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*Original Research*

# Nectin4 as a Promising Target for CAR-NK Cell Therapy in Breast Cancer Treatment

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**Abstract**

**Background:** Breast cancer continues to be a major global health burden, with a significant proportion of patients developing metastatic or treatment-resistant disease. Novel therapeutic strategies are urgently needed. Nectin cell adhesion molecule 4 (Nectin4), an adhesion molecule overexpressed in aggressive breast cancer subtypes, represents a promising target for immunotherapy due to its restricted expression in normal tissues. This study investigated the therapeutic potential of Nectin4-directed chimeric antigen receptor natural killer (CAR-NK) cell therapy against triple-negative breast cancer (TNBC) at the preclinical stage. **Methods:** Human natural killer (NK) cells obtained from peripheral blood from healthy donors were engineered to express an anti-Nectin4 chimeric antigen receptor (CAR) via lentiviral transduction, expanded *ex vivo*, and then used for functional studies. Cytotoxic activity was assessed *in vitro* against Nectin4-high-expressing breast cancer cells using luciferase-based killing assays at various effector-to-target ratios. *In vivo* efficacy was evaluated in NOD/SCID/IL2rγnull (NSG) mice bearing subcutaneous breast cancer tumors treated with CAR-NK cells, compared to untransduced NK cells and phosphate-buffered saline controls. Tumor growth was monitored using bioluminescence imaging and harvested tumors were subsequently analyzed for Nectin4 expression using immunohistochemistry. **Results:** CAR-NK cells potently and specifically lysed Nectin4-positive breast cancer cells *in vitro*, with up to 95% target cell lysis at an effector-to-target ratio of 5:1. *In vivo*, CAR-NK treatment reduced tumor burden and prolonged survival compared with controls. Immunohistochemical analysis revealed a marked reduction in Nectin4 expression within tumors from the CAR-NK-treated group, consistent with targeted tumor cell elimination. **Conclusions:** CAR-NK cells targeting Nectin4 demonstrated potent cytotoxic activity *in vitro* and effectively suppressed tumor growth *in vivo*, supporting the further evaluation of Nectin4 as a therapeutic target for CAR-NK-based immunotherapy in TNBC, particularly for aggressive subtypes with limited treatment options.

**Keywords:** breast cancer; Nectin4; CAR-NK cell therapy; immunotherapy; targeted therapy

## 1. Introduction

Breast cancer remains a leading global health threat among women. Globally, it has overtaken lung cancer as the most frequently diagnosed female malignancy, accounting for roughly one-quarter of all female cancer cases and ranking among the top causes of cancer-related deaths [1]. Despite advances in early detection and multimodal therapies—including surgery, chemotherapy, and targeted agents like human epidermal growth factor receptor 2 (HER2) inhibitors [2]—metastatic or treatment-resistant disease persists in about 30% of patients [3]. Triple-negative breast cancer (TNBC) comprises approximately 10%–20% of all breast cancers and is particularly common in women under 40 years of age. This aggressive cancer lacks the receptors targeted by current therapies, thereby restricting treatment options. It also carries substantial risks of recurrence and distant metastasis, which contribute to poor survival outcomes, thus underscoring the need for novel therapeutic strategies [4,5].

Chimeric antigen receptor (CAR)-engineered immune cells have reshaped cancer therapy by enabling tumor-specific precision. CAR-T cell therapy, in which CARs act as synthetic receptors that redirect lymphocytes (primarily T cells) to recognize and eliminate target antigen-expressing cells, has achieved remarkably effective and durable clinical responses. Despite their notable success in hematologic malignancies, CAR-T cells encounter major hurdles in solid tumor settings, such as on-target/off-tumor toxicity and an immunosuppressive microenvironment [6]. Compared to CAR-T cells, CAR-natural killer (NK) cells offer inherent tumor homing, reduced risk of severe cytokine release syndrome, and allogeneic "off-the-shelf" feasibility. As innate effectors, NK cells naturally eliminate cells with downregulated human leukocyte antigen (HLA) class I or stress markers, while CAR engineering

provides antigen-specific precision. Allogeneic NK cells require no full HLA matching for safe use, avoiding individualized CAR production and showing proven safety in adoptive cancer immunotherapy [7].

Nectin Cell Adhesion Molecule 4 (Nectin4), an immunoglobulin superfamily adhesion molecule, is frequently overexpressed in aggressive breast cancer subtypes, particularly triple-negative and HER2-positive tumors, where it promotes metastasis and correlates with poor patient prognosis [8]. A study has validated Nectin4 as both a biologically significant and therapeutically targetable molecule in breast cancer [9]. Its expression in normal adult tissues is largely restricted to skin, bladder, and esophagus, making it an attractive and relatively tumor-specific target for immunotherapy [10]. The therapeutic potential of targeting Nectin4 is exemplified by the antibody-drug conjugate enfortumab vedotin, which has shown clinical activity in cancers expressing Nectin4 [11]. Building on this, we hypothesize that redirecting NK cell cytotoxicity via a Nectin4-specific CAR could provide a potent and targeted approach for breast cancer treatment. This work aimed to evaluate Nectin4-targeted CAR-NK cells against TNBC, assessing their efficacy both *in vitro* and *in vivo*.

## 2. Materials and Methods

### *2.1 Isolation and Transduction of NK Cells*

This study involving human samples was conducted in accordance with the Declaration of Helsinki. The ethics approval number for this study is KY-2024-231-01, issued by the Ethics Committee of the Affiliated Huaian No.1 People's Hospital of Nanjing Medical University. Informed consent was obtained from all healthy donors. Peripheral blood mononuclear cells (PBMCs) were separated from healthy donor leukapheresis products via Ficoll-Paque PLUS density gradient centrifugation at the Affiliated Huaian No.1 People's Hospital of Nanjing Medical University, Huaian, in 2024. NK cells were subsequently enriched using an NK cell isolation kit (Stemcell Technologies) and cultured in serum-free KBM581 medium supplemented with 5% human AB serum, 500 IU/mL recombinant human IL-2, and 20 ng/mL IL-15. After 4 days of activation, cells were transduced via a lentiviral vector encoding a second-generation anti-Nectin4 CAR at a multiplicity of infection (MOI) of 10. Transduction efficiency was assessed 72 hours post-transduction by flow cytometry using a biotinylated protein L/ streptavidin-phycoerythrin (PE) detection strategy.

### *2.2 CAR Constructs and Flow Cytometry*

The Nectin4-targeting CAR construct comprised an scFv derived from a human Nectin4-specific antibody, a CD8α-derived leader peptide, a 4-1BB costimulatory domain, and the CD3ζ signaling domain [9]. For immunophenotyping, NK or CAR-NK cells were stained with fluorochrome-conjugated antibodies against human CD3, CD56, and relevant CAR detection reagents for 20 minutes at 4 °C in the dark. Surface Nectin4 expression on tumor cells was detected using an Alexa Fluor® 647-conjugated anti-human Nectin4 antibody. Surface expression of NKG2D ligands was assessed by using PE-conjugated antibodies specific for MICA/B, ULBP1, ULBP2/5/6, ULBP3, and ULBP4 [12]. Cells were first gated on forward scatter (FSC) and side scatter (SSC) to exclude debris, followed by doublet discrimination using FSC-A/FSC-H and SSC-A/SSC-H. Viable cells were then gated based on the expression of the indicated markers. Positive populations were defined using fluorescence-minus-one (FMO) controls and isotype-matched control antibodies for each fluorochrome. Data were collected on a BD FACS Aria™ III flow cytometer. Subsequent analysis was performed with FlowJo software (version 10.8.1; FlowJo LLC, Ashland, OR, USA).

### *2.3 Cell Lines*

All cell lines were sourced from Shanghai FuHeng Biology (2405281, Shanghai, China), authenticated via short tandem repeat (STR) profiling, and routinely confirmed to be mycoplasma-freeRPMI-1640 or DMEM, each containing 10% FBS and 1% penicillin-streptomycin, served as the culture medium, with cells kept at 37 °C under 5% $CO_2$. A PCR-based mycoplasma detection kit (MP0050, Sigma-Aldrich, St. Louis, MO, USA) was used to routinely examine all cell lines every 2–3 weeks, and the most recent test for all cell lines used in this study was performed and all results were negative.

### *2.4 Cellular Cytotoxicity Assays*

The cytotoxic activity of CAR-NK and control NK cells was evaluated against firefly luciferase-expressing MDA-MB-231-Luc-GFP target cells, which exhibit high Nectin4 expression. We plated target cells ($1 \times 10^4$ per well) in 96-well plates and added effector cells at E:T ratios of 1:1, 2:1, and 5:1 for an 8-hour incubation. Following incubation, D-luciferin substrate was added, and luminescence was measured. Spontaneous (targets only in medium) and maximum lysis (targets lysed with $ddH_2O$) controls were included. Specific lysis (%) was calculated as: (Luminescence Max – Luminescence Sample) / (Luminescence Max – Luminescence Min) × 100% [13].

### *2.5 Animal Experiments*

The Institutional Animal Care and Use Committee of Wenzhou Medical University (wydw2022-0447) approved all animal procedures, which were conducted in compliance with institutional guidelines. Under 100% oxygen, animals received isoflurane at 3% for induction and 1.5%–2% for maintenance of anesthesia in an induction chamber; the duration of anesthesia was kept within 10–15 min for each procedure, and the depth of anesthesia was continuously monitored by the pedal withdrawal reflex every 3–5 min, with additional maintenance doses adjusted if the reflex re-

