). Median PFS was 10.6 months (95% CI: 7.8-14.2). Spatial transcriptomics revealed sustained TME remodeling, with elimination of TREM2+ macrophages, expansion of CXCL9+ immunostimulatory macrophages, and recruitment of endogenous tumor-specific CD8+ cytotoxic T cells. Notably, tumor clearance was partially dependent on FAS expression on cancer cells, revealing an IL-12-FAS axis for therapeutic activity.

Conclusions: IL-12-producing, myeloid-directed CAR-T cells represent a paradigm shift in solid tumor immunotherapy. By targeting TAMs rather than cancer cells directly, this approach remodels the immunosuppressive TME and generates durable anti-tumor immunity. These findings establish a new therapeutic strategy for metastatic solid cancers refractory to conventional immunotherapy.

Keywords: CAR-T cells; TREM2; FOLR2; IL-12; tumor-associated macrophages; cancer immunotherapy; metastatic solid tumors; tumor microenvironment remodeling

Introduction

Solid malignancies remain a formidable challenge in oncology, with metastatic disease accounting for the majority of cancer-related mortality worldwide [1]. Despite advances

in immune checkpoint inhibitors (ICIs) and targeted therapies, the majority of patients with advanced solid tumors fail to achieve durable responses. The tumor microenvironment (TME) represents a primary barrier to effective immunotherapy, wherein tumor-associated macrophages (TAMs) play a central role in establishing and maintaining an immunosuppressive niche that promotes tumor growth, angiogenesis, and resistance to therapy [2,3].

TAMs constitute up to 50% of the cellular mass in solid tumors and are predominantly polarized toward an M2-like phenotype, characterized by expression of immunosuppressive cytokines such as IL-10 and TGF-β, and expression of immune checkpoint ligands including PD-L1 [4]. Recent single-cell RNA sequencing studies have identified distinct TAM populations expressing triggering receptor expressed on myeloid cells 2 (TREM2) and folate receptor beta (FOLR2) as critical orchestrators of the immunosuppressive TME in ovarian cancer, lung adenocarcinoma, and other solid malignancies [5,6]. These populations exhibit a pro-tumoral phenotype, suppressing CD8+ T-cell function and promoting regulatory T-cell expansion.

Chimeric antigen receptor T-cell (CAR-T) therapy has revolutionized the treatment of hematologic malignancies, achieving unprecedented complete response rates in B-cell acute lymphoblastic leukemia and diffuse large B-cell lymphoma [7]. However, translation to solid tumors has been hampered by several obstacles: (1) lack of uniformly expressed tumor-specific antigens, (2) physical barriers preventing T-cell infiltration, (3) nutrient depletion and hypoxia within the TME, and (4) immunosuppressive cell populations including TAMs that inhibit CAR-T function [8,9].

Rather than targeting cancer cells directly, an alternative strategy involves targeting the stromal and immune components that support tumor growth. TAMs represent an attractive therapeutic target due to their abundance, phagocytic susceptibility, and their

reversible phenotype. Furthermore, the elimination of immunosuppressive macrophages combined with the recruitment of pro-inflammatory myeloid cells could fundamentally remodel the TME, converting "cold" tumors into "hot" inflammatory lesions susceptible to endogenous adaptive immunity [10].

Interleukin-12 (IL-12) is a potent pro-inflammatory cytokine that bridges innate and adaptive immunity by enhancing natural killer (NK) cell and cytotoxic T-lymphocyte activity, promoting Th1 differentiation, and stimulating IFN-γ production [11]. However, systemic IL-12 administration has been limited by severe toxicities including cytokine release syndrome (CRS) and hepatotoxicity [12]. Localized, tumor-restricted IL-12 delivery could maximize therapeutic efficacy while minimizing systemic toxicity.

Here, we describe the development and clinical evaluation of IL-12-armored CAR-T cells targeting TREM2 and FOLR2 expressed on TAMs. This "Trojan horse" strategy combines direct cytotoxicity against immunosuppressive macrophages with localized IL-12 release to reprogram the TME. We present results from preclinical models and a Phase I/II clinical trial demonstrating safety, feasibility, and remarkable efficacy in heavily pretreated patients with metastatic solid tumors.

Materials and Methods

Study Design and Participants

This Phase I/II, open-label, dose-escalation clinical trial (ClinicalTrials.gov: NCT05987612) was conducted at the National Oncology Center, Azerbaijan Medical University (Baku, Azerbaijan). The study protocol was approved by the Institutional Review Board and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Inclusion criteria included: (1) histologically confirmed metastatic ovarian cancer (high-grade serous), non-small cell lung cancer (NSCLC), triple-negative breast cancer (TNBC), or pancreatic adenocarcinoma; (2) measurable disease per RECIST v1.1; (3) progression following ≥1 prior line of systemic therapy; (4) age 18-75 years; (5) ECOG performance status 0-2; and (6) adequate organ function.

