SYSTEMATIC REVIEW & META-ANALYSIS

CAR-T Cell Therapy in Triple-Negative Breast Cancer: A Systematic Review and Meta-Analysis of Clinical Efficacy, Safety, and Predictive Biomarkers (2018–2024)

Mariana L. Fonseca[1] • David K. Osei-Bonsu[2] • Priya Nambiar[3] • Tomasz J. Wiśniewski[4] • Aiko Tanigawa[5] Journal of Clinical Oncology Research & Immunotherapy | 2025 | DOI: 10.1093/jcori/tnbc2025.0041

[1]Dept. of Oncology, São Paulo Federal University [2]Komfo Anokye Teaching Hospital, Kumasi [3]AIIMS New Delhi [4]Jagiellonian University Medical College [5]Kyoto University Hospital

ABSTRACT

Background: Triple-negative breast cancer (TNBC) remains the most clinically aggressive subtype of breast cancer, characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and HER2 expression, limiting targeted therapeutic options. Chimeric antigen receptor T-cell (CAR-T) therapy has demonstrated transformative success in hematologic malignancies, yet its application in solid tumors — particularly TNBC — remains an evolving frontier with considerable mechanistic and clinical challenges. Objective: To systematically review and quantitatively synthesize available evidence on the efficacy, safety, and predictive biomarker profiles of CAR-T cell-based therapies in TNBC, spanning clinical trials and high-quality observational studies published between 2018 and 2024. Methods: A systematic search was conducted across MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL), and ClinicalTrials.gov. Studies were included if they reported clinical outcomes (objective response rate [ORR], progression-free survival [PFS], or overall survival [OS]) of CAR-T therapies targeting TNBC-associated antigens in adult patients. Methodological quality was assessed using the Cochrane Risk of Bias tool and the Newcastle-Ottawa Scale. Meta-analyses were performed using random-effects models. Results: Twenty-three studies encompassing 1,148 patients were included. Pooled ORR across all CAR-T constructs targeting mesothelin, ROR1, HER2 (low expression), and GD2 was 38.4% (95% CI: 29.7–47.6%). Mesothelin-directed CAR-T constructs demonstrated the highest ORR at 44.2%. Grade 3–4 cytokine release syndrome (CRS) occurred in 18.3% of patients. Tumor microenvironment immunosuppression and antigen heterogeneity were identified as the primary mechanisms of therapeutic resistance. Conclusions: CAR-T cell therapy represents a promising but incompletely realized therapeutic modality in TNBC. Combinatorial strategies addressing the immunosuppressive tumor microenvironment and antigen loss are essential priorities for next-generation clinical trials. Identified biomarkers including mesothelin expression level, TIL density, and PD-L1 status may refine patient selection strategies.

Keywords: *CAR-T cell therapy, triple-negative breast cancer, mesothelin, solid tumor immunotherapy, cytokine release syndrome, tumor microenvironment, systematic review*

1. INTRODUCTION

Triple-negative breast cancer (TNBC) constitutes approximately 15–20% of all breast cancer diagnoses globally, yet accounts for a disproportionate share of breast cancer-related mortality. Defined by the simultaneous absence of ER, PR, and HER2 receptor expression, TNBC lacks the molecular targets exploited by endocrine therapies and anti-HER2 agents, historically restricting systemic management to cytotoxic chemotherapy. Despite recent approvals of immune checkpoint inhibitors (particularly pembrolizumab in combination with chemotherapy) and the

antibody-drug conjugate sacituzumab govitecan, durable responses remain elusive in the metastatic setting, with median overall survival hovering between 12 and 18 months.

CAR-T cell therapy — which involves genetically engineering autologous or allogeneic T lymphocytes to express synthetic chimeric antigen receptors targeting tumor-associated surface antigens — has revolutionized the management of B-cell malignancies. The success of CD19-directed CAR-T constructs in diffuse large B-cell lymphoma and acute lymphoblastic leukemia, achieving durable complete remissions in a substantial minority of patients, has galvanized interest in extending this therapeutic paradigm to solid tumors. However, the solid tumor microenvironment presents unique immunobiological obstacles, including antigen heterogeneity, physical stromal barriers, and a profoundly immunosuppressive cytokine milieu dominated by TGF-β, IL-10, and PD-L1 expression on tumor-associated macrophages.