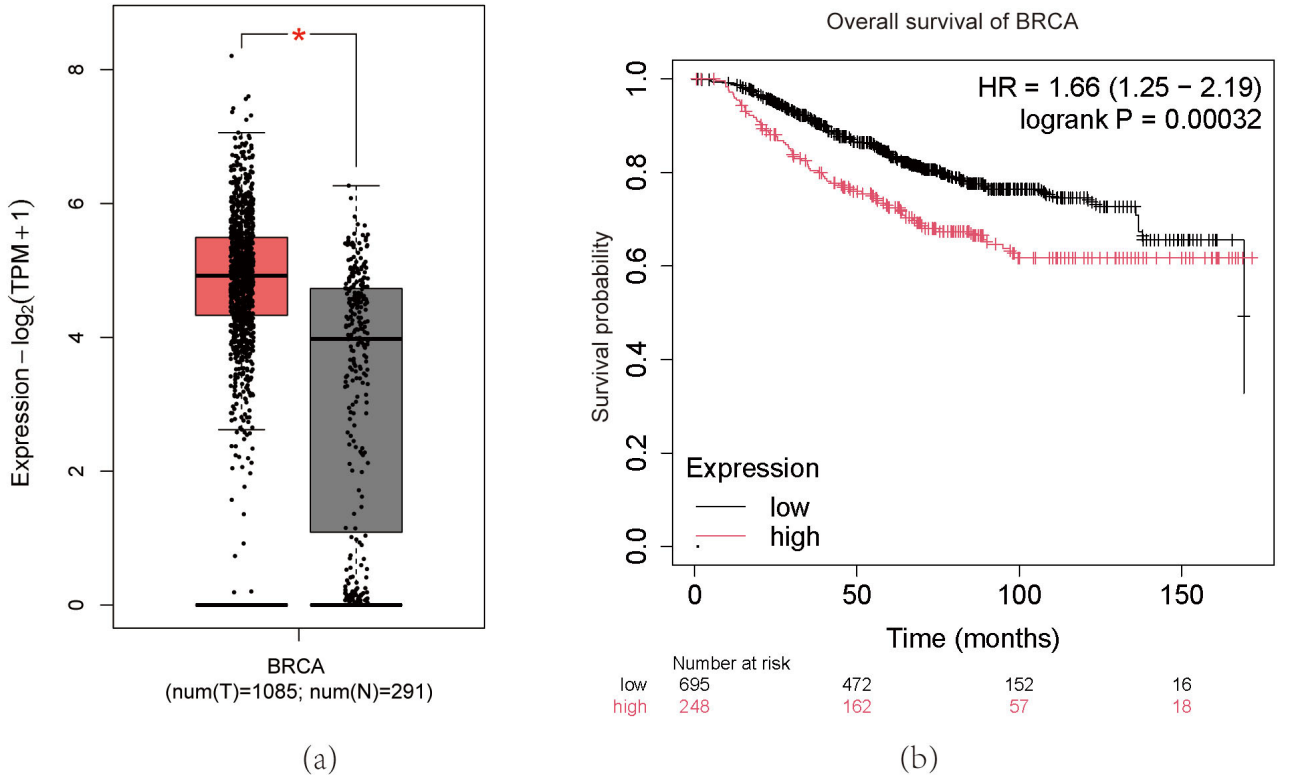


**Fig. 1. Nectin4 upregulation in breast cancer (BRCA) tissues and its correlation with poor prognosis**. (a) Nectin4 mRNA levels were compared between BRCA and normal breast tissues using The Cancer Genome Atlas and Genotype-Tissue Expression databases; *$p < 0.05$, Student's *t*-test. (b) Statistical analysis of the association between Nectin4 expression and survival probability in patients with BRCA, KMPLOT survival analysis.

turned. The depth of anesthesia was confirmed by loss of the pedal reflex prior to any invasive procedure. Five-week-old male NOD-SCID IL2rγnull (NSG) mice were subcutaneously injected with $3 \times 10^6$ MDA-MB-231-Luc-GFP cells. On day 7 post-inoculation, when tumors were established, mice were randomized into three treatment groups (n = 4 per group): (1) phosphate-buffered saline (PBS) control, (2) untransduced NK cells ($6 \times 10^6$ cells/ injection), and (3) anti-Nectin4 CAR-NK cells ($6 \times 10^6$ cells/ injection). Treatments were administered via tail vein injection on days 7 and 13. Tumor growth was assessed weekly via bioluminescence imaging (BLI) using an IVIS Spectrum system following intraperitoneal D-luciferin injection. At the endpoint (day 32), mice were euthanized by $CO_2$ inhalation (20% chamber volume/min until respiratory arrest) followed by cervical dislocation; tumors were then excised, weighed, and processed for staining.

### *2.6 Immunohistochemical Staining*

Nectin4 expression was performed by immunohistochemistry (IHC) on formalin-fixed, paraffin-embedded tissue sections. Sections were deparaffinized in xylene and rehydrated in graded ethanol. Antigen retrieval was then carried out by microwave heating. After quenching endogenous peroxidase with 3% $H_2O_2$ and blocking with 20% goat serum, the slides were incubated with Nectin4 antibody overnight at 4 °C. An enhancer and horseradish peroxidase (HRP)-conjugated secondary antibody were then applied sequentially for 20 min each at room temperature. Signal was visualized using 3,3′-diaminobenzidine (DAB), and sections were counterstained with hematoxylin. After dehydration and clearing, sections were mounted with neutral balsam and examined under a light microscope.

### *2.7 Statistical Analysis*

Statistical comparisons between groups were made with Student's *t*-test or one-way analysis of variance (ANOVA). Normality and equal variance assumptions were verified using Shapiro–Wilk and Levene's tests; when applicable, multiple comparisons were adjusted with Bonferroni or Tukey corrections. The log-rank test was applied to survival data, with significance set at $p < 0.05$.

## 3. Results

### *3.1 Expression and Clinical Significance of Nectin4 in Breast Cancer (BRCA)*

Analysis of publicly available data from The Cancer Genome Atlas (TCGA) revealed that Nectin4 mRNA expression was significantly higher in breast cancer tissues compared to adjacent normal breast tissues ($p <$

