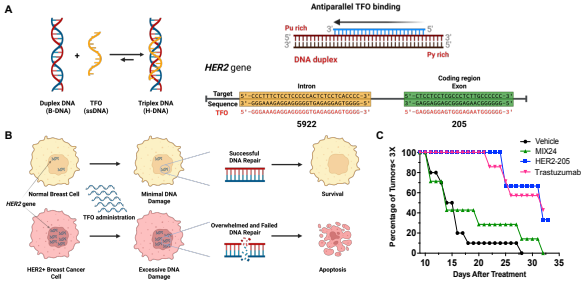
Direct targeting of amplified *HER2* gene activates immune signaling through DNA damage response

Adam Krysztofiak, Ayjha Brown, Anthony Minnah, and Faye A. Rogers
Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, CT

Background

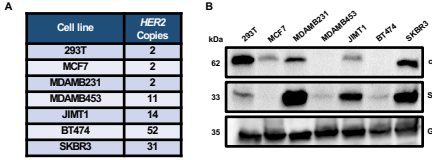
Human epidermal growth factor receptor 2 (HER2), which gene is often amplified in breast cancer (BC), promotes metastatic phenotype and confers aggressive tumor behavior. Progress made in recent years by therapeutic strategies using monoclonal antibodies has increased survival rates of HER2-positive BC patients. However, current clinical trials in BC combining immunotherapy (inhibition of PD-1/PD-L1) with anti-HER2 antibodies, resulted only in a relatively modest overall response rate. Intriguingly, recent studies provide strong evidence that genomic DNA damage leads to activation of the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway and facilitates antitumor immune responses. Therefore, the crosstalk between stimulation of the innate immune signaling and DNA damage response opens an interesting perspective of combination therapy strategies focusing on both DNA-damaging and immune system-activating therapies in BC. In this work we discover how a novel; technology able to directly target amplified gene loci in HER2-positive BC using triplex-forming oligonucleotides (TFOs) activates innate immune signaling through DNA damage response. Our work will aid in the design of novel combination therapeutic approaches to increase responsiveness of BCs to immune-targeted treatments through activation of DNA damage response and ultimately combating therapy resistance mechanisms.

Direct targeting of amplified *HER2* gene using TFOs



Direct targeting of amplified cancer genes to invoke apoptosis through the activation of DNA damage response offers an alternative mechanism to induce tumor-specific cell death. (**A**) The technology recently developed in our laboratory utilizes triplex-forming oligonucleotides (TFOs) to form triplex structures at amplified oncogenic loci. (**B**) Excessive distortion to the helical DNA structure caused by the TFOs (205 and 5922, targeting coding and intronic sequences respectively) leads to overwhelming DNA damage and apoptosis, but only in response to the formation of multiple triplex structures. As a result, normal cells having only two copies of the gene can efficiently handle a low level of triplex-induced DNA damage by employing nucleotide excision repair (NER). (**C**) This phenomenon provides an opportunity to specifically target and induce apoptosis in BC tumors with amplifications of the *HER2* gene with efficacy comparable to standard-of-care trastuzumab in a preclinical orthotopic mouse model [1].

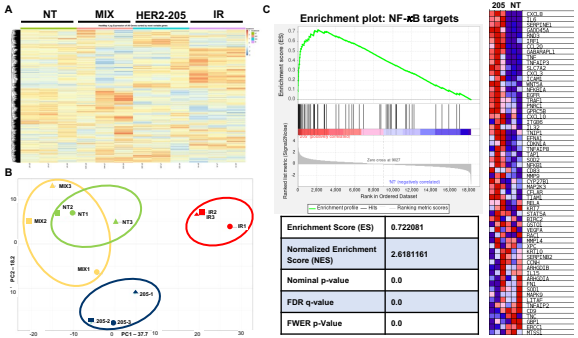
cGAS-STING landscape in breast cancer cell lines



Cell line	*HER2* Copies
293T	2
MCF7	2
MDAMB231	2
MDAMB453	11
JIMT1	14
BT474	52
SKBR3	31

(**A**, **B**) Landscape of *HER2* gene amplification status and expression of cGAS and STING in various breast cancer cell line models. SKBR3 cells were chosen as an experimental model based on a high *HER2* copy number and expression of both cGAS and STING as an experimental model. Copy number variation data from depmap.org [2].

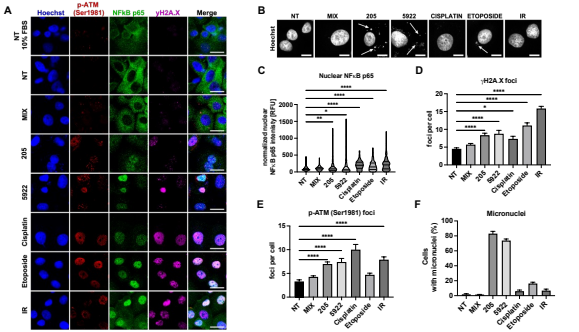
TFOs treatment leads to transcriptional induction of NF-κB signaling



Enrichment Score (ES)	0.722081
Normalized Enrichment Score (NES)	2.6181161
Nominal p-value	0.0
FDR q-value	0.0
FWER p-Value	0.0

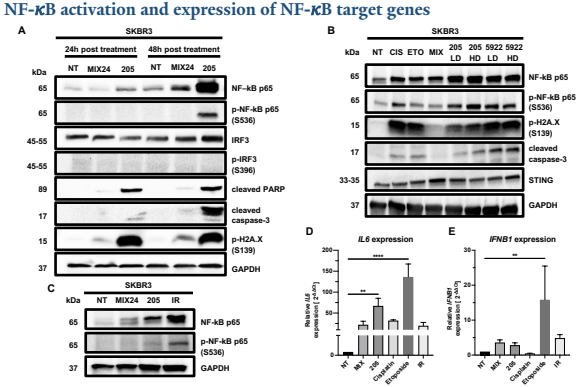
(**A**) RNA-seq analysis of 17,233 differentially expressed genes in SKBR3 HER2-positive breast cancer cells revealed that treatment with TFOs leads to significant changes in transcription patterns. (**B**) Principal Component Analysis (PCA) analysis showed that the HER2-205-driven gene expression also highly differs from treatