



Active-Conformation-Specific EGFR CAR T Cells Target TKI-Naïve and -Resistant HER2-Mutant and -Amplified NSCLC

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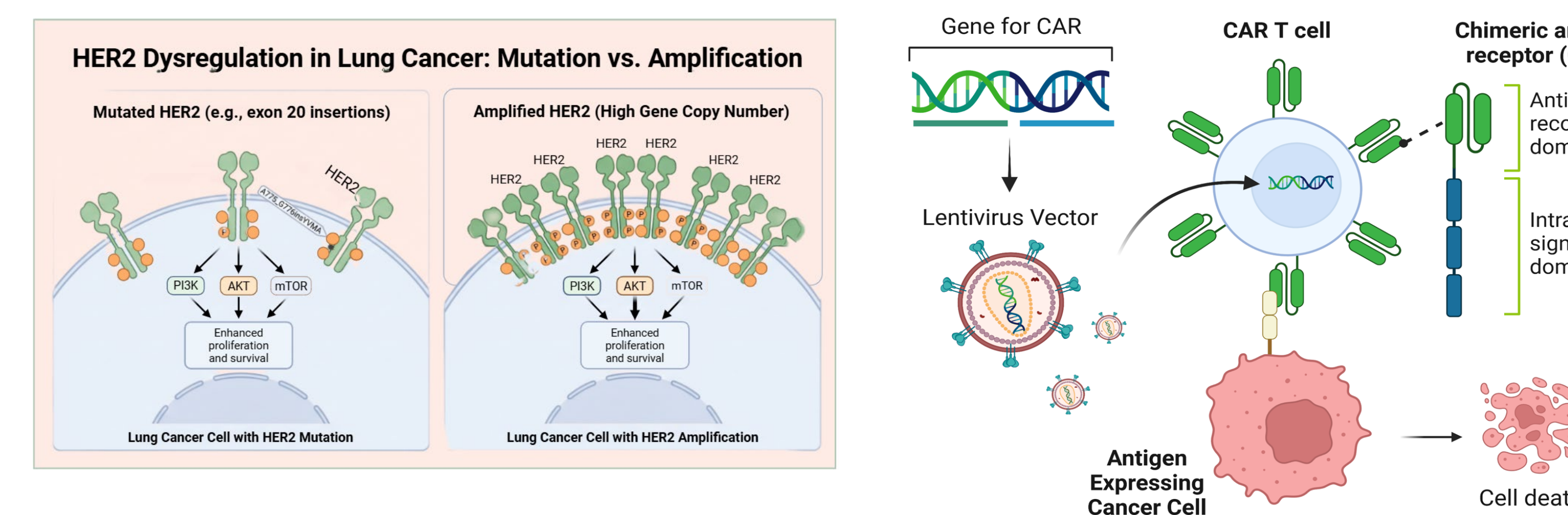
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INTRODUCTION

Non-Small Cell Lung Cancers (NSCLC) represent about 85% of lung cancers and frequently harbor targetable oncogenic mutations.