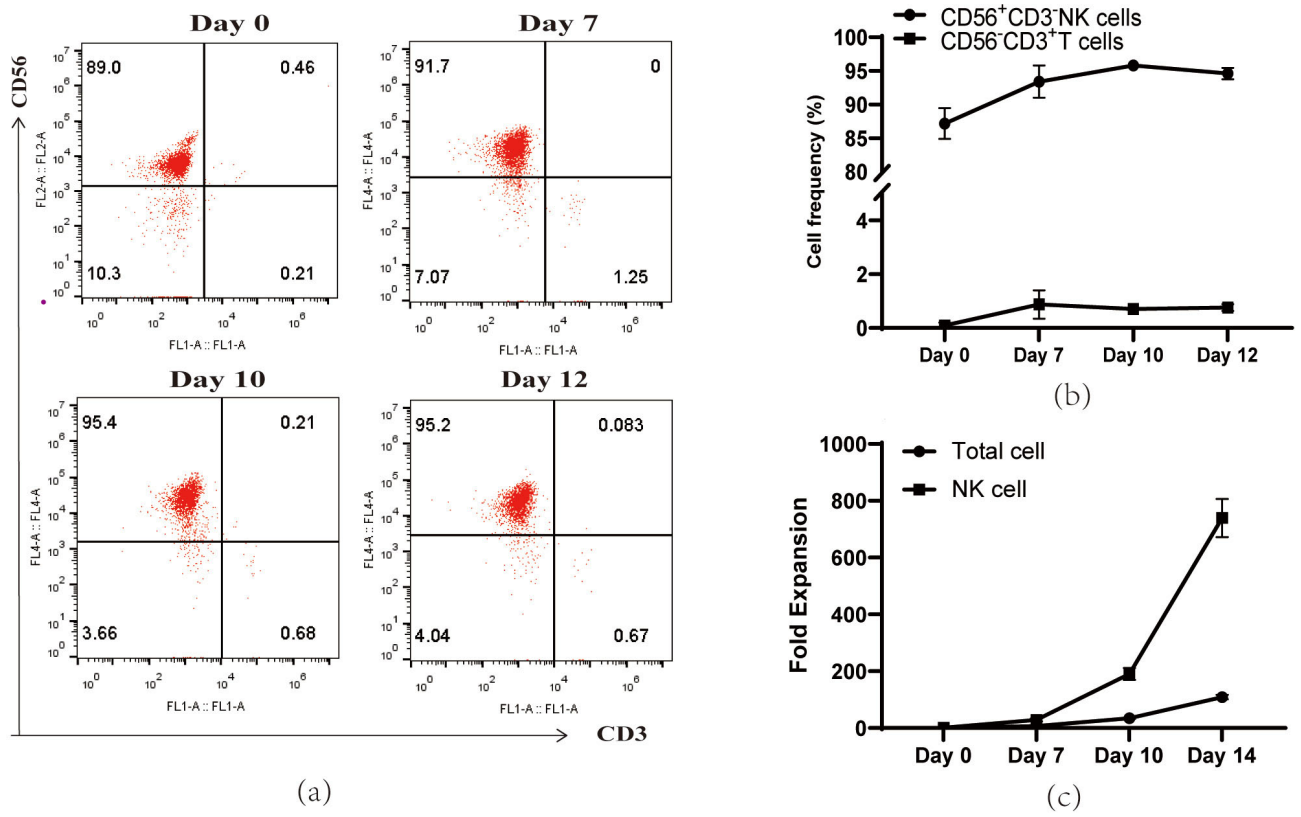


**Fig. 2. Isolation and expansion of NK cells from PBMCs**. (a) Proportion of NK cells at different time points after isolation from PBMCs. (b) Statistical analysis of the percentages of NK and T cells, respectively. (c) Statistical analysis of NK cell and total cell amplification. PBMCs, peripheral blood mononuclear cells; NK, natural killer.

0.05, Fig. 1a). Kaplan-Meier survival analysis using the Kaplan-Meier Plotter database further demonstrated that high Nectin4 expression was significantly associated with worse overall survival in breast cancer patients (hazard ratio [HR] = 1.66, 95% confidence interval [CI]: 1.25–2.19, $p = 0.00032$, Fig. 1b).

### *3.2 Isolation and Expansion of NK Cells*

NK cells were successfully isolated and expanded from PBMCs. The purities on days 0, 7, 10, and 12 were 87.17 ± 2.89%, 93.10 ± 1.78%, 95.80 ± 0.57%, and 94.60 ± 0.85%, respectively (Fig. 2a,b). The fold expansion of NK cells was calculated by combining initial cell counts with flow cytometry results. NK cell proliferation accelerated after day 7, reaching an 800-fold increase relative to the initial number by day 14 (Fig. 2c).

### *3.3 The Anti-Tumor Effects of Nectin4-CAR-NK Cells Against Breast Cancer Cells In Vitro*

NK cells mediate target cell killing through NKG2D ligands—eight cell-associated glycoproteins from the MIC and ULBP families. The MDA-MB-231-Luc-GFP target cells were confirmed to have high Nectin4 expression and low expression of NKG2D ligands including MICA/B, ULBP1, ULBP2/5/6, ULBP3, and ULBP4 (Fig. 3a). Lentiviral transduction yielded a CAR expression efficiency of approximately 40–54% (Fig. 3b). *In vitro* cytotoxicity assays demonstrated that anti-Nectin4 CAR-NK cells exhibited potent, dose-dependent killing of Nectin4-high MDA-MB-231-Luc-GFP target cells. At an E:T ratio of 5:1, CAR-NK cells mediated nearly 95% specific lysis, significantly outperforming untransduced NK cells ($p < 0.001$, Fig. 3c).

### *3.4 The Anti-Tumor Effects of Nectin4-CAR-NK Cells In Vivo*

The therapeutic efficacy of Nectin4-CAR-NK cells was evaluated in an subcutaneously breast cancer xenograft model (Fig. 4a). Bioluminescence imaging showed that tumor burden increased steadily in PBS-treated control mice, leading to morbidity in half of the animals by day 17 (Fig. 4b,d). Treatment with untransduced NK cells resulted in a modest but non-significant difference in body weight compared to the PBS group (Fig. 4c). In contrast, mice treated with Nectin4-CAR-NK cells displayed a profound and sustained suppression of tumor growth (Fig. 4d), which translated into a significant survival benefit (Fig. 4e).