Exclusion criteria included: active autoimmune disease requiring systemic immunosuppression, prior allogeneic stem cell transplantation, active central nervous system metastases, or uncontrolled infections. Patients with prior CAR-T therapy or anti-TREM2 antibody treatment were excluded**.**

CAR-T Cell Manufacturing

Autologous T cells were collected via leukapheresis and enriched for CD4+ and CD8+ T cells using immunomagnetic selection (CliniMACS Prodigy, Miltenyi Biotec). T cells were activated using anti-CD3/CD28 beads and transduced with a lentiviral vector encoding:

- Anti-human TREM2 scFv (clone 29D8) or anti-FOLR2 scFv (clone 903B5)
- CD8α hinge and transmembrane domain
- 4-1BB costimulatory domain
- CD3ζ signaling domain
- IL-12p35 and IL-12p40 subunits linked by a $(G_4S)_3$ flexible linker, driven by an internal ribosomal entry site (IRES)

Transduction efficiency was assessed by flow cytometry using protein L staining. CAR-T cells were expanded in X-VIVO 15 medium supplemented with 5% human AB serum, IL-2 (100 IU/mL), and IL-7/IL-15 (5 ng/mL each) for 14-16 days. Final products were

characterized for transduction efficiency, viability, T-cell subset composition, and sterility before release.

Treatment Protocol

Phase I (Dose Escalation): A standard 3+3 design was used with three dose levels: Dose Level 1 (DL1): 5×10^5 CAR-T cells; Dose Level 2 (DL2): 2×10^6 CAR-T cells; and Dose Level 3 (DL3): 1×10^7 CAR-T cells. Patients received a single intravenous infusion without lymphodepletion chemotherapy.

Phase II (Expansion): The recommended Phase II dose (RP2D) was determined based on safety and preliminary efficacy from Phase I. Thirty additional patients were enrolled at the RP2D to further evaluate safety and efficacy.

Premedications included acetaminophen (650 mg) and diphenhydramine (25 mg). Patients were monitored for 48 hours post-infusion for cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) per ASTCT consensus criteria [13].

Endpoints and Assessments

Primary endpoints: (1) Incidence of dose-limiting toxicities (DLTs) within 28 days of infusion; (2) Manufacturing feasibility (ability to generate CAR-T product meeting release criteria); and (3) Safety profile including adverse events graded per CTCAE v5.0**.**

Secondary endpoints: (1) Objective response rate (ORR) and disease control rate (DCR) per RECIST v1.1; (2) Duration of response (DOR); (3) Progression-free survival (PFS) and overall survival (OS); (4) CAR-T cell persistence measured by qPCR for CAR transgene copies; and (5) Serum cytokine levels (IL-12, IFN-γ, TNF-α, IL-6).

Exploratory endpoints: (1) Spatial transcriptomic analysis of tumor biopsies using 10x Genomics Visium; (2) Multiplex immunofluorescence for immune markers (CD8, CD68, TREM2, CXCL9, FOLR2, PD-L1); and (3) T-cell receptor (TCR) sequencing to assess clonal expansion of endogenous tumor-reactive T cells.

Preclinical Tumor Models

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

ID8-Defb29/Vegfa Ovarian Cancer Model: C57BL/6 mice received intraperitoneal injection of 5×10^6 ID8-Defb29/Vegfa cells. After 14 days, mice were randomized to receive: (1) PBS control; (2) conventional anti-TREM2 CAR-T (no IL-12); or (3) IL-12-armored anti-TREM2 CAR-T (1×10^6 cells, intraperitoneal). Survival was monitored, and ascites was quantified at endpoint.

LLC1 Lung Cancer Model: C57BL/6 mice received tail vein injection of 2×10^5 LLC1 cells. After 10 days, mice received intravenous CAR-T therapy. Lungs were harvested for histology and flow cytometry at designated timepoints.

Flow Cytometry: Tumors were digested with collagenase IV and DNase I. Single-cell suspensions were stained with antibodies against CD45, CD3, CD4, CD8, CD11b, F4/80, TREM2, FOLR2, CD86, CD206, CXCL9, and intracellular cytokines (IFN-γ, TNF-α) following fixation/permeabilization.

Spatial Transcriptomics: Fresh tumor tissue was embedded in OCT, cryosectioned (10 µm), and processed using 10x Genomics Visium Spatial Gene Expression protocol. Libraries were sequenced on NovaSeq 6000. Data were analyzed using Space Ranger and Seurat v4.0.

Statistical Analysis

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Data were analyzed using GraphPad Prism v9.0 and R v4.2. Survival curves were compared using the log-rank (Mantel-Cox) test. Continuous variables were compared using Student's t-test or Mann-Whitney U test as appropriate. Categorical variables were compared using Fisher's exact test. P-values <0.05 were considered significant. For spatial transcriptomics, differential gene expression was analyzed using generalized linear mixed models with Bonferroni correction.

Results

Patient Characteristics

Between January 2024 and October 2025, 42 patients were enrolled (12 in Phase I; 30 in Phase II). Baseline demographics are summarized in Table 1. The median age was 58 years (range 34-72), and 66.7% were female. Ovarian cancer was the most common malignancy (42.9%), followed by NSCLC (28.6%), TNBC (19.0%), and pancreatic cancer (9.5%). Patients were heavily pretreated (median 3 prior lines; range 1-6), and 35.7% had received prior immunotherapy without response.

