HER2-Displaying M13 Bacteriophages induce Therapeutic Immunity against Breast Cancer

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HER2-Displaying M13 Bacteriophages induce Therapeutic Immunity against Breast Cancer

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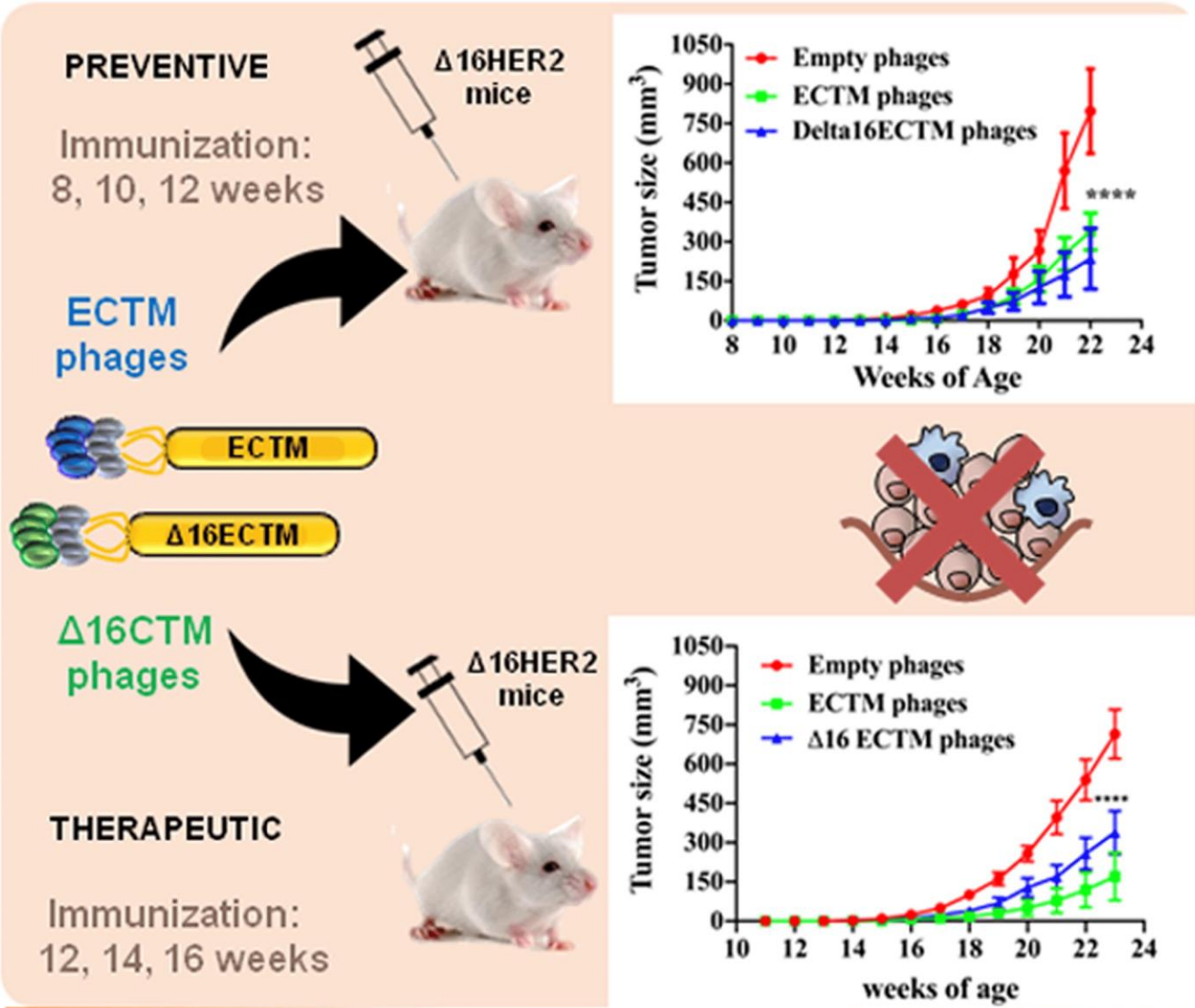
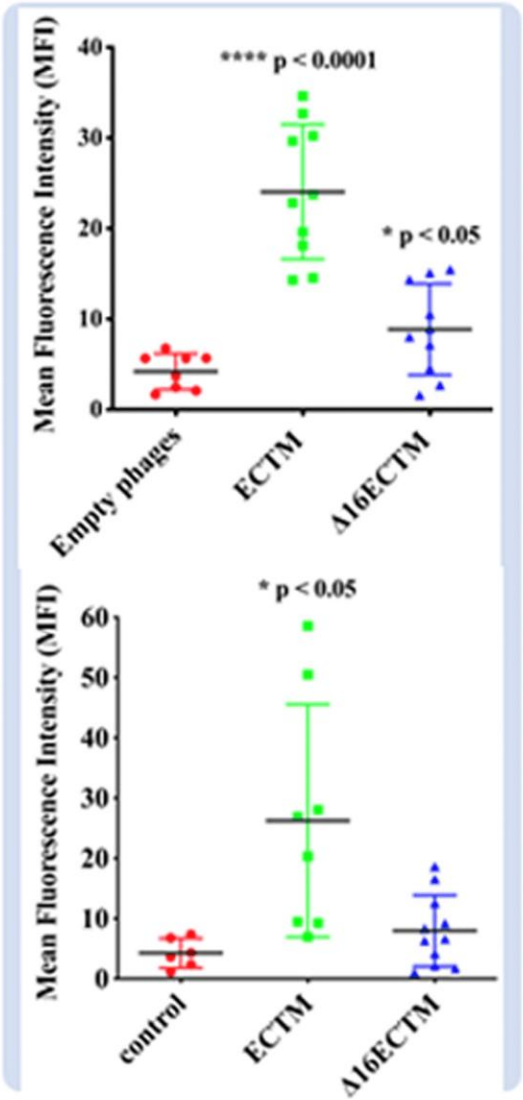
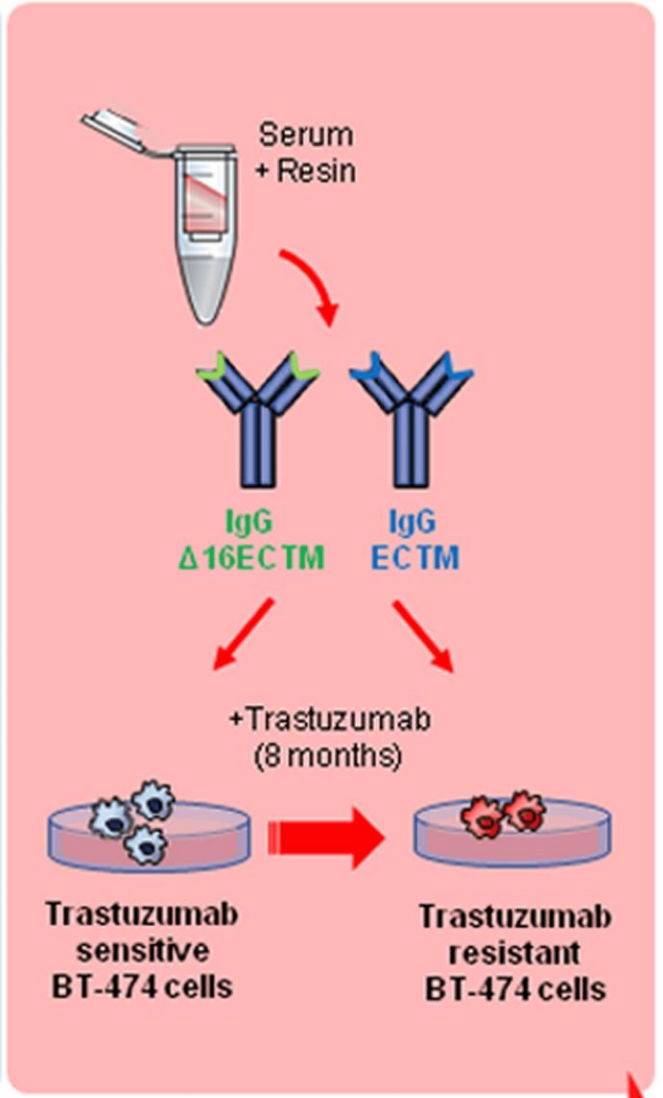
Abstract

- **Problem Statement**: HER2-positive breast cancer accounts for 20–30% of cases and is associated with poor outcomes. Although trastuzumab significantly improves prognosis, its benefits are often limited by resistance and side effects, such as cardiotoxicity.
- **Proposed Solution**: Phage-based vaccines using M13 bacteriophages displaying HER2 or Δ16HER2 (a more aggressive HER2 isoform) domains offer an alternative by stimulating the immune system to produce anti-HER2 antibodies.
- **Key Results**:
  - Preventive and therapeutic vaccination in transgenic mice delayed tumor onset and reduced tumor growth.
  - The vaccines induced strong anti-HER2 antibody responses and impaired HER2 signaling, even in trastuzumab-resistant cancer cells.
- **Conclusion**: Phage-based vaccines could serve as a promising immunotherapeutic approach for HER2-positive breast cancer, addressing resistance issues and providing long-term immunity.







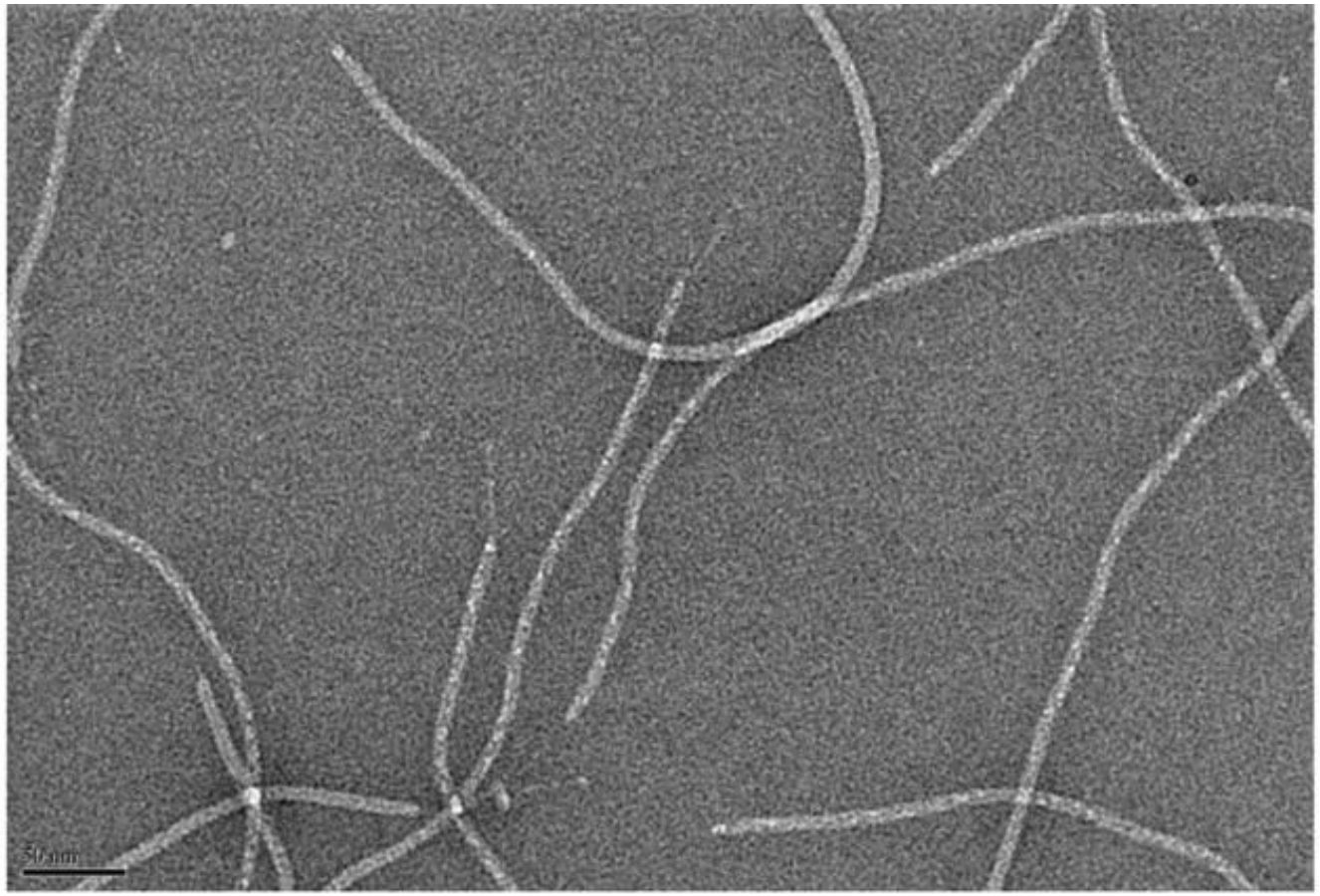
Anti-HER2 phage-based vaccination

Ab response

Antibody Characterization

Introduction

a)



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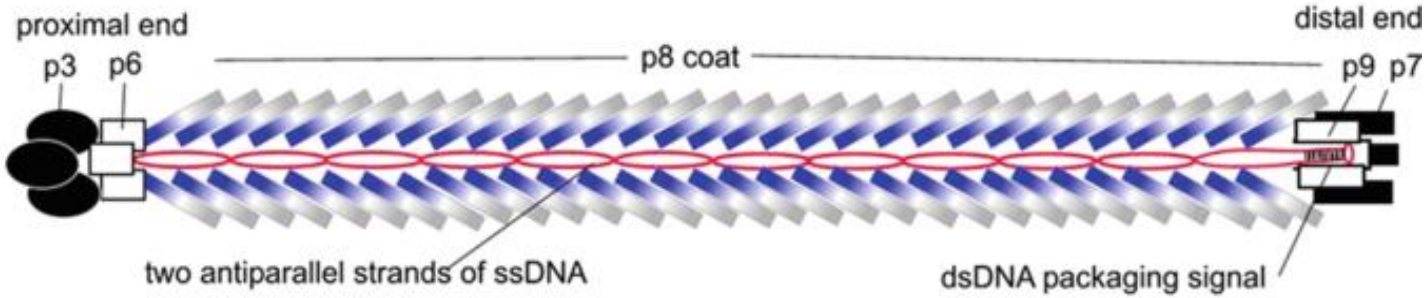
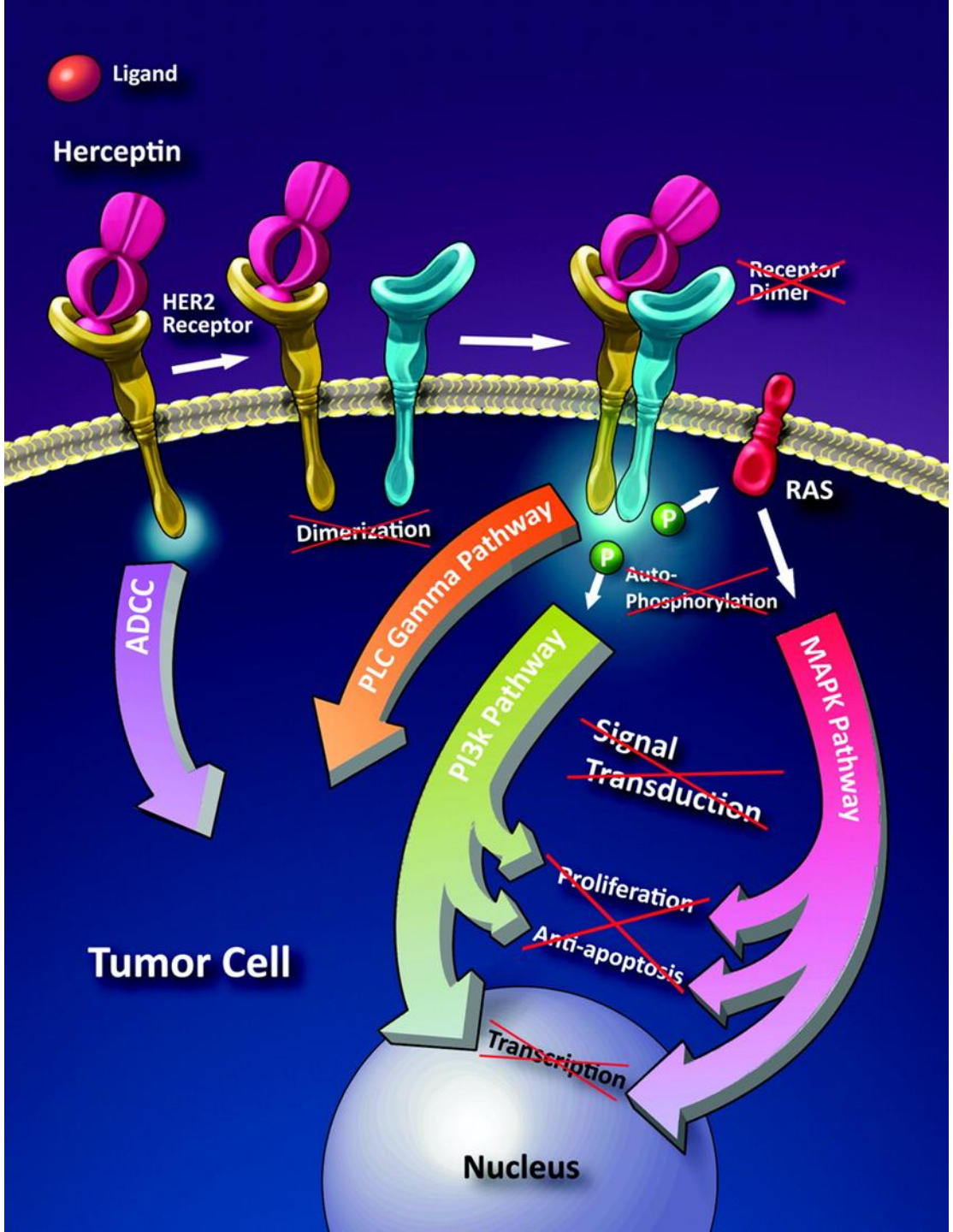
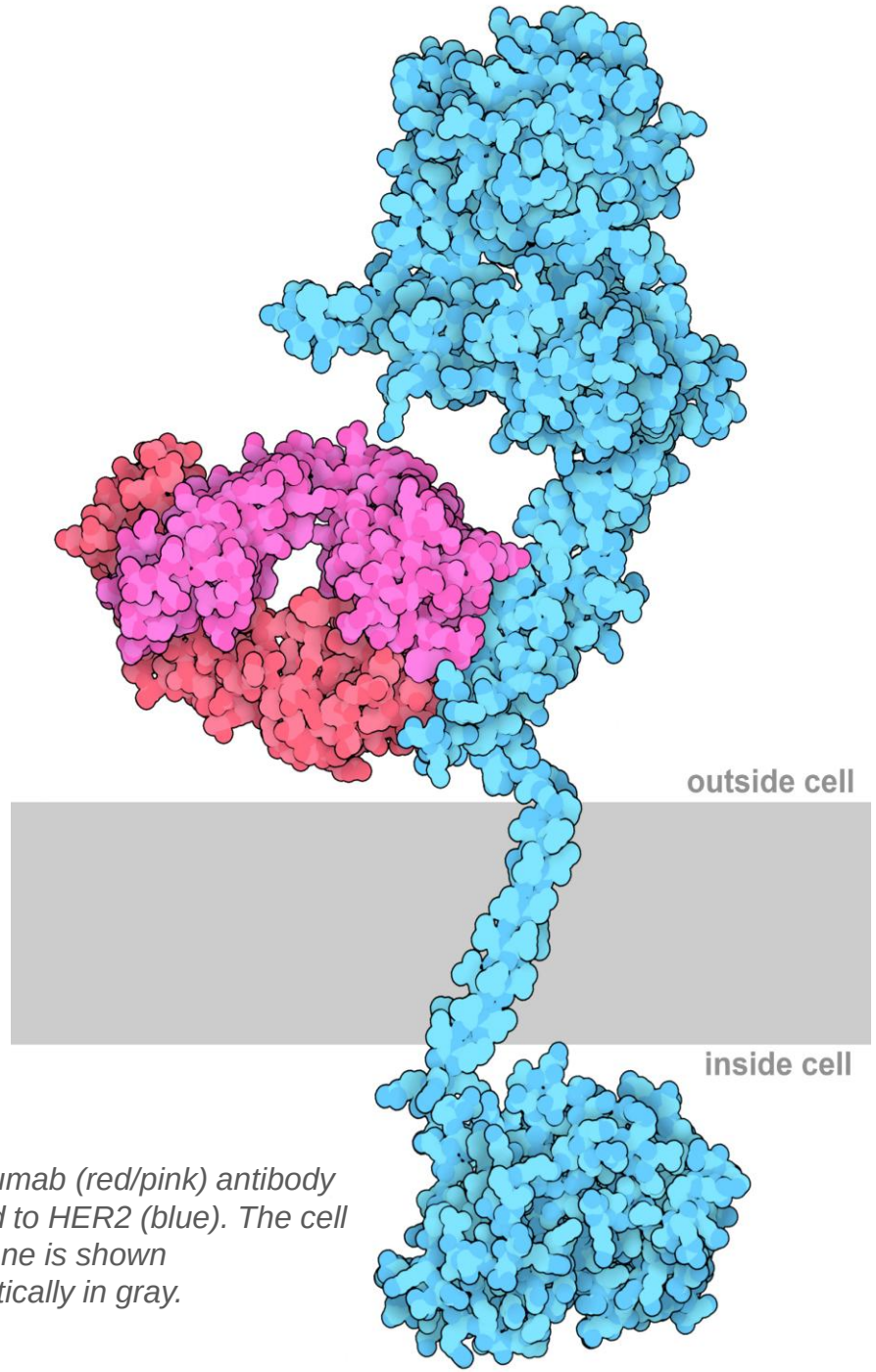