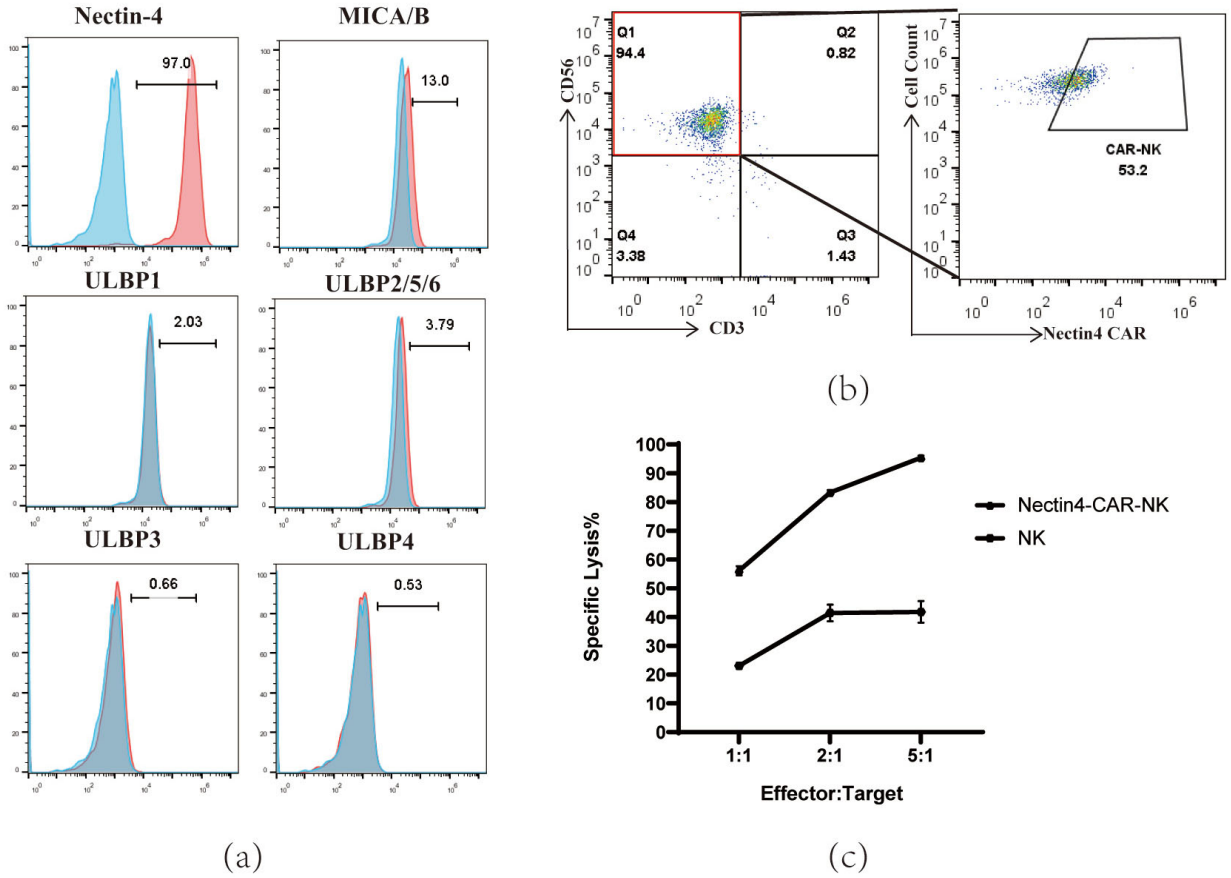


**Fig. 3. Characterization of Nectin4 expression and CAR-NK cell transduction**. (a) Flow cytometry analysis confirming high Nectin4 expression and low NKG2D ligand expression on MDA-MB-231-Luc-GFP target cells. (b) Transduction efficiency of anti-Nectin4 CAR in NK cells as assessed by flow cytometry. (c) *In vitro* cytotoxicity of anti-Nectin4 CAR-NK cells against Nectin4-high MDA-MB-231-Luc-GFP cells at various effector-to-target (E:T) ratios. CAR, chimeric antigen receptor.

### *3.5 Tumor Analysis and Nectin4 Expression Post-Treatment*

At the experimental endpoint (day 32), tumor weights were significantly lower in the CAR-NK treatment group compared to both the NK cell ($p < 0.05$, Fig. 5a,b). Immunohistochemical staining of excised tumor tissues revealed a substantial reduction in Nectin4 protein expression in tumors from the CAR-NK-treated mice compared to controls ($p < 0.001$, Fig. 5c), suggesting effective targeting and elimination of Nectin4-positive tumor cells *in vivo*.

## 4. Discussion

Nectin4 belongs to the nectin family of immunoglobulin-like adhesion molecules and is classified as an oncofetal antigen due to its re-expression in various carcinomas, including breast cancer [14,15]. Its role extends beyond cell adhesion; soluble Nectin4, shed by metalloproteases like tumor necrosis factor-α converting enzyme (TACE)/a disintegrin and metalloproteinase 17 (ADAM-17), can promote tumor angiogenesis by engaging integrin-β4 on endothelial cells, thereby activating pro-angiogenic signaling pathways [16,17]. Furthermore, Nectin4 overexpression has been linked to the induction of epithelial-mesenchymal transition (EMT), metastasis, and activation of cancer stem cell-related pathways such as Wingless-related integration site (Wnt)/β-catenin via the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) axis [15,16]. Moreover, Nectin4 cis-interacted with ErbB2 to enhance its homodimerization and activation through a novel mechanism, resulting in PI3K-AKT pathway activation for DNA synthesis, while also potentiating p95-ErbB2-induced Janus kinase (JAK)-signal transducer and activator of transcription 3 (STAT3) signaling [17]. These pleiotropic roles in tumor progression make it a compelling therapeutic target. Our findings demonstrate that Nectin4 is not only a prognostic biomarker associated with poor survival in breast cancer but also a functionally relevant and druggable target for cellular immunotherapy. The potent *in vitro* and *in vivo* anti-tumor activity of Nectin4-targeted CAR-NK cells supports this premise.