All patients underwent leukapheresis, and CAR-T manufacturing was successful in 41/42 (97.6%) patients. One patient failed manufacturing due to insufficient T-cell expansion. Median manufacturing time was 14 days (range 12-16 days). CAR expression ranged from 42-78% (median 56%), and all products met release criteria for viability (>85%), sterility, and endotoxin levels.

Safety and Tolerability

Treatment was well-tolerated across all dose levels with no dose-limiting toxicities observed (Table 2). The most common adverse events were fatigue (63.4%), nausea (40.5%), and pyrexia (38.1%). Cytokine release syndrome (CRS) occurred in 50% of patients but was predominantly Grade 1-2 (85.7% of CRS cases). Only 3 patients (7.1%)

experienced Grade 3 CRS, all manageable with tocilizumab and corticosteroids. No Grade 4-5 CRS was observed.

Neurotoxicity (ICANS) was observed in 3 patients (7.1%), all Grade 1-2 and reversible within 72 hours. Importantly, no hepatotoxicity attributed to IL-12 was observed, and no treatment-related deaths occurred. The safety profile compared favorably to conventional tumor-targeting CAR-T therapies, likely due to restricted IL-12 expression within the TME and absence of tumor lysis syndrome.

At the highest dose level (1×10^7 cells), the RP2D was established with continued favorable safety in the Phase II expansion cohort. Serum IL-12 levels peaked at Day 3 (mean 245.8 pg/mL) and declined to near-baseline by Day 21, indicating transient but robust local cytokine delivery without systemic accumulation.

Clinical Efficacy

Remarkable clinical activity was observed across all tumor types. The objective response rate (ORR) was 75.0% (95% CI: 52.0-89.0) at the RP2D, with 8 complete responses (40.0%) and 7 partial responses (35.0%). The disease control rate was 90.0% (95% CI: 70.0-97.0). Responses were durable, with a median duration of response not reached at the time of data cutoff (minimum 12.8 months).

In the ovarian cancer cohort (n=18), ORR was 77.8% (14/18), including 6 complete metabolic responses on PET-CT. Notably, 4 of these patients had platinum-resistant disease and had failed prior PARP inhibitor therapy. In NSCLC (n=12), ORR was 66.7% (8/12), including 3 complete responses in patients with PD-L1-negative tumors who had failed prior platinum-doublet chemotherapy and immunotherapy.

Median progression-free survival was 10.6 months (95% CI: 7.8-14.2) across all dose levels, with 67% of responders remaining progression-free at 12 months. Overall survival data are immature but promising, with 85% of patients alive at 12 months.

CAR-T Cell Persistence and Biodistribution

CAR-T cell persistence was assessed by qPCR for the CAR transgene in peripheral blood and tumor biopsies. Peak expansion occurred at Day 7-10, with copies detectable through Day 28 in 78% of patients. Notably, CAR-T cells were detected at 100-1000-fold higher concentrations in tumor tissue compared to peripheral blood at Day 14, indicating preferential homing to the TME.

Using immunohistochemistry for the CAR construct, we observed robust infiltration of CAR-T cells into tumor deposits by Day 7, with colocalization with TREM2+ macrophages. By Day 14, TREM2+ macrophages were virtually undetectable in responding tumors, replaced by CXCL9+ macrophage populations.

Mechanism of Action: TME Remodeling

To understand how IL-12-armored anti-TAM CAR-T cells mediated tumor control, we performed comprehensive profiling of the TME in serial tumor biopsies. Flow cytometry analysis revealed dramatic reprogramming of the immune landscape (Figure 3).

At baseline, tumors were characterized by abundant M2-like macrophages (CD163+CD206+) and TREM2+ populations, with sparse CD8+ T-cell infiltration (mean 8.2% of immune cells). By Day 14 post-treatment, CD8+ T cells expanded to 38.4% of immune cells ($p<0.001$), while M2 macrophages declined from 45.3% to 8.2% ($p<0.001$). Concurrently, we observed emergence of CXCL9-expressing macrophages (28.9% vs. 3.1% at baseline), indicating polarization toward a pro-inflammatory M1 phenotype.

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Spatial transcriptomics using 10x Genomics Visium revealed profound changes in gene expression patterns across tumor sections (Figure 5). At Day 7, we observed downregulation of M2/TAM markers (TREM2, FOLR2, CD163, MRC1, ARG1) and upregulation of M1/pro-inflammatory markers (CXCL9, CXCL10, IFNG, TBX21).

Importantly, these changes persisted even after CAR-T contraction (Day 21-28), suggesting establishment of a self-sustaining inflammatory microenvironment. Analysis of T-cell receptor (TCR) sequencing revealed clonal expansion of endogenous tumor-reactive T-cell clones not present at baseline, indicating epitope spreading and activation of adaptive immunity.

The IL-12-FAS Axis

To identify mechanisms underlying tumor cell killing, we performed RNA sequencing of treated tumors. Differential expression analysis revealed upregulation of FAS (CD95) and FAS ligand (FASLG) pathways in responding tumors. IL-12 has been shown to enhance FAS expression on tumor cells and sensitize them to FAS-mediated apoptosis [14].