1. HER2 in Breast Cancer

- HER2 (Human Epidermal Growth Factor Receptor 2) is overexpressed in a subset of breast cancers and contributes to:
  - Tumor cell proliferation, survival, and migration.
  - Activation of key pathways like MAPK/ERK and PI3K/Akt.
- Trastuzumab, a monoclonal antibody targeting HER2, has significantly improved survival but is limited by:
  - **De Novo and Acquired Resistance**: Many patients either fail to respond initially or develop resistance over time.
  - **Side Effects**: Notably, an increased risk of cardiotoxicity, especially in older patients.





Trastuzumab (red/pink) antibody is bound to HER2 (blue). The cell membrane is shown schematically in gray.

2. Need for New Therapies

- Cancer vaccines are proposed as a long-term immunotherapeutic strategy that could:
  - Induce durable immune responses against HER2.
  - Overcome immune tolerance to HER2, a self-antigen.
- Despite promising preclinical results, no anti-HER2 cancer vaccine has been approved for clinical use.

3. Phage Display Technology

- **M13 Bacteriophages**:
  - Non-lytic viruses that infect *E. coli*.
  - Can be engineered to display antigenic proteins (e.g., HER2 domains) on their surface.
  - Intrinsically immunogenic, stimulating both innate and adaptive immune responses.
- **Advantages of Phage-Based Vaccines**:
  - Safe and easy to produce.
  - Stable for transport and storage.
  - Capable of breaking immune tolerance and inducing specific cytotoxic T-cell responses.

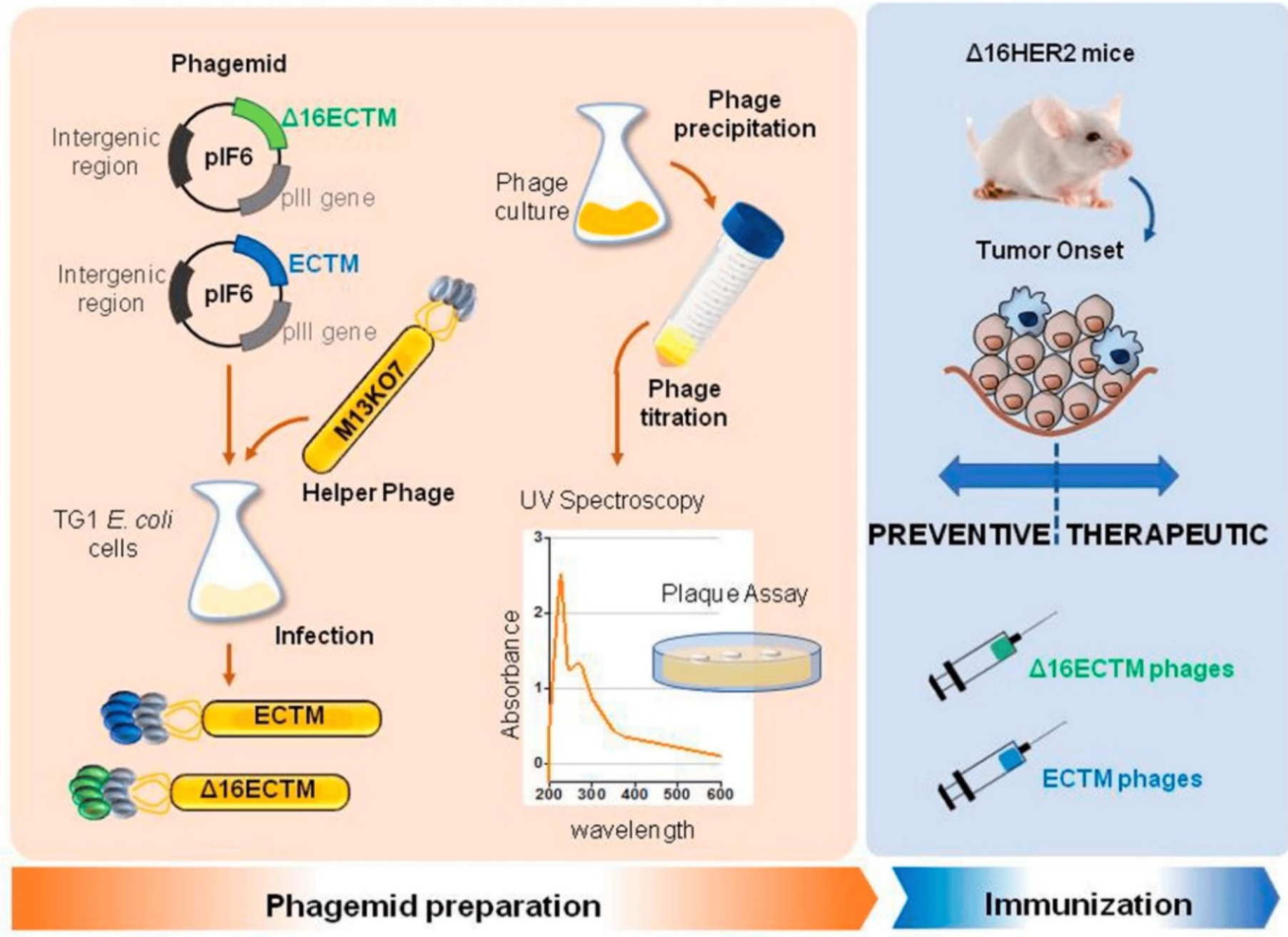
4. Δ16HER2 Variant

- Δ16HER2 is a splice variant of HER2 that:
  - Lacks exon 16, leading to constitutively active homodimers.
  - Is associated with more aggressive and metastatic disease.
  - Presents a unique therapeutic challenge due to its heightened oncogenic potential.