Nectin4 is reactivated in various malignancies, including breast cancer, and is therefore categorized as an oncofetal antigen [14,15]. Beyond its conventional role in intercellular adhesion, Nectin4 contributes to tumor pro-

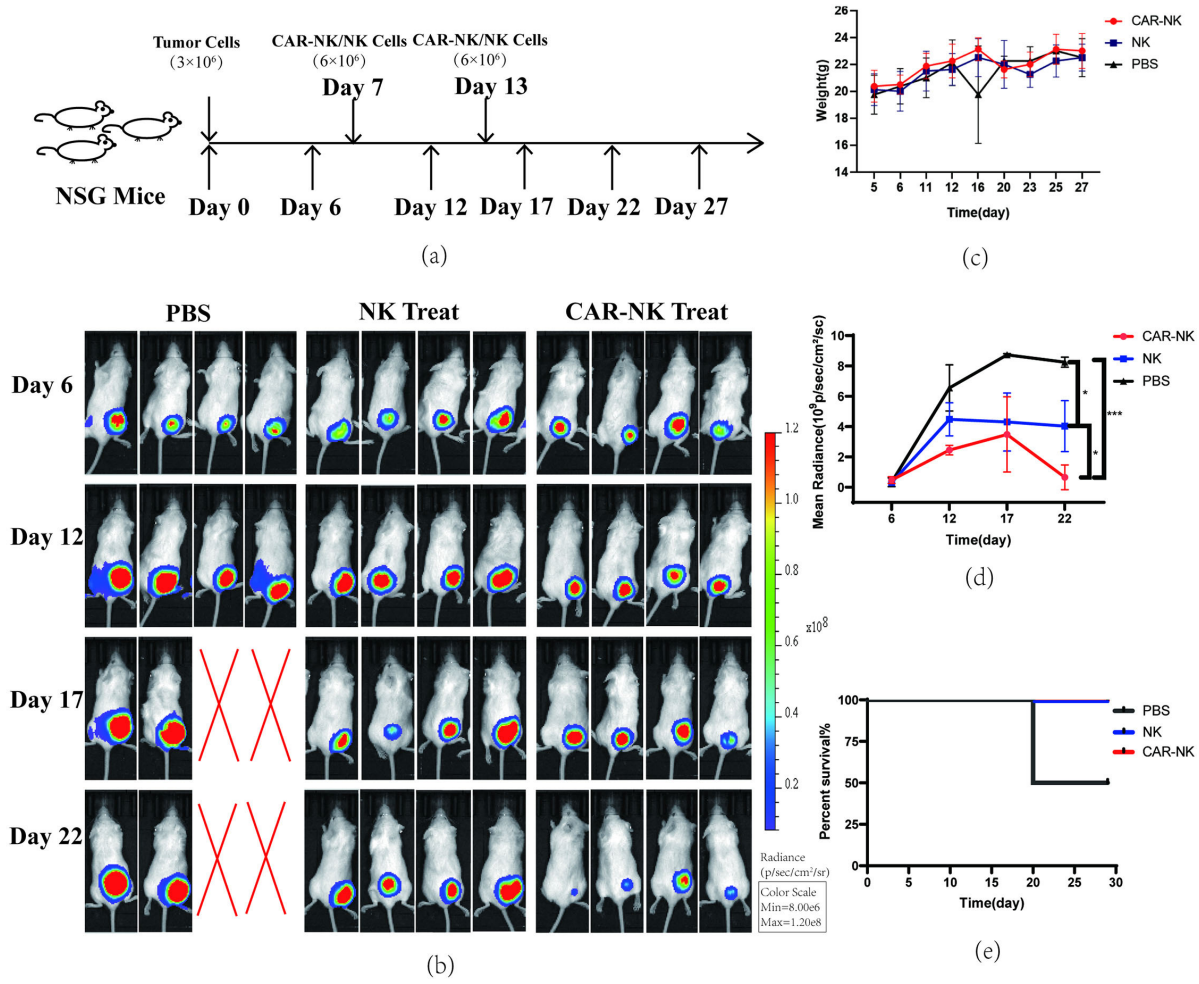


**Fig. 4.** ***In vivo*** **anti-tumor efficacy of Nectin4-CAR-NK cells in a breast cancer xenograft model**. (a) Schematic of the animal experiment timeline. (b) Representative bioluminescence images showing tumor burden in NSG mice at different time points. (c) Mice weight at different time points. (d) Average bioluminescent radiance for each treatment group over time (mean ± SD); *$p < 0.05$, ***$p < 0.001$. (e) Survival curves of mice in different treatment groups.