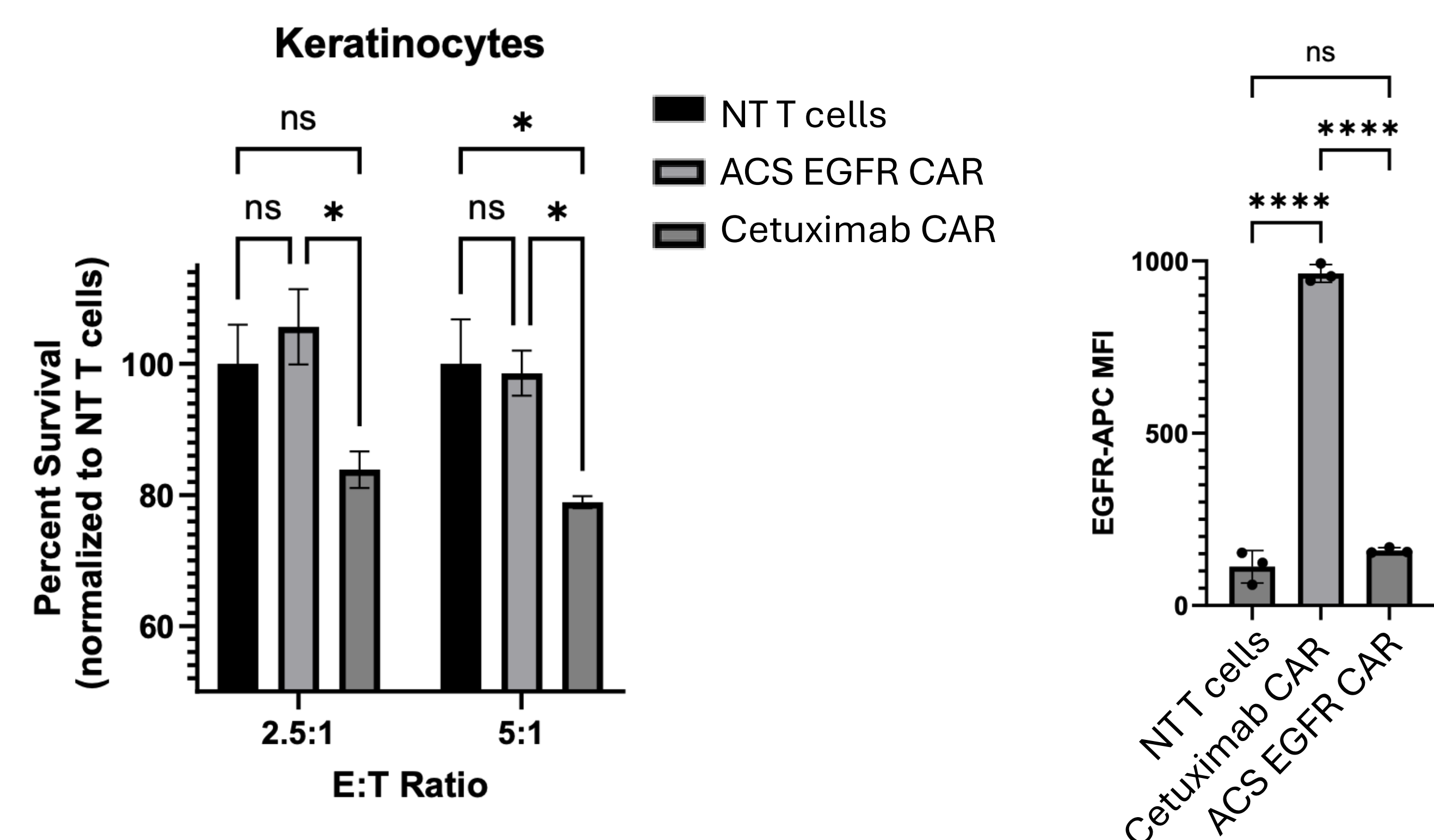
A subset of NSCLC is defined by HER2 mutations (2–4%) and amplifications (3–5%), which function as oncogenic drivers and confer sensitivity to tyrosine kinase inhibitors (TKIs).

Zongertinib is a third-generation highly selective HER2 TKI approved in 2026 for front-line treatment of HER2-mutant NSCLC which has shown preclinical efficacy against HER2-amplified tumors. While this treatment yields a median progression-free survival of 12.4 months, patients universally progress and additional treatments are urgently needed.



Cellular therapies have demonstrated curative potential in B cell malignancies but remain underdeveloped in lung cancer.

In collaboration with the Fecci laboratory at the University of Colorado, we identified that an active-conformation-specific (ACS) EGFR-targeting CAR T cell can spare inactive EGFR on normal keratinocytes and does not bind to monomeric ligand-free EGFR.

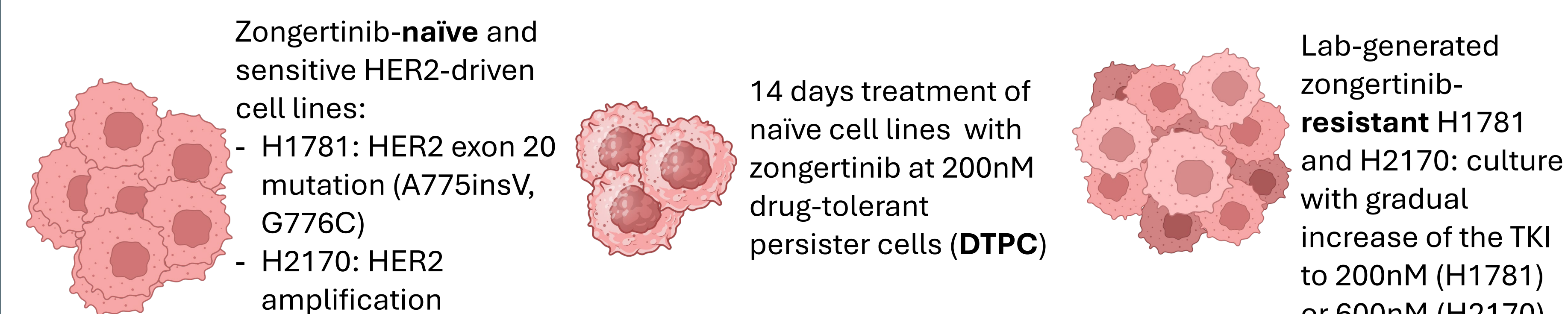


Hypothesis: We hypothesized that HER2-driven tumors maintain EGFR in an active conformation and therefore are sensitive to ACS-EGFR CAR T cell killing.

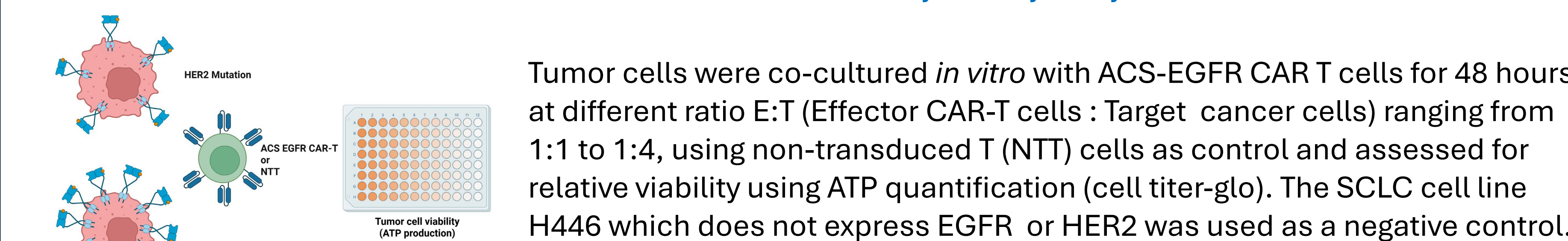
To test this hypothesis, we have evaluated the expression of EGFR in HER2-driven NSCLCs and tested the cytotoxicity of the ACS EGFR CAR-T cell therapy on TKI-sensitive and -resistant HER2-mutant and -amplified NSCLC models.

METHODOLOGY

We sought to define the dynamic changes of EGFR cell surface receptors exhibited by HER2-driven NSCLC cell lines at the naïve stage or after different *in vitro* treatments with zongertinib to generate DTPC or resistant models.

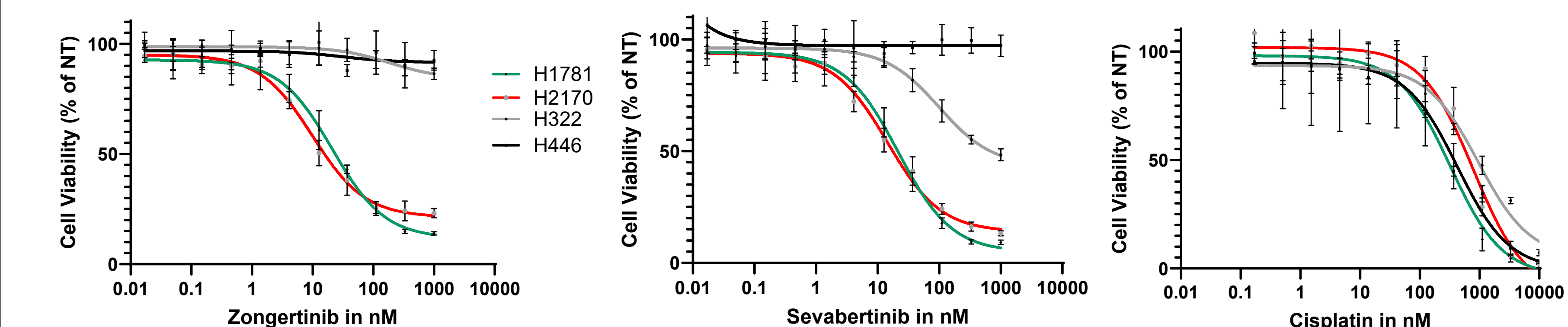


Comparison of expression markers at the surface by extracellular stain followed by flow cytometry profiles of selected surface NSCLC-related cytometry analysis