5. Study Objectives

- To evaluate the efficacy of M13 bacteriophages displaying HER2 and Δ16HER2 domains as preventive and therapeutic vaccines in a transgenic mouse model of HER2-positive breast cancer.
- To assess the immune responses elicited by these vaccines and their impact on trastuzumab-sensitive and -resistant cancer cells.

Materials and Methods



1. Production and Purification of Phages

- **Engineering**: HER2 and Δ16HER2 domains were fused to the M13 phage pIII coat protein via recombinant phagemid vectors (pIF6). These phagemids were introduced into *Escherichia coli* TG1 cells.
- **Superinfection**: After reaching the log phase of bacterial growth (OD600 ~0.4–0.5), cells were infected with M13K07 helper phages.
- **Induction**: Cultures were treated with IPTG and grown overnight at 30°C.
- **Purification**: Phages were precipitated using polyethylene glycol (PEG)-NaCl, centrifuged, and filtered through a 0.22 µm filter. The final phage concentration was determined by UV absorption.

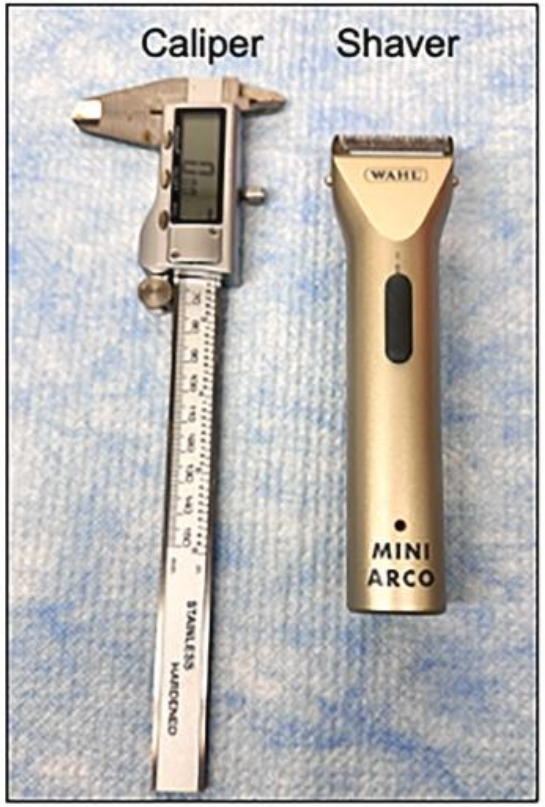
2. Animal Model and Vaccination

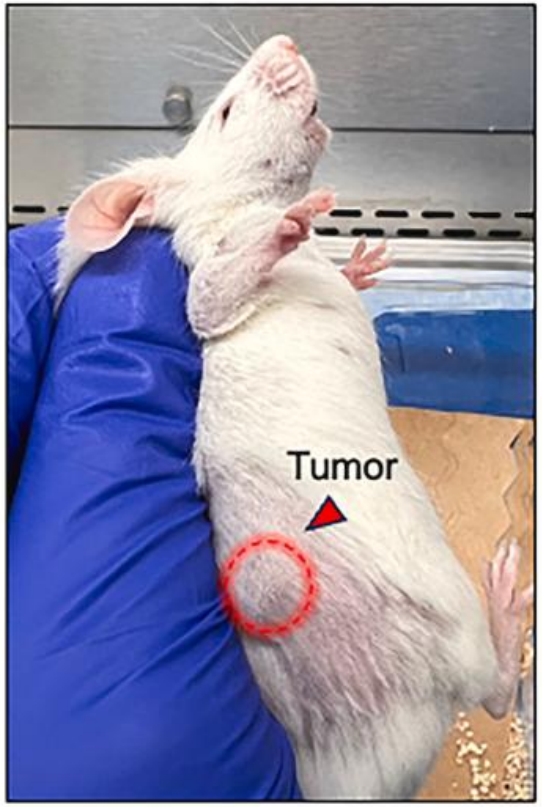
- **Mice**: Δ16HER2 transgenic and FVB mice were used. These mice mimic human HER2-positive breast cancer and are tolerant to HER2 antigens.
- **Housing**: Mice were kept under standard conditions (12-hour light/dark cycle, 20°C temperature) with food and water provided.
- **Immunization Protocol**:
  - **Preventive Vaccination**: Administered intraperitoneally (i.p.) at weeks 8, 10, and 12 (prior to tumor onset).
  - **Therapeutic Vaccination**: Administered i.p. at weeks 12, 14, and 16 (after tumor initiation).
  - Sera were collected post-immunization for antibody analysis.

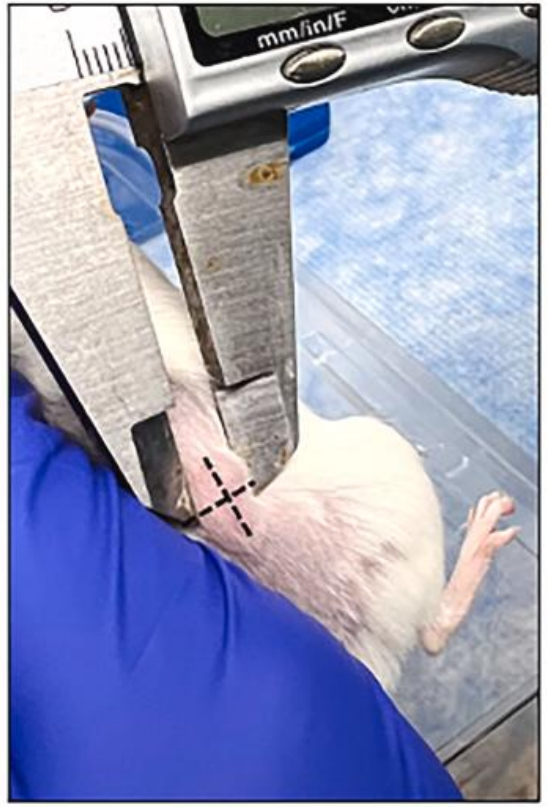
3. Tumor Monitoring

Tumor onset and growth were assessed weekly by palpation.

Tumor size was calculated using caliper measurements.