gression through multiple mechanisms: the proteolytically shed soluble form engages endothelial integrin-β4 to promote angiogenesis [16,17]; its overexpression drives EMT, facilitates metastatic dissemination, and activates stemness-related Wnt/β-catenin signaling, partially via the PI3K/Akt axis [18,19]; additionally, cis-association with ErbB2 reinforces ErbB2 homodimerization and activation, thereby stimulating PI3K-AKT-mediated DNA synthesis and augmenting p95-ErbB2-triggered JAK-STAT3 signals [20]. The PI3K/Akt/mTOR and Ras/Raf/MEK/ERK cascades are pivotal in relaying proliferative and survival cues, and their constitutive activation is strongly associated with malignant progression, chemoresistance, and unfavorable clinical outcomes [21]. Nectin4-driven signals converge on these pathways, particularly through PI3K/Akt, and accumulating evidence indicates that concomitant blockade of Nectin4 and its downstream kinase relays may elicit synergistic antitumor efficacy [19]. Given the intricate crosstalk and reciprocal feedback loops between these two kinase networks, dual or sequential inhibition has attracted growing interest [22]; Nectin4-directed cellular immunotherapy, exemplified by CAR-NK cells, may provide a distinctive modality that could overcome certain drawbacks of small-molecule kinase inhibitors while still lending itself to rational combination regimens. Collectively, our results establish Nectin4 as both a prognostic indicator of poor survival in breast cancer and a functionally validated, druggable target for immune-based intervention. The robust *in vitro* and *in vivo* antitumor activity of Nectin4-specific CAR-NK cells highlights the translational promise of this approach and strongly supports future investigations into its combination with pathway-targeted agents to counteract resistance and improve patient outcomes.

While antibody-drug conjugates like enfortumab vedotin have validated Nectin4 as a target, Concurrent advances in Nectin4-targeted positron emission tomography/computed tomography (PET/CT) imaging for patient stratification [23], next-generation ADC development with

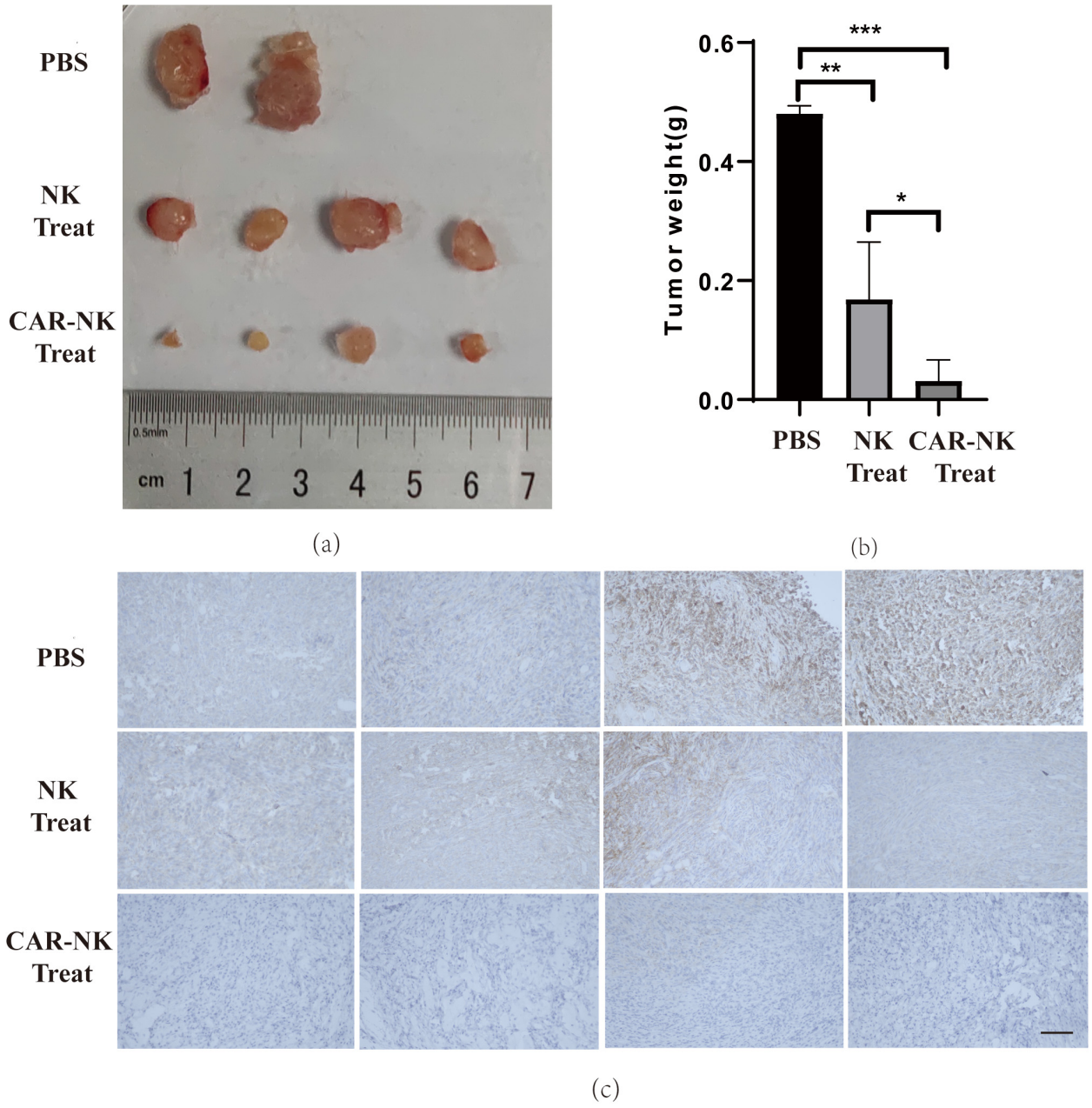


**Fig. 5. Tumor analysis after CAR-NK cell therapy**. (a) Representative images of excised tumors from MDA-MB-231-Luc-GFP-bearing mice treated with PBS, untransduced NK cells, or Nectin4-CAR-NK cells. (b) Tumor weights at the experimental endpoint. (c) Immunohistochemical staining of Nectin4 expression in tumor tissues from each treatment group. Scale bar: 100 µm. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. PBS, phosphate-buffered saline.

improved safety profiles [24], and covalent radioligand strategies for enhanced tumor retention [25] further underscore the growing potential of Nectin4-directed precision medicine and provide complementary tools that may synergize with CAR-NK cell therapy in future clinical paradigms. CAR-based cellular therapies offer a distinct mechanism of action with potential for long-term immune memory. The choice of the NK cell platform is strategic. CAR-NK cells may offer safety advantages over CAR-T cells, including a lower risk of severe cytokine release syndrome and graft-versus-host disease, facilitating allogeneic application [7]. Our data show that Nectin4-CAR-NK cells can efficiently kill breast cancer cells *in vitro* and mediate significant tumor control *in vivo*, resulting in prolonged survival.

The observed reduction in tumor Nectin4 expression following CAR-NK therapy, as seen by IHC, is a critical finding. It suggests that the treatment effectively eliminated the Nectin4-high tumor cell population, potentially disrupting a key axis of tumor maintenance and progression. This "antigen editing" or selective pressure is a documented phenomenon in targeted therapies and underscores the po-

tency of the approach. However, this selective pressure also raises the concern of potential antigen escape, which could compromise long-term therapeutic efficacy. Possible mechanisms include downregulation of Nectin4 surface expression, loss of heterozygosity, or the emergence of Nectin4-negative tumor clones. To mitigate this risk, future combination strategies may be considered, such as pairing CAR-NK cells with agents that upregulate Nectin4 expression or with immune checkpoint inhibitors to counteract the immunosuppressive tumor microenvironment. These approaches warrant further investigation to optimize the Nectin4-directed CAR-NK therapy, as combining HER2-targeted antibody-drug conjugates produces synergistic antitumor effects in cancers co-expressing both targets [26].

Despite the promising results, several limitations of this study should be acknowledged. First, our *in vitro* experiments were confined to a single breast cancer cell line (MDA-MB-231), which may not adequately capture the molecular heterogeneity across different breast cancer subtypes. Second, the absence of a Nectin4-negative cell line precludes a definitive assessment of antigen specificity and leaves room for potential off-target effects. Third, the modest sample size in the *in vivo* study (n = 4 per group) limits the statistical power and generalizability of our conclusions. Fourth, we did not evaluate the persistence, expansion, or biodistribution of the infused CAR-NK cells, all of which are critical parameters influencing therapeutic efficacy. Fifth, long-term toxicity and potential damage to normal tissues expressing Nectin4 were not examined. Furthermore, intratumoral heterogeneity in Nectin4 expression may pose a risk of immune evasion, underscoring the need for future combination strategies—such as pairing CAR-NK cells with agents that upregulate Nectin4 expression or with immune checkpoint inhibitors—to counteract the immunosuppressive tumor microenvironment. Optimizing CAR architecture (e.g., by incorporating cytokine signaling domains) and refining manufacturing protocols to improve NK cell persistence and *in vivo* functionality will also be essential for advancing this approach toward clinical application.

## 5. Conclusions

In conclusion, this study provides proof-of-concept for Nectin4 as a viable CAR-NK cell target in breast cancer. We successfully generated Nectin4-specific CAR-NK cells that exhibited potent cytotoxicity against Nectin4-positive breast cancer cells *in vitro* and significantly suppressed tumor growth while prolonging survival in a xenograft model. Beyond efficacy, our work confirms Nectin4 as a biologically relevant and druggable target in TNBC, with mechanistic links to key oncogenic signaling networks. Translationally, Nectin4-directed CAR-NK therapy holds promise as a novel immunotherapeutic strategy for TNBC and other Nectin4-expressing tumors; future optimization of CAR design, combination approaches, and validation in clinically relevant models will be essential to advance this strategy toward the clinic. Collectively, these findings support continued development of Nectin4-targeted CAR-NK therapy for aggressive, Nectin4-positive breast cancer, especially in patients with limited treatment options.

## Availability of Data and Materials

The original data presented in this study are included in the article. Further inquiries can be directed to the corresponding authors. All figures and tables presented in this manuscript are original works created by the authors, and no previously published materials have been used.

## Author Contributions

Conceptualization, JG, AZ, CW and JL; Methodology, JL, XL, TX, TZ and XH; Investigation, JL, TX, TZ and XH; Data Curation, JL; Writing – Original Draft Preparation, JL; Writing – Review & Editing, JG, AZ and CW; Supervision, JG and CW; Funding Acquisition, JG. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki. The use of human samples was approved by the Ethics Committee of the Affiliated Huaian No.1 People's Hospital of Nanjing Medical University (KY-2024-231-01). The animal study protocol was approved by the Institutional Animal Care and Use Committee of Wenzhou Medical University (wydw2022-0447). All animal experiments were conducted in accordance with relevant animal welfare guidelines, including the UK Animals (Scientific Procedures) Act 1986, EU Directive 2010/63/EU, and the guidelines of the local Animal Care and Use Committee, and in compliance with the principles of the 3Rs (Replacement, Reduction, and Refinement). The study was reported following the ARRIVE guidelines for animal research. Informed consent was obtained from all healthy donors involved in the study.

## Acknowledgment

Not applicable.

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## Conflicts of Interest

The authors declare no conflicts of interest.

## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBL54654.

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