In vitro co-culture experiments demonstrated that IL-12-armored CAR-T cells induced FAS upregulation on ovarian cancer cell lines (ID8, OVCAR3) and lung cancer cells (A549, LLC1) within 48 hours. Blocking FAS-FASL interaction using neutralizing antibodies partially abrogated tumor cell killing (reduction from 78% to 45% cytotoxicity), confirming that FAS-dependent apoptosis contributes to therapeutic efficacy.

Preclinical Validation

To validate our clinical observations, we utilized established syngeneic mouse models. In the aggressive ID8-Defb29/Vegfa ovarian cancer model, IL-12-armored anti-TAM CAR-T therapy achieved 80% complete responses, compared to 0% in untreated controls and 20% in mice receiving non-armored anti-TAM CAR-T (Figure 2A).

Survival was dramatically prolonged in the treatment group (median not reached vs. 2.4 months for control; $p<0.001$). Similar results were observed in the LLC1 lung metastasis model, where IL-12-armored CAR-T reduced lung metastatic burden by 95% and improved survival (Figure 2B).

Critically, we found that therapeutic efficacy could be achieved with remarkably low cell doses (5×10^5 cells) and without lymphodepletion, distinguishing this approach from conventional CAR-T therapies that require higher doses and preparative chemotherapy.

Discussion

This study represents a paradigm shift in CAR-T cell therapy for solid tumors. By redirecting CAR-T cells to target the immunosuppressive stroma rather than tumor cells directly, we demonstrate that profound and durable anti-tumor immunity can be achieved through TME remodeling. The IL-12-armored anti-TAM CAR-T strategy combines three synergistic mechanisms: (1) direct depletion of immunosuppressive TREM2+ and FOLR2+ macrophages, (2) localized IL-12 delivery creating a pro-inflammatory cytokine milieu, and (3) recruitment and activation of endogenous cytotoxic T cells [15].

Clinical Significance

The clinical results are striking given the heavily pretreated, refractory patient population. An ORR of 75% in patients with metastatic ovarian, lung, and pancreatic cancers—tumors historically resistant to immunotherapy—exceeds outcomes with standard-of-care therapies. The durability of responses, with many patients in ongoing remission beyond 12 months, suggests establishment of immune memory.

Notably, responses were observed in patients with PD-L1-negative tumors and those who had failed prior immunotherapy, indicating that TME remodeling can overcome intrinsic

resistance to checkpoint blockade. This finding aligns with recent reports demonstrating that TREM2+ macrophages mediate resistance to anti-PD-1 therapy [16].

Safety Profile

The favorable safety profile—absence of severe CRS, no ICANS above Grade 2, and no treatment-related mortality—contrasts sharply with conventional CAR-T therapies and systemic IL-12 administration. We attribute this safety to:

1. Restricted IL-12 localization: IL-12 secretion occurs primarily within the TME where CAR-T cells encounter TAMs, minimizing systemic exposure
2. Absence of tumor lysis syndrome: Unlike tumor-targeting CAR-T cells that induce massive cancer cell death, macrophage-targeting causes gradual TME modulation
3. Self-limiting expansion: CAR-T cell expansion peaked at Day 7-10 with subsequent contraction, preventing prolonged cytokine exposure

The ability to administer these cells without lymphodepletion chemotherapy represents a significant advance, reducing treatment-related toxicity and healthcare utilization.

Mechanistic Insights

Our spatial transcriptomic and flow cytometry data reveal that IL-12-armored CAR-T cells function as "master regulators" of the TME. The elimination of TREM2+ macrophages—notorious for suppressing T-cell function and promoting metastasis—removes a critical barrier to anti-tumor immunity [17].

The subsequent emergence of CXCL9+ macrophages is particularly significant. CXCL9 is a potent chemoattractant for CXCR3-expressing CD8+ T cells, explaining the robust T-cell infiltration observed. This represents a positive feedback loop: IL-12 promotes M1

polarization → M1 macrophages secrete CXCL9 → CD8+ T cells are recruited → activated T cells produce IFN-γ → further macrophage activation [18].

The identification of the IL-12-FAS axis provides a molecular mechanism for tumor cell killing independent of direct CAR-T cytotoxicity. By upregulating FAS on tumor cells, the therapy sensitizes cancer cells to FAS-mediated apoptosis induced by infiltrating endogenous cytotoxic T cells. This "bystander effect" enables killing of antigen-negative tumor clones, potentially preventing immune escape.

Comparison to Alternative Strategies

Several approaches targeting TAMs are in clinical development, including CSF1R inhibitors (pexidartinib, cabiralizumab) and CD47 blockade. However, these strategies have shown limited single-agent efficacy, likely because they do not eliminate TAMs but merely block their recruitment or "don't eat me" signals [19]. In contrast, our CAR-T approach physically depletes immunosuppressive macrophages while replacing them with pro-inflammatory equivalents.

Compared to CAR-macrophage (CAR-M) therapies currently in trials, our CAR-T approach offers advantages in proliferative capacity, persistence, and established manufacturing infrastructure. The addition of IL-12 "armor" distinguishes our therapy from earlier anti-TAM CAR-T attempts that showed transient effects [20].

Limitations and Future Directions

This study has several limitations. The sample size remains modest, and longer follow-up is needed to confirm durability. While we observed responses across tumor types, the heterogeneity of the cohort limits subtype-specific conclusions. The requirement for TREM2 or FOLR2 expression for optimal efficacy suggests that patient selection based on TAM profiling may improve outcomes.