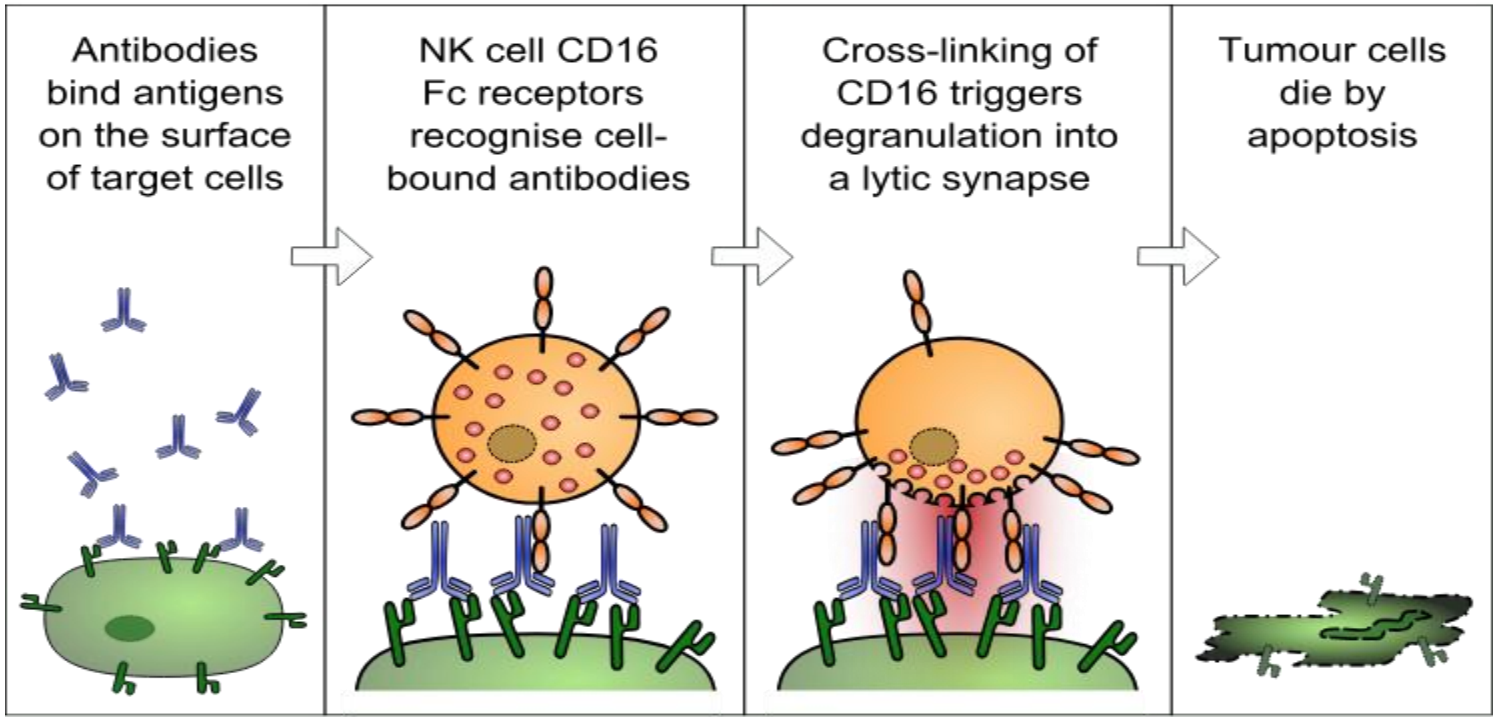






4. Immunological Assays

- **Antibody Detection**: Flow cytometry was used to detect HER2-specific antibodies in sera using Δ16HER2-expressing HEK293 cells.
- **Cytotoxicity**: Antibody-dependent cellular cytotoxicity (ADCC) and T-cell cytotoxicity were evaluated by incubating splenocytes from vaccinated mice with Δ16HER2-positive tumor cells.



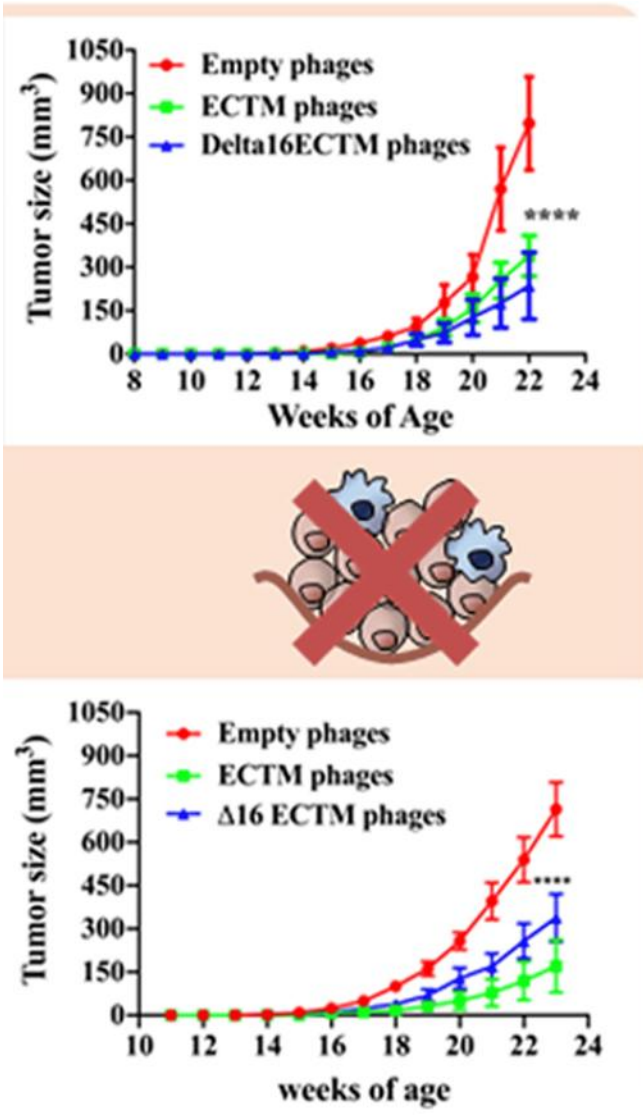
5. Cell Culture and Viability Assays

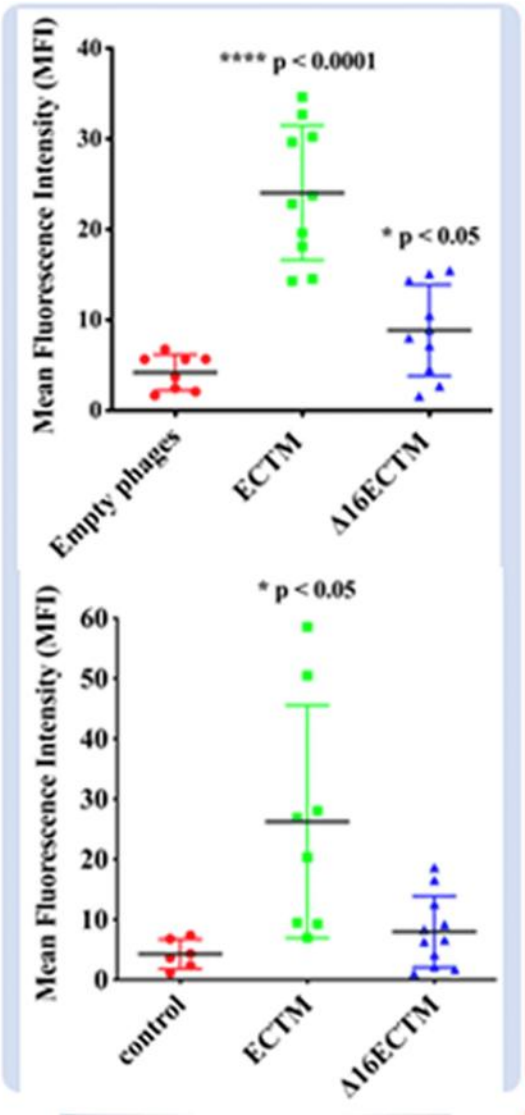
- **Cell Lines**:
  - Human BT-474 cells (trastuzumab-sensitive) were cultured in DMEM with 10% fetal bovine serum (FBS).
  - A trastuzumab-resistant variant (BT-474.R) was developed by prolonged exposure to trastuzumab.
  - Mouse CAM6 and CAM3 cells were derived from Δ16HER2-positive tumors.
- **MTT Assay**: Cell viability was measured after 72 hours of treatment with immune sera-derived IgG or trastuzumab. Results were analyzed by spectrophotometry at 540 nm.

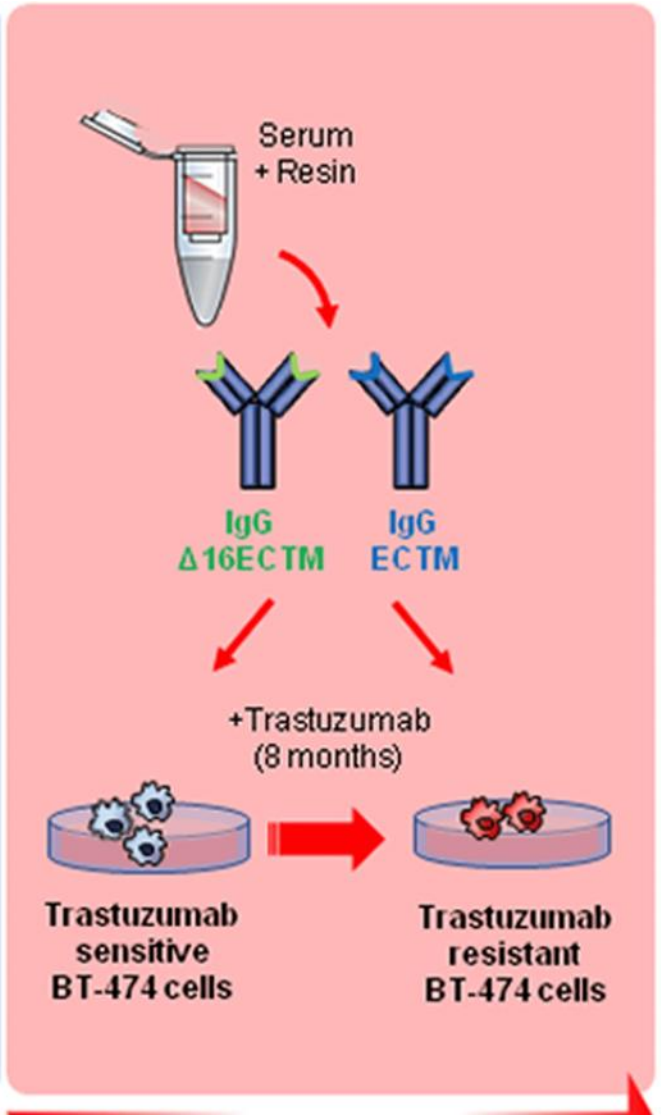
6. Molecular and Histological Analyses

- **Western Blot**: Protein expression and phosphorylation levels (HER2, ERK, AKT, and RB) were analyzed. Lysates were resolved on SDS-PAGE and transferred to PVDF membranes, probed with specific antibodies, and visualized using chemiluminescence.
- **Immunohistochemistry**:
  - Tumor sections were stained for proliferating cell nuclear antigen (PCNA) and phosphorylated ERK (p-ERK).
  - Staining intensity and marker distribution were quantified using image analysis software.