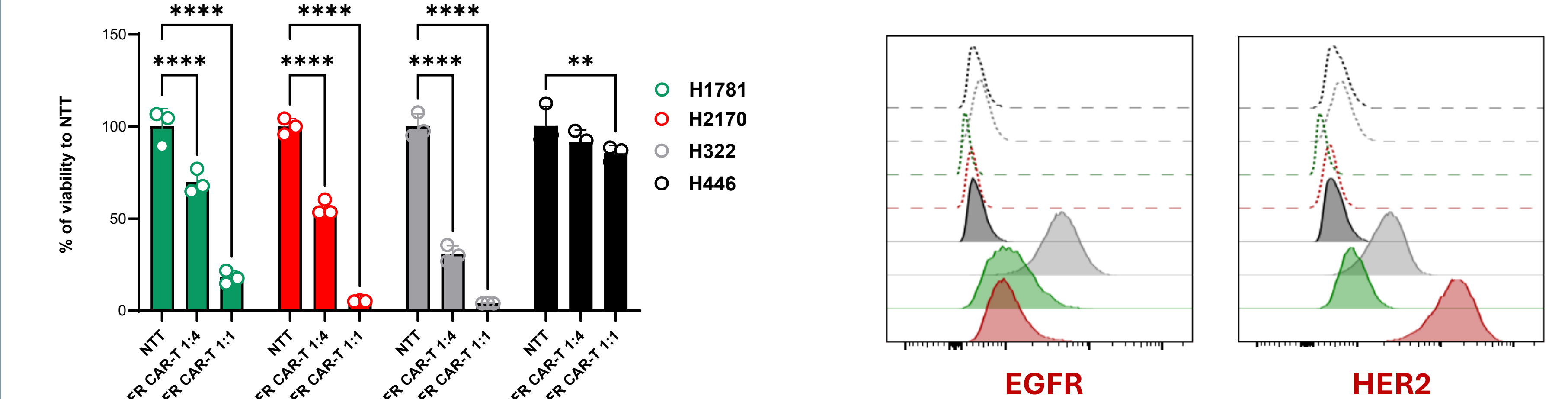


Comparison of cytotoxicity of ACS EGFR CAR T cells and efficacy at eradicating tumor cells *in vitro*