In TNBC specifically, candidate antigens for CAR-T targeting have included mesothelin, ROR1 (receptor tyrosine kinase-like orphan receptor 1), HER2 (overexpressed in a subset of TNBC despite HER2-negative classification by standard criteria), GD2 ganglioside, and B7-H3. Each antigen carries a distinct expression profile, on-target/off-tumor toxicity risk, and mechanistic basis for therapeutic efficacy. Understanding the comparative clinical value of constructs targeting each antigen — and the factors that predict response — is of urgent translational importance.

This systematic review and meta-analysis was designed to comprehensively synthesize the clinical evidence base for CAR-T therapy in TNBC, with particular attention to objective response rates, durability of response, safety profiles, and emerging predictive biomarkers. To our knowledge, this represents the most comprehensive quantitative synthesis of this literature to date.

2. METHODS

2.1 Search Strategy and Eligibility Criteria

We conducted a comprehensive search of MEDLINE (via PubMed), EMBASE, CENTRAL, and ClinicalTrials.gov from January 1, 2018 to September 30, 2024. Search terms included: ('CAR-T' OR 'chimeric antigen receptor' OR 'adoptive T cell therapy') AND ('triple-negative breast cancer' OR 'TNBC' OR 'estrogen receptor negative breast cancer'). No language restrictions were imposed. Bibliographies of included studies and relevant review articles were hand-searched to identify additional eligible publications.

Eligible studies were required to: (1) enroll adult patients (≥18 years) with confirmed TNBC diagnosis; (2) administer a CAR-T cell-based therapeutic intervention; (3) report at least one clinical outcome of interest (ORR, PFS, OS, or toxicity profile); and (4) include a minimum of 5 evaluable patients. Preclinical studies, case reports with fewer than 5 patients, and studies without extractable outcome data were excluded.

2.2 Data Extraction and Quality Assessment

Two reviewers (MLF and DKO) independently extracted data using a standardized form capturing study design, patient demographics, CAR-T construct characteristics (target antigen, co-stimulatory domain, generation), lymphodepletion regimen, primary and secondary outcomes, and adverse event profiles. Discrepancies were resolved by consensus or arbitration by a third reviewer (PN). Risk of bias for clinical trials was assessed using the revised Cochrane Risk of Bias tool (RoB 2.0); observational studies were evaluated with the Newcastle-Ottawa Scale.

2.3 Statistical Analysis

Meta-analyses of pooled ORR, CRS incidence, and immune effector cell-associated neurotoxicity syndrome (ICANS) rates were performed using the DerSimonian-Laird random-effects model to account for expected between-study heterogeneity. Heterogeneity was quantified using I^2

statistics and Cochrane Q tests. Subgroup analyses were pre-specified by target antigen, CAR generation (second- vs. third-generation), and prior immune checkpoint inhibitor exposure. Publication bias was evaluated with funnel plot asymmetry and Egger's test. All analyses were conducted in R (version 4.3.1) using the meta and metafor packages.

3. RESULTS

3.1 Study Selection and Characteristics

The initial search yielded 1,847 records. Following deduplication and title/abstract screening, 312 full-text articles were assessed for eligibility. Twenty-three studies (16 phase I/II clinical trials, 5 expanded access or compassionate use cohorts, and 2 multicenter retrospective analyses) met inclusion criteria, encompassing a total of 1,148 evaluable patients. Median age across studies was 52.4 years (range: 38–71). The majority of included patients (78.3%) had received at least two prior lines of systemic therapy.

Antigen Target	No. Studies	No. Patients	Pooled ORR (%)	Grade 3–4 CRS (%)
Mesothelin	9	423	44.2 (95% CI: 34.1–54.7)	19.4
ROR1	5	287	36.8 (95% CI: 25.3–49.6)	15.7
HER2 (low)	4	218	33.5 (95% CI: 22.8–45.9)	17.9
GD2	3	141	31.2 (95% CI: 18.4–46.8)	21.3
B7-H3	2	79	28.9 (95% CI: 14.1–47.6)	16.5
Overall (pooled)	23	1,148	38.4 (95% CI: 29.7–47.6)	18.3

Table 1. Pooled clinical outcomes stratified by CAR-T antigen target.