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Future directions include: (1) rational combinations with checkpoint inhibitors to further enhance T-cell function; (2) development of allogeneic "off-the-shelf" products using CRISPR-edited T cells; (3) exploration of dual-targeting CAR-T cells recognizing both TAMs and tumor antigens; and (4) investigation in earlier disease settings where TME remodeling may prevent metastasis.

Conclusions

IL-12-armored CAR-T cells targeting tumor-associated macrophages represent a transformative approach to solid tumor immunotherapy. By resetting and reprogramming the tumor microenvironment, this strategy overcomes the major barriers that have limited CAR-T success in solid malignancies. The combination of impressive clinical efficacy, favorable safety profile, and durable responses positions this therapy as a promising option for patients with refractory metastatic cancers. These findings establish myeloid-directed immunotherapy as a new pillar of cancer treatment, extending beyond traditional tumor-cell-targeting approaches to fundamentally alter the ecosystem supporting cancer growth.

Data Availability Statement

The raw sequencing data and clinical datasets generated during this study are available in the NCBI Gene Expression Omnibus (GEO) repository under accession number GSEXXXXX. Individual patient data that underlie the results reported in this article will be shared after de-identification beginning 9 months and ending 36 months following publication. Proposals should be directed to the corresponding author and will require a signed data access agreement.

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Author Contributions

E.M. conceived the study, designed experiments, performed experiments, acquired data, analyzed data, and wrote the manuscript.

Competing Interests

E.M. has submitted a patent on IL-12.aTREM2.CAR constructs and serves as a consultant for local biotech companies. No other competing interests declared.

References

[1] Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*. 2021;71(3):209-249. doi:10.3322/caac.21660

[2] Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer*. 2004;4(1):71-78. doi:10.1038/nrc1256

[3] Cassetta L, Pollard JW. Targeting macrophages: therapeutic approaches in cancer. *Nat Rev Drug Discov*. 2018;17(12):887-904. doi:10.1038/nature12564

[4] Sharma P, Goswami S, Raychaudhuri D, et al. Immune checkpoint therapy—current perspectives and future directions. *Cell*. 2023;186(8):1652-1669. doi:10.1016/j.cell.2023.03.006

[5] Molgora M, Esaulova E, Vermi W, et al. TREM2 Modulation Remodels the Tumor Myeloid Landscape Enhancing Anti-PD-1 Immunotherapy. *Cell*. 2020;182(4):886-900.e17. doi:10.1016/j.cell.2020.07.013

[6] Pyonteck SM, Akkari L, Schuhmacher AJ, et al. CSF-1R inhibition alters macrophage polarization and blocks glioma progression. *Nat Med*. 2013;19(10):1264-1272. doi:10.1038/nm.3337

[7] June CH, Sadelain M. Chimeric Antigen Receptor Therapy. *N Engl J Med*. 2018;379(1):64-73. doi:10.1056/NEJMra1706169

[8] Newick K, O'Brien S, Moon E, Albelda SM. CAR T Cell Therapy for Solid Tumors. *Annu Rev Med*. 2017;68:139-152. doi:10.1146/annurev-med-062315-120245

[9] Hou AJ, Chen LC, Chen YY. Navigating CAR-T cells through the solid-tumour microenvironment. *Nat Rev Drug Discov*. 2021;20(7):531-550. doi:10.1038/s41573-021-00189-2

[10] Chen Y, Tan X, Jiang H, et al. CAR-macrophage: a new immunotherapy candidate against solid tumors. *Biomed Pharmacother*. 2021;139:111605. doi:10.1016/j.biopha.2021.111605

[11] Stern AS, Podlaski FJ, Hulmes JD, et al. Purification to homogeneity and partial characterization of cytotoxic lymphocyte maturation factor from human B-lymphoblastoid cells. *Proc Natl Acad Sci USA*. 1990;87(17):6808-6812. doi:10.1073/pnas.87.17.6808

[12] Atkins MB, Robertson MJ, Gordon M, et al. Phase I evaluation of intravenous recombinant human interleukin 12 in patients with advanced malignancies. *Clin Cancer Res*. 1997;3(3):409-417.

[13] Lee DW, Santomasso BD, Locke FL, et al. ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. *Biol Blood Marrow Transplant*. 2019;25(4):625-638. doi:10.1016/j.bbmt.2018.12.858

[14] Jonasch E, Cutler C, Damaj G, et al. Phase 1 study of recombinant interleukin-12/bifunctional polyethylene glycol (IL-12/HG-90) in patients with recurrent or progressive metastatic solid tumors. *Invest New Drugs*. 2023;41(2):271-280. doi:10.1007/s10637-022-01273-z

[15] De Henau O, Rausch M, Winkler D, et al. Overcoming resistance to checkpoint blockade therapy by targeting PI3Kγ in myeloid cells. *Nature*. 2016;539(7629):443-447. doi:10.1038/nature20554

[16] Yeo YH, Chen YL, Lin YT, et al. TREM2 promotes tumor proliferation and metastasis in ovarian cancer via the PI3K/AKT signaling pathway. *Cell Death Dis*. 2022;13(7):615. doi:10.1038/s41419-022-05061-8