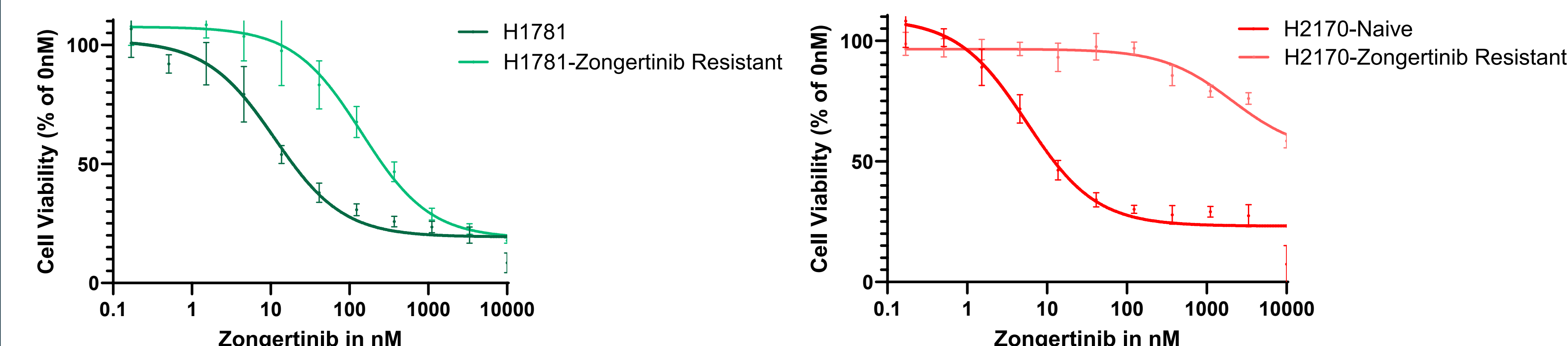
RESULTS



NSCLC cell lines H1781 and H2170 are sensitive to zongertinib *in vitro*

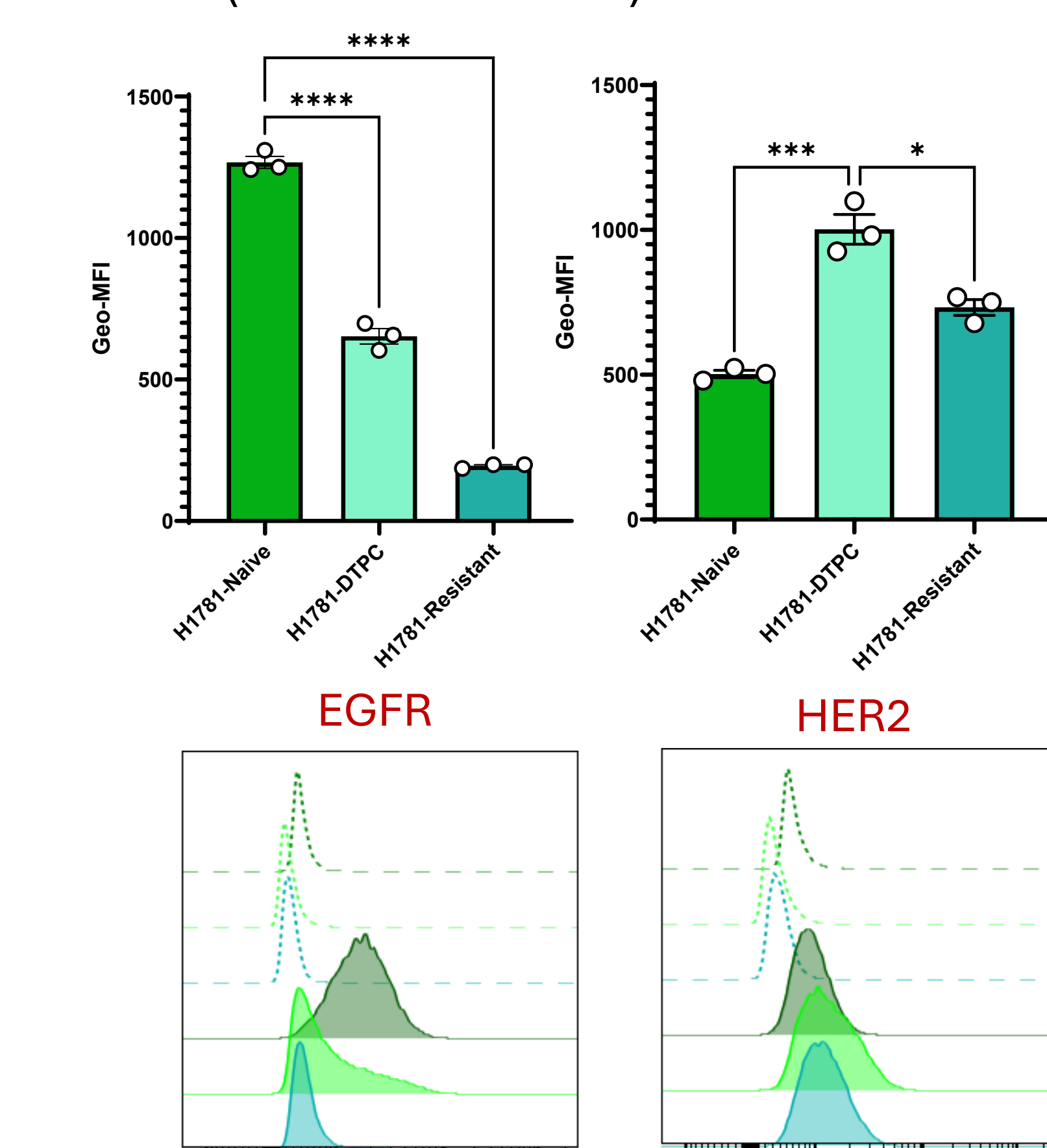


EGFR expression at the surface of HER2-driven NSCLC cell lines sensitizes them to the cytotoxic activity of ACS EGFR CAR T cells