Results and Discussion





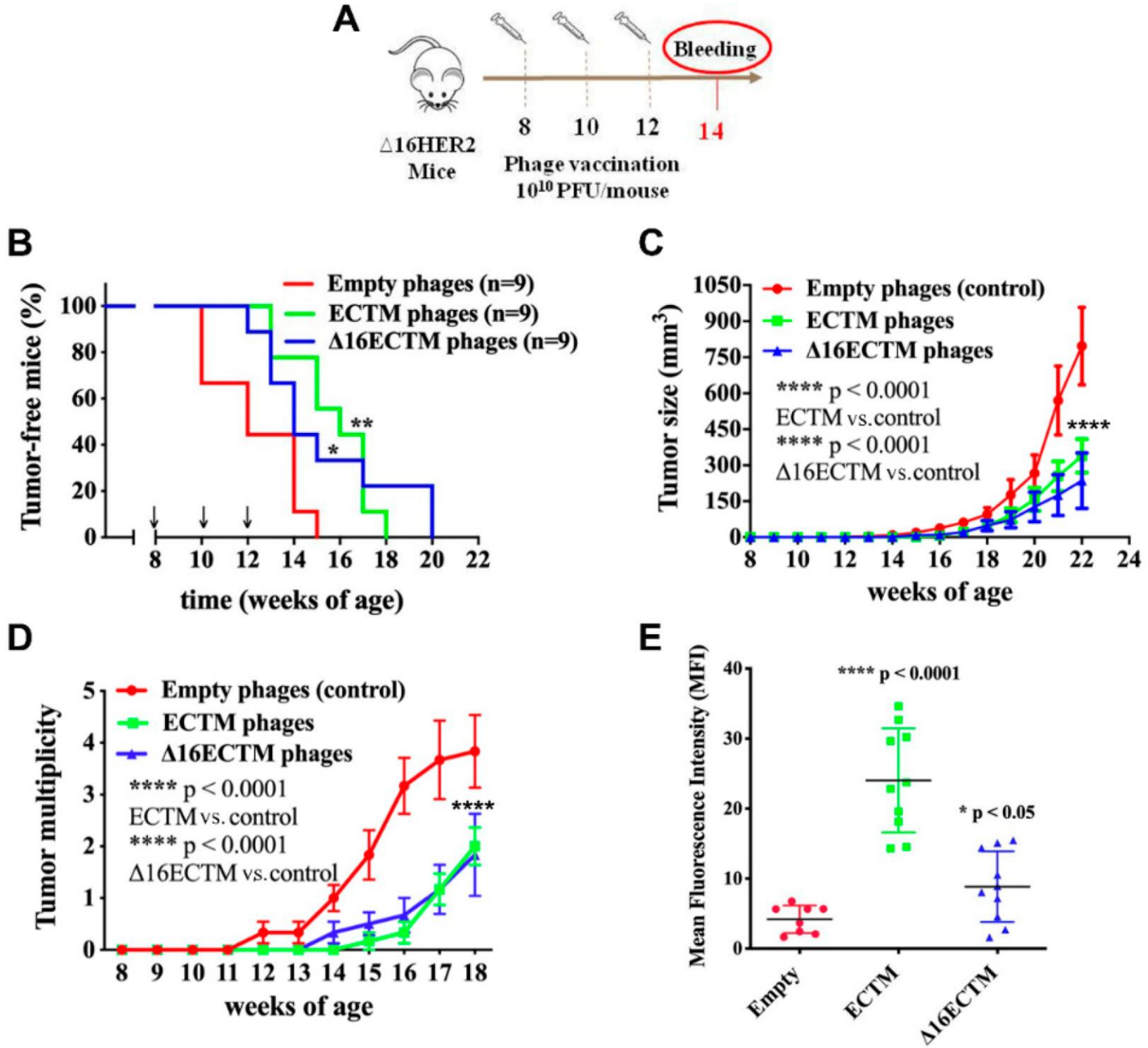


1. Preventive Vaccination

- **Experimental Setup**:
  - Mice were vaccinated with HER2-displaying phages (ECTM and Δ16ECTM) at 8, 10, and 12 weeks of age before tumor onset.
  - Empty phages served as controls.
- **Key Findings**:
  - **Delayed Tumor Onset**:
    - 75% of mice vaccinated with ECTM phages and 40% with Δ16ECTM phages were tumor-free at 15 weeks, compared to 0% in controls.
  - **Reduced Tumor Multiplicity and Growth**:
    - Vaccinated mice developed significantly fewer and smaller tumors than controls.
    - By week 20, vaccinated mice had an average of fewer than one tumor per mouse, compared to three in controls.

- **Antibody Response**:
  - Both vaccines induced HER2-specific antibodies, with ECTM phages producing higher antibody titers.
- **T-cell Cytotoxicity**:
  - Splenocytes from vaccinated mice demonstrated cytotoxic activity against Δ16HER2-positive tumor cells.

- **Discussion**:
  - Preventive vaccination effectively primed the immune system to recognize and target HER2-positive cells, likely through both antibody-mediated and T-cell responses.
  - The stronger efficacy of ECTM phages may be due to higher immunogenicity or enhanced antibody production.