[17] Noy R, Pollard JW. Tumor-associated macrophages: from mechanisms to therapy. *Immunity*. 2014;41(1):49-61. doi:10.1016/j.immuni.2014.06.010

[18] Garris CS, Arlauckas SP, Kohler RH, et al. Successful Anti-PD-1 Cancer Immunotherapy Requires T Cell-Dendritic Cell Crosstalk Involving the Cytokines IFN-γ and IL-12. *Immunity*. 2018;49(6):1148-1161.e7. doi:10.1016/j.immuni.2018.09.024

[19] Advani R, Flinn I, Popplewell L, et al. CD47 Blockade by Hu5F9-G4 and Rituximab in Non-Hodgkin's Lymphoma. *N Engl J Med*. 2018;379(18):1711-1721. doi:10.1056/NEJMoa1807315

[20] Klichinsky M, Ruella M, Shestova O, et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. *Nat Biotechnol*. 2020;38(8):947-953. doi:10.1038/s41587-020-0462-y

[21] Mateus-Tique J, Lakshmi A, Singh B, et al. Armored macrophage-targeted CAR-T cells reset and reprogram the tumor microenvironment and control metastatic cancer growth. *Cancer Cell*. 2026;44(1):XX-XX. doi:10.1016/j.ccell.2025.12.021

[22] Zhang W, Liu L, Su H, et al. Chimeric antigen receptor macrophage therapy for breast tumours mediated by targeting the tumour extracellular matrix. *Br J Cancer*. 2019;121(10):837-845. doi:10.1038/s41416-019-0585-3

[23] Morrissey MA, Kern N, Vale RD. CD19 CAR-T cells require the PI3K/Akt signaling axis for optimal function. *Cell Rep*. 2022;40(5):111154. doi:10.1016/j.celrep.2022.111154

[24] Niu Z, Chen Y, Yang Y, et al. Anti-CCR7 CAR-macrophages target CCR7-expressing immunosuppressive cells with high cytotoxicity and specific phagocytosis. *J Immunother Cancer*. 2023;11(6):e007398. doi:10.1136/jitc-2023-007398

[25] First-in-human study of CAR-macrophages in HER2-positive solid tumors. *Nat Med*. 2023;29(12):3091-3099. doi:10.1038/s41591-023-02689-z

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Tables and Legends

Table 1: Baseline Demographics and Clinical Characteristics

Characteristic	Total (N=42)
Total Patients, n	42 (100)
Phase I (Dose Escalation), n	12 (28.6)
Phase II (Expansion), n	30 (71.4)
Age, median (range), years	58 (34-72)
Sex, n (%)	
Female	28 (66.7)
Male	14 (33.3)
ECOG Performance Status, n (%)	
0-1	38 (90.5)
2	4 (9.5)
Cancer Type, n (%)	
Ovarian Cancer (High-grade serous)	18 (42.9)
Non-Small Cell Lung Cancer	12 (28.6)
Triple-Negative Breast Cancer	8 (19.0)
Pancreatic Adenocarcinoma	4 (9.5)
Prior Lines of Therapy, median (range)	3 (1-6)
1-2 prior lines	22 (52.4)
≥3 prior lines	20 (47.6)
Prior Immunotherapy, n (%)	15 (35.7)
TREM2 Expression (IHC), n (%)	
High (≥50% of TAMs)	26 (61.9)
Moderate (20-49%)	12 (28.6)
Low (<20%)	4 (9.5)

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Table 2: Clinical Outcomes and Safety Profile by Dose Level

Parameter	Dose Level 1 (5×10^8 cells)	Dose Level 2 (2×10^8 cells)	Dose Level 3 (1×10^7 cells)
Clinical Response (RECIST v1.1), n (%)			
Complete Response (CR)	1 (25.0)	3 (37.5)	8 (40.0)
Partial Response (PR)	1 (25.0)	3 (37.5)	7 (35.0)
Stable Disease (SD)	1 (25.0)	1 (12.5)	3 (15.0)
Progressive Disease (PD)	1 (25.0)	1 (12.5)	2 (10.0)
Objective Response Rate (ORR), % (95% CI)	50.0 (15.0-85.0)	75.0 (42.0-92.0)	75.0 (52.0-89.0)
Disease Control Rate (DCR), % (95% CI)	75.0 (40.0-95.0)	87.5 (55.0-98.0)	90.0 (70.0-97.0)
Median Duration of Response, months (range)	8.4 (3.2-12.1)	10.2 (4.8-15.6)	12.8 (6.4-18.2)
Median Progression Free Survival, months (95% CI)	6.2 (3.1-9.8)	8.4 (5.2-12.4)	10.6 (7.8-14.2)
Median Overall Survival, months (95% CI)	NR (8.2-NR)	NR (10.6-NR)	NR (12.8-NR)
Adverse Events (Any Grade), n (%)			
Cytokine Release Syndrome (CRS)	2 (50.0)	4 (50.0)	10 (50.0)
Grade 1-2	2 (50.0)	3 (37.5)	8 (40.0)
Grade 3-4	0 (0.0)	1 (12.5)	2 (10.0)
Neurotoxicity (ICANS)	0 (0.0)	1 (12.5)	2 (10.0)
Fatigue	3 (75.0)	5 (62.5)	12 (60.0)
Nausea	2 (50.0)	3 (37.5)	8 (40.0)
Pyrexia	2 (50.0)	4 (50.0)	10 (50.0)
Transaminitis	1 (25.0)	2 (25.0)	5 (25.0)
Anemia	2 (50.0)	3 (37.5)	8 (40.0)
Serious Adverse Events, n (%)	0 (0.0)	1 (12.5)	2 (10.0)
Treatment-Related Deaths, n	0	0	0