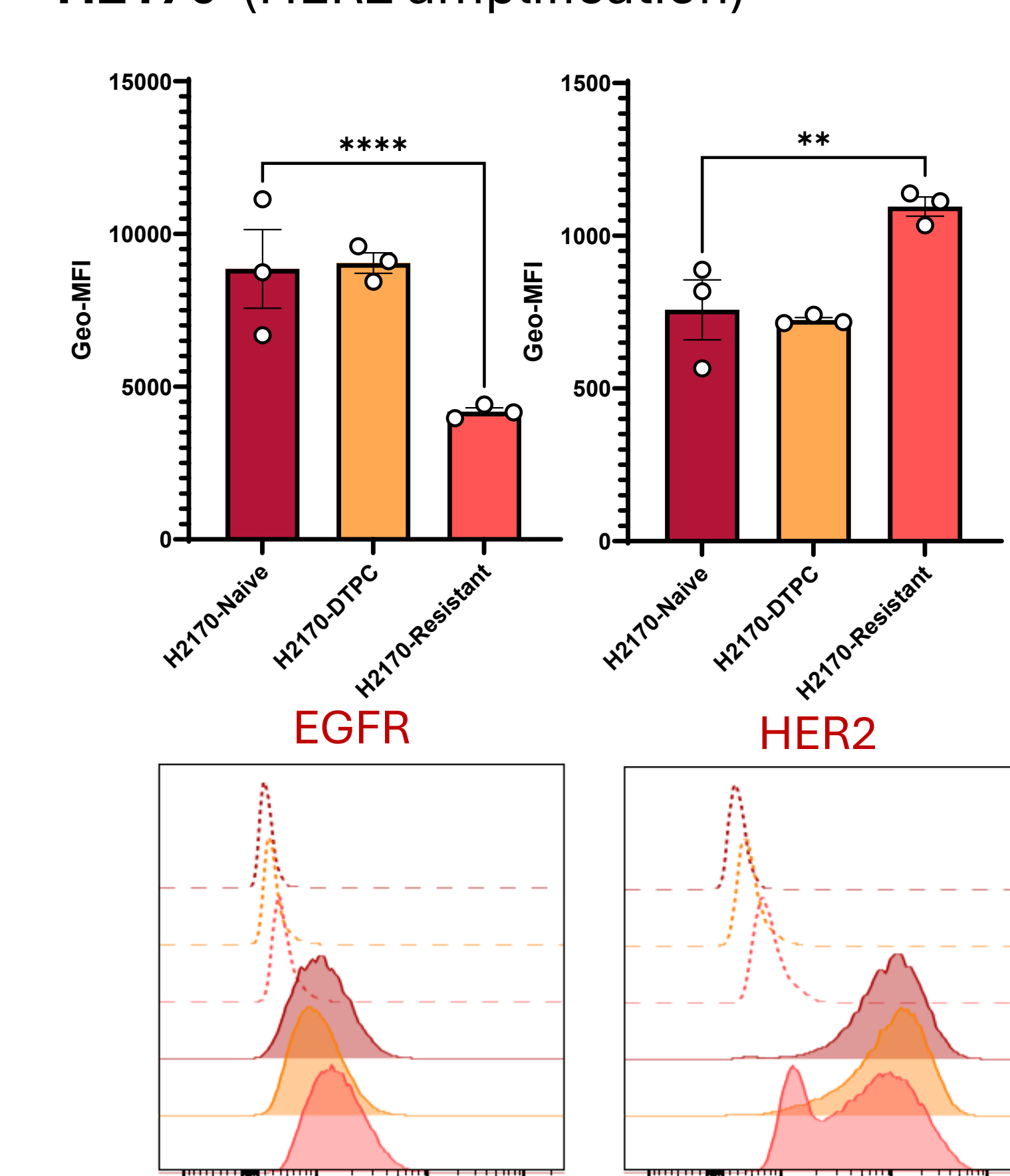


Zongertinib-resistant cell lines have been successfully generated from the zongertinib-sensitive H1781 (HER2-mutated) and H2170 (HER-amplified) cell lines