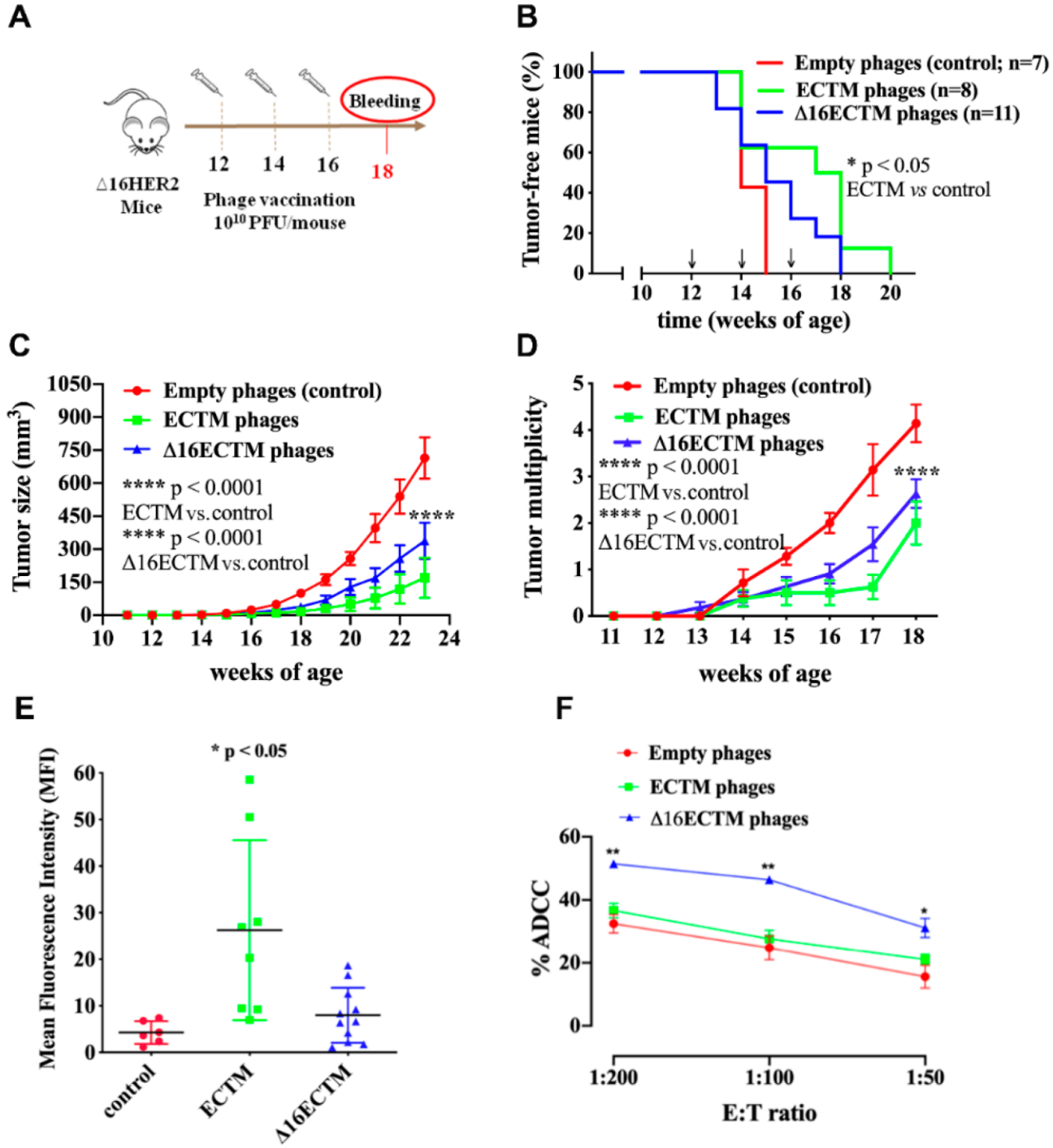

A
Bleeding
8
10
12
14
Δ16HER2
Mice
Phage vaccination
10^10 PFU/mouse
B
Empty phages (n=9)
ECTM phages (n=9)
Δ16ECTM phages (n=9)
Tumor-free mice (%)
time (weeks of age)
C
Empty phages (control)
ECTM phages
Δ16ECTM phages
**** p < 0.0001
ECTM vs.control
**** p < 0.0001
Δ16ECTM vs.control
Tumor size (mm3)
weeks of age
D
Empty phages (control)
ECTM phages
Δ16ECTM phages
**** p < 0.0001
ECTM vs. control
**** p < 0.0001
Δ16ECTM vs.control
Tumor multiplicity
weeks of age
E
**** p < 0.0001
* p < 0.05
Mean Fluorescence Intensity (MFI)
Empty
ECTM
Δ16ECTM

2. Therapeutic Vaccination

- **Experimental Setup**:
  - Mice were vaccinated at 12, 14, and 16 weeks of age, when tumors were already initiated.
- **Key Findings**:
  - **Prolonged Tumor Latency**:
    - Vaccination with ECTM phages significantly delayed tumor onset compared to controls.
    - Δ16ECTM phages also showed latency extension but were less effective than ECTM.
  - **Tumor Growth Suppression**:
    - Both vaccines significantly reduced tumor growth and multiplicity.

- **Antibody-Mediated Effects**:
  - Anti-HER2 antibodies from vaccinated mice reduced cell viability in vitro, with ECTM-IgG showing greater efficacy.
- **ADCC**:
  - Sera from vaccinated mice mediated ADCC against Δ16HER2-positive cells.

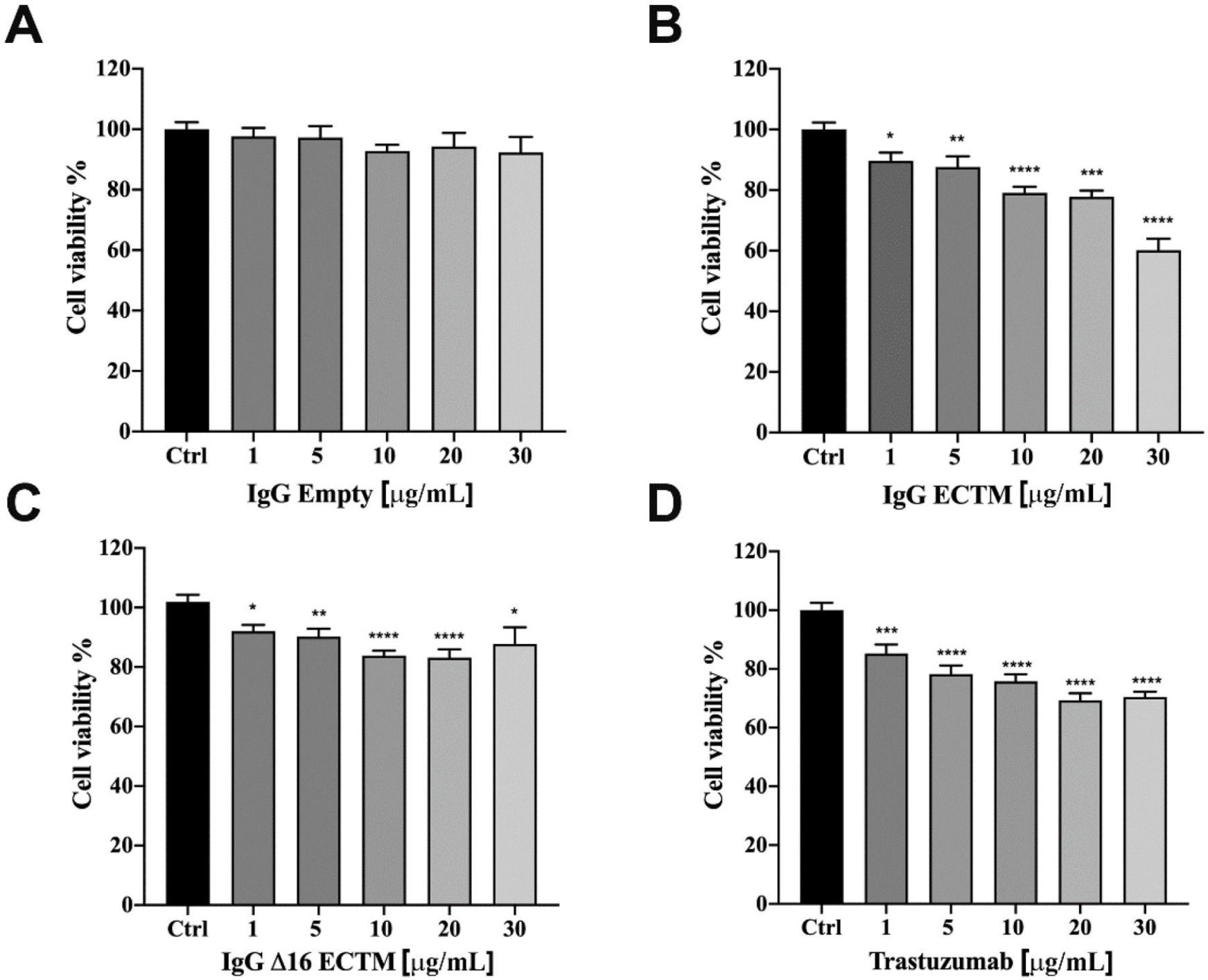
- **Discussion**:
  - Therapeutic vaccination demonstrated efficacy in controlling established tumors, supporting its potential for patients with existing HER2-positive cancers.
  - Variability in antibody titers and immune responses suggests room for optimization, particularly for Δ16HER2 vaccines.


A
Bleeding
12
14
16
18
Δ16HER2
Mice
Phage vaccination
10^10 PFU/mouse
B
Empty phages (control; n=7)
ECTM phages (n=8)
Δ16ECTM phages (n=11)
* p < 0.05
ECTM vs control
Tumor-free mice (%)
time (weeks of age)
C
Empty phages (control)
ECTM phages
Δ16ECTM phages
**** p < 0.0001
ECTM vs.control
**** p < 0.0001
Δ16ECTM vs.control
Tumor size (mm3)
weeks of age
D
Empty phages (control)
ECTM phages
Δ16ECTM phages
**** p < 0.0001
ECTM vs.control
**** p < 0.0001
Δ16ECTM vs.control
Tumor multiplicity
weeks of age
E
* p < 0.05
Mean Fluorescence Intensity (MFI)
control
ECTM
Δ16ECTM
F
Empty phages
ECTM phages
Δ16ECTM phages
% ADCC
E:T ratio
1:200
1:100
1:50