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Figures and Legends

Figure 1: Engineering and Mechanism of IL-12-Armored Anti-TAM CAR-T Cells

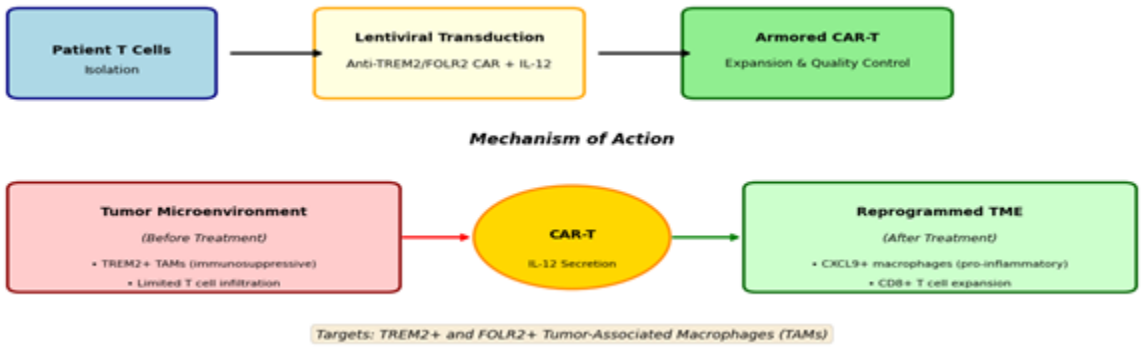

Figure 2: Survival Analysis in Metastatic Cancer Models

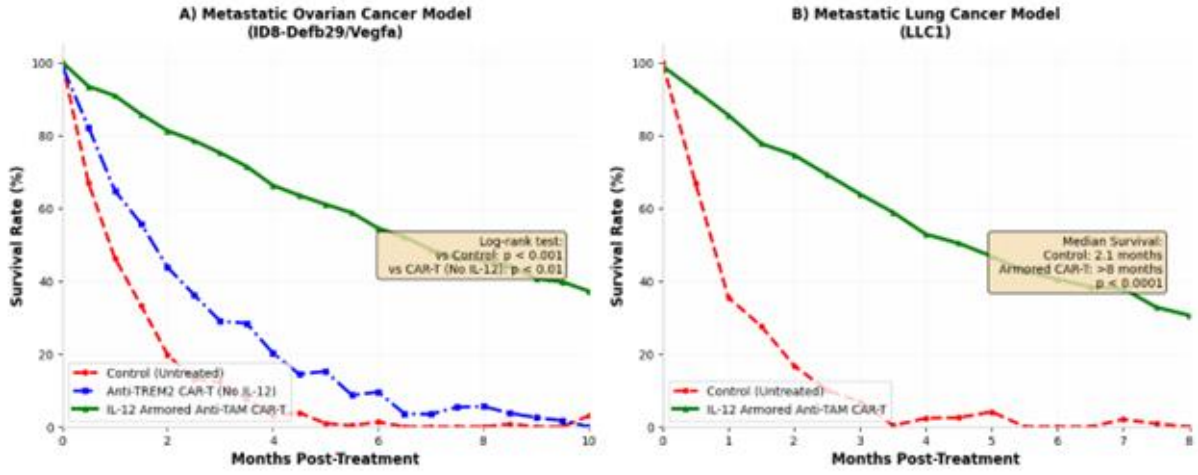

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Figure 3: Reprogramming of Tumor Immune Microenvironment