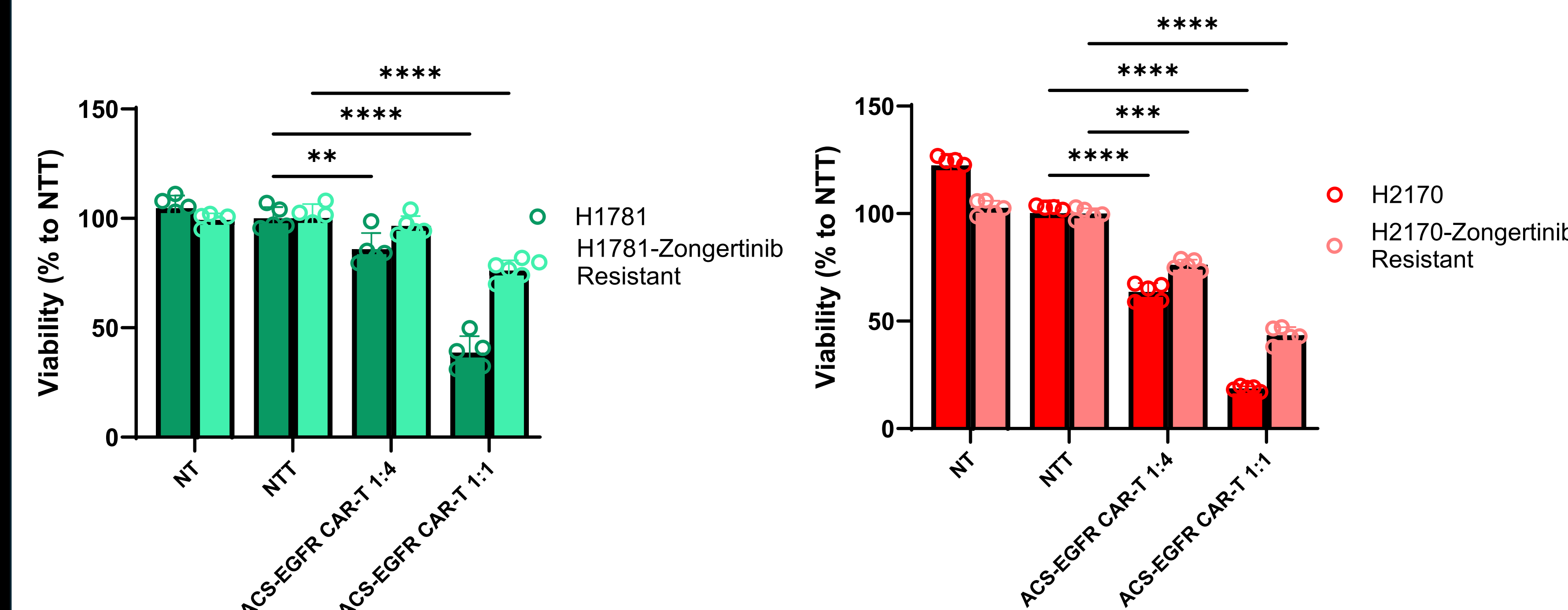
H1781 (HER2 mutations)



H2170 (HER2 amplification)



Dynamic changes in surface EGFR expression occur through the progression from a naïve state to DTPC or resistance after treatment with zongertinib



HER2-driven NSCLC cell lines can remain sensitive to ACS EGFR CAR T after zongertinib acquired resistance. Changes in EGFR expression below a threshold predict efficacy of ACS CAR T cells.

CONCLUSIONS

HER2-driven NSCLC cell lines demonstrated consistent expression of EGFR and HER2, including at the cell surface.

EGFR and HER2 receptors show dynamic changes at the surface of NSCLCs after developing resistance to zongertinib with some retaining their profile and other cell models concomitantly losing HER2 and EGFR during adaptation to the TKI.

ACS EGFR CAR T cells induced robust cytotoxicity across models with sufficient EGFR expression at the cell membrane.

CAR T cell efficacy was preserved in cell lines with primary or acquired resistance to HER2 TKI therapy exhibiting sufficient EGFR expression. In contrast, cell lines with minimal expression of EGFR were less sensitive to the cytotoxic effect of the CAR T mediated cytotoxicity, supporting target specificity.

Acknowledgements

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