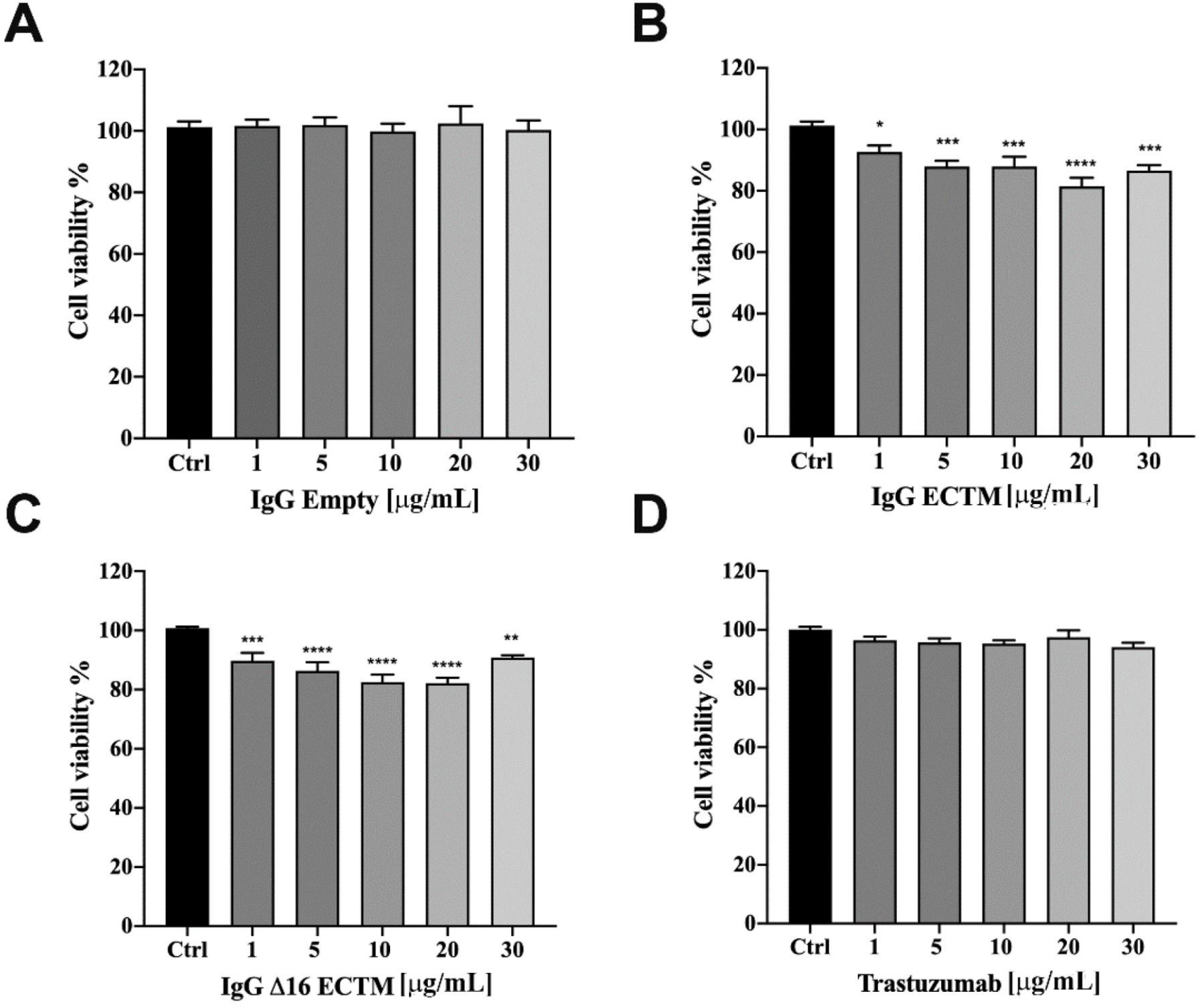
3. Molecular Mechanisms of Vaccine Efficacy

- **Key Findings**:
  - **Inhibition of HER2 Signaling**:
    - Immune-IgG antibodies reduced phosphorylation of ERK and reactivated retinoblastoma (RB) protein in HER2-positive cancer cells.
    - This led to reduced cell viability and proliferation in both trastuzumab-sensitive and -resistant BT-474 cells.

trastuzumab-sensitive BT-474 cells.

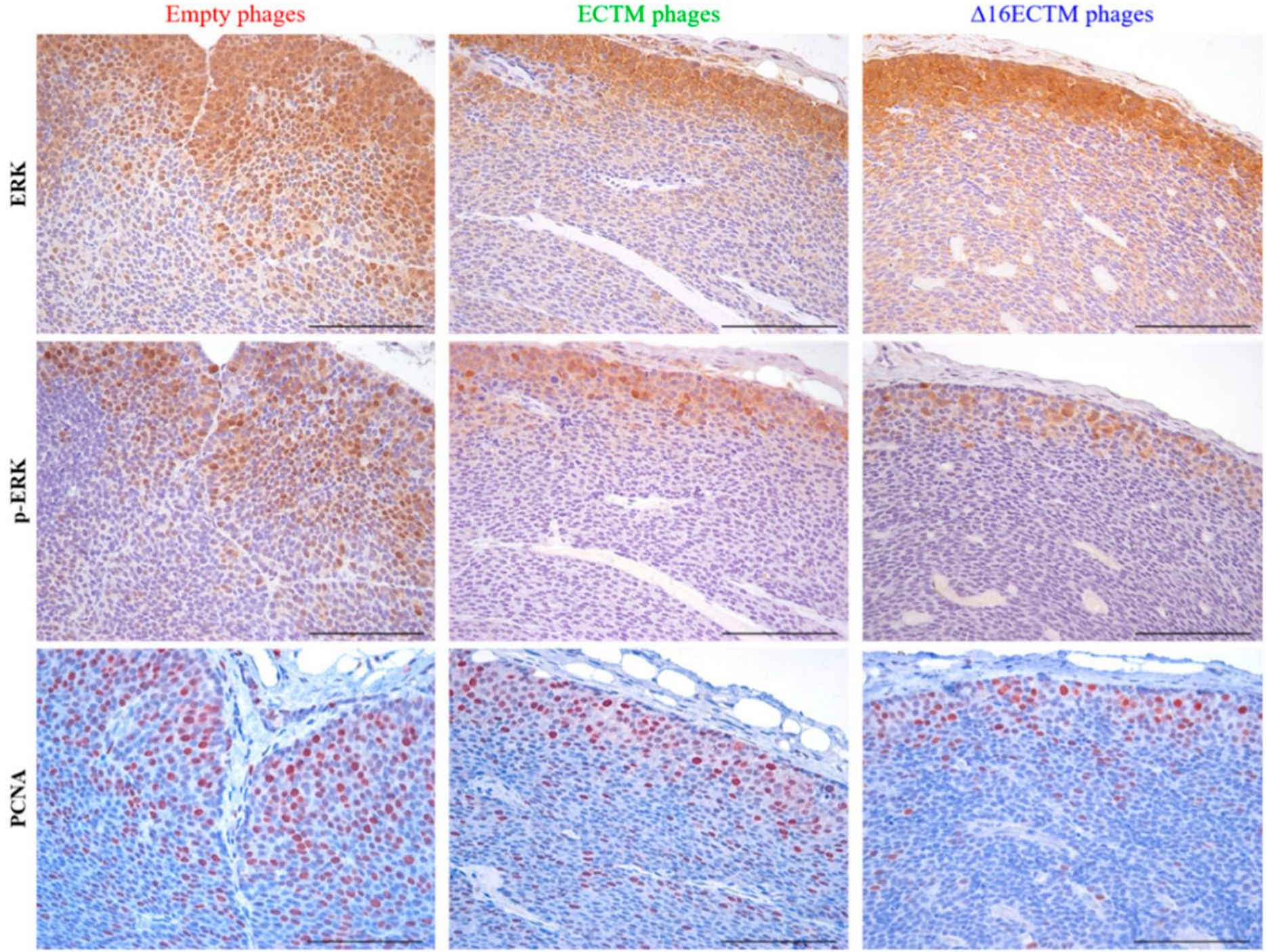


trastuzumab-resistant BT-474 cells.



- **Effects on Trastuzumab-Resistant Cells**:
  - Vaccinated mice’s sera impaired ERK activation and restored RB function in trastuzumab-resistant cells, suggesting a potential alternative for overcoming resistance.
- **Histological Analysis**:
  - Tumors from vaccinated mice showed lower levels of PCNA and p-ERK, indicating reduced proliferation and MAPK pathway activity.

- **Discussion**:
  - The downregulation of HER2 signaling by vaccine-induced antibodies highlights a dual mechanism of action: direct interference with tumor cell proliferation and enhancement of immune-mediated tumor killing.
  - The ability to target trastuzumab-resistant cells is particularly significant for addressing a major clinical challenge.


Empty phages
ECTM phages
Δ16ECTM phages
ERK
p-ERK
PCNA

4. Comparative Efficacy of ECTM vs. Δ16ECTM Vaccines

- **Key Points**:
  - ECTM phages consistently outperformed Δ16ECTM in delaying tumor onset, reducing growth, and eliciting higher antibody titers.
  - Δ16ECTM antibodies exhibited specific efficacy in mediating ADCC and showed unique benefits in targeting Δ16HER2-positive tumors.
- **Discussion**:
  - The differential efficacy reflects structural or immunological differences in the displayed HER2 epitopes.
  - Combining epitopes or enhancing Δ16HER2 immunogenicity could improve vaccine performance.

5. Clinical Implications

- **Potential Benefits**:
  - Ability to overcome trastuzumab resistance addresses a significant unmet need in HER2-positive breast cancer treatment.
  - Vaccines offer a long-term, cost-effective alternative to monoclonal antibodies like trastuzumab.
  - Phage stability, safety, and ease of production make them attractive candidates for clinical translation.
- **Challenges and Future Directions**:
  - Further optimization is needed to balance immunogenicity and tolerance-breaking properties.
  - Combination therapies with checkpoint inhibitors or adjuvants may enhance efficacy.
  - Clinical trials are required to confirm safety and effectiveness in humans.

Conclusion

Phage-based HER2 vaccines represent an innovative and potentially transformative strategy for combating HER2-positive breast cancer. By addressing key challenges such as trastuzumab resistance and recurrence, these vaccines could significantly expand the therapeutic options available to patients. With further research and clinical validation, phage-based vaccines may become a cornerstone of immunotherapy, offering safer, more durable, and cost-effective treatment solutions for this aggressive cancer subtype.

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