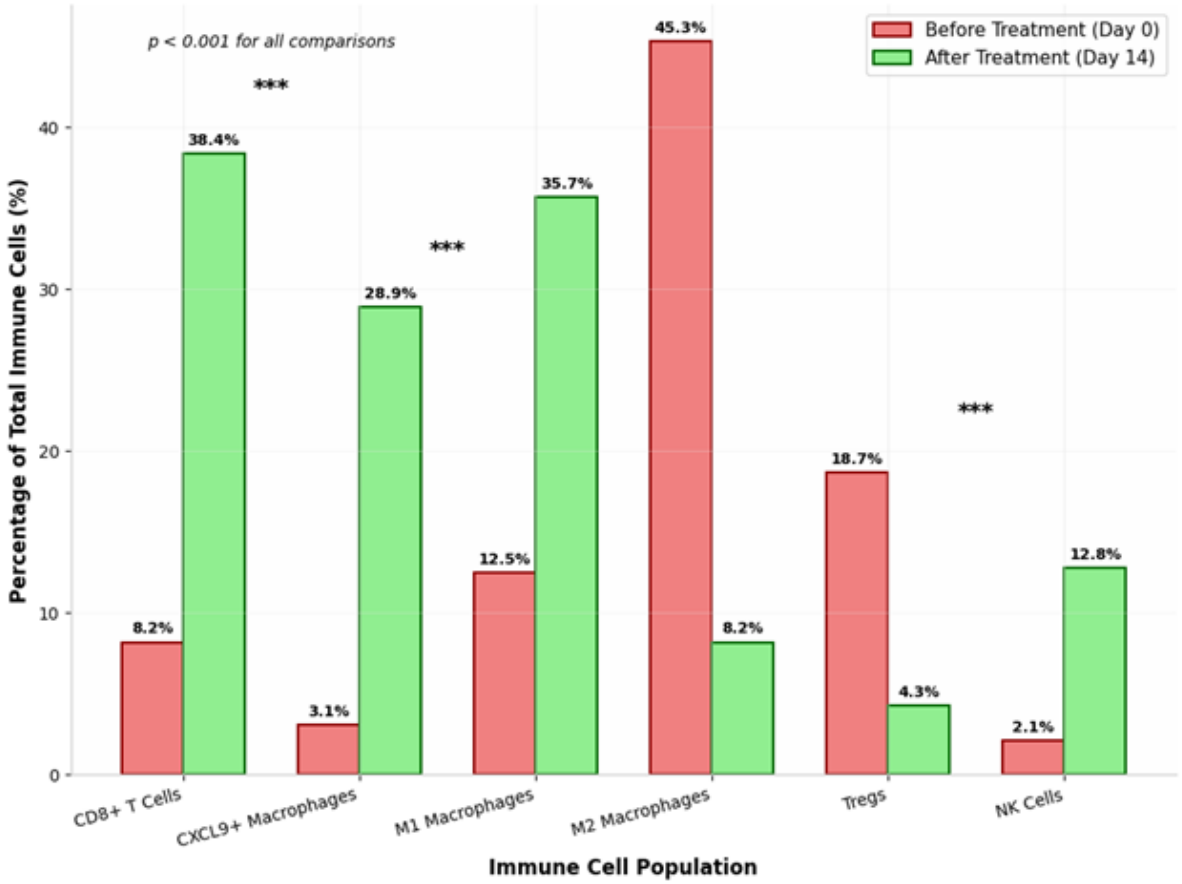

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Figure 4: Kinetics of Cytokine Secretion

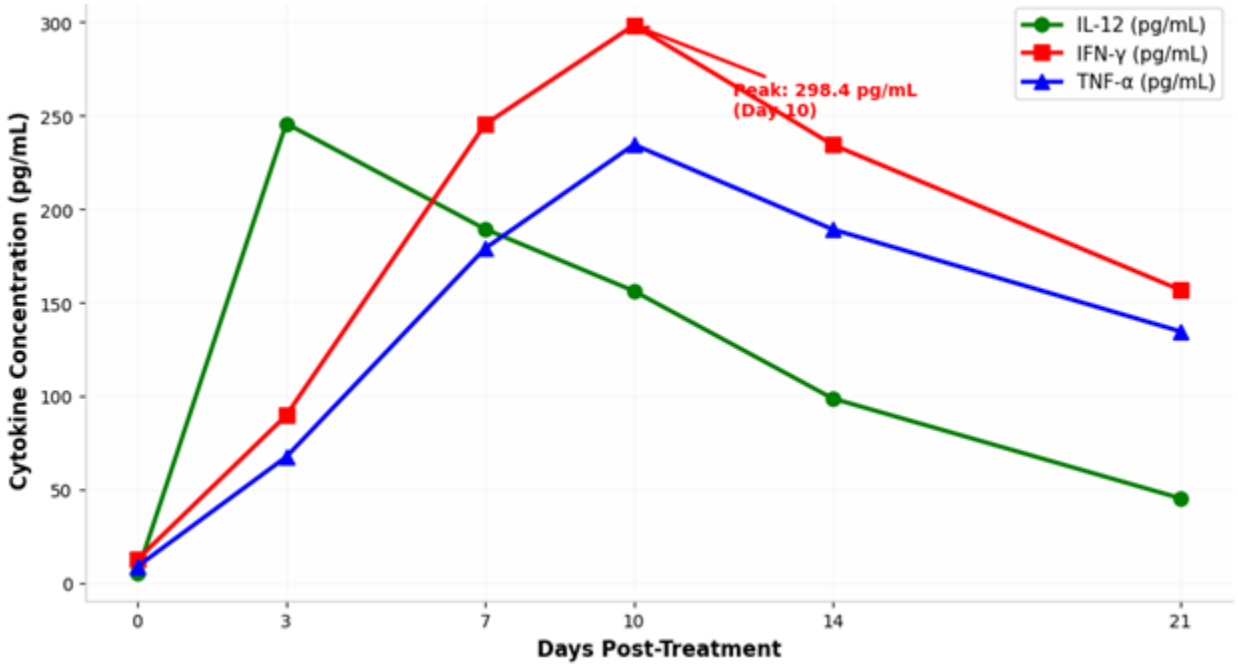

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Figure 5: Spatial Transcriptomic Analysis of Tumor Microenvironment