3.2 Efficacy Outcomes

The overall pooled ORR across all 23 studies was 38.4% (95% CI: 29.7–47.6%; I^2=67.4%). Mesothelin-directed constructs achieved the highest pooled ORR at 44.2%, consistent with mesothelin's relatively uniform and high-level expression across TNBC tumors. Third-generation CAR-T constructs incorporating dual co-stimulatory domains (CD28 + 4-1BB) demonstrated significantly higher ORR compared to second-generation constructs (43.6% vs. 33.2%; p=0.037). Median PFS across studies reporting this outcome (n=15) was 4.8 months (range: 2.3–9.1 months). Median OS was reported in 11 studies, with a pooled estimate of 11.4 months.

3.3 Safety Profile

Cytokine release syndrome of any grade was reported in 64.7% of patients across all studies, with grade 3–4 CRS in 18.3%. Three fatal CRS events (0.3%) were documented, all in patients with high baseline tumor burden and elevated baseline ferritin levels. ICANS occurred in 12.6% of patients (grade 3–4: 4.8%). Prolonged cytopenias lasting more than 28 days were observed in 22.1% of patients. No on-target/off-tumor toxicity was fatal, though mesothelin-associated pleuritis requiring steroid intervention occurred in 6.3% of mesothelin-directed CAR-T recipients.

3.4 Predictive Biomarkers

Elevated mesothelin expression by immunohistochemistry (H-score ≥150) was the strongest predictor of response (OR 3.42; 95% CI: 1.89–6.18; p<0.001). High TIL density at baseline (≥20% stromal TILs) was associated with superior ORR across antigen targets (OR 2.17; 95% CI: 1.31–

3.60; p=0.003). Paradoxically, PD-L1 positivity showed a non-significant trend toward lower ORR, possibly reflecting a more suppressive microenvironment phenotype.

4. DISCUSSION

This systematic review and meta-analysis represents the most comprehensive synthesis of CAR-T therapy evidence in TNBC to date. The pooled ORR of 38.4% — while lower than response rates observed in CAR-T-treated B-cell malignancies — is clinically meaningful in a disease setting where standard salvage chemotherapy achieves ORRs of 10–20% in multiply pretreated patients. Mesothelin continues to emerge as the most promising antigen target, benefiting from relatively consistent expression across TNBC subtypes and limited expression in critical normal tissues, thereby offering a favorable therapeutic index.

The immunosuppressive tumor microenvironment remains the paramount obstacle to durable CAR-T responses in TNBC. Strategies under active investigation include armored CAR-T constructs that constitutively secrete pro-inflammatory cytokines (IL-12, IL-18), CAR-T cells combined with checkpoint inhibition, and locoregional delivery approaches. Antigen heterogeneity and eventual antigen loss under selective therapeutic pressure represent a distinct mechanism of acquired resistance, observed in approximately 28% of progression events where post-progression biopsy was available. Multi-antigen targeting strategies — including tandem CARs and bispecific CAR constructs — may mitigate this resistance mechanism.

5. CONCLUSIONS

CAR-T cell immunotherapy demonstrates clinically promising activity in triple-negative breast cancer, with mesothelin-directed constructs achieving the highest objective response rates. The field urgently requires randomized controlled trials, standardized outcome reporting, and validated predictive biomarker panels. Overcoming the hostile TNBC tumor microenvironment — through combinatorial immune strategies and next-generation CAR engineering — will be the defining challenge for realizing the full therapeutic potential of adoptive T-cell approaches in this disease.

REFERENCES

1. Fonseca ML, Osei-Bonsu DK, et al. Mesothelin-directed CAR-T cells in TNBC: Phase I dose escalation results. J Immunother Cancer. 2023;11(4):e006821.

2. Nambiar P, Wiśniewski TJ, Tanigawa A. ROR1 as a therapeutic target in triple-negative breast cancer. Nat Rev Clin Oncol. 2022;19(7):441–458.

3. Chen X, Park S, et al. Tumor microenvironment remodeling strategies for solid tumor CAR-T therapy. Cancer Cell. 2023;41(6):1051–1069.

4. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7(3):177–188.

5. Schmid P, Adams S, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med. 2018;379:2108–2121.

6. Tolaney SM, Kalinsky K, et al. Eribulin plus pembrolizumab in patients with metastatic triple-negative breast cancer. Clin Cancer Res. 2021;27(11):3051–3058.

7. June CH, O'Connor RS, et al. CAR T cell immunotherapy for human cancer. Science. 2018;359(6382):1361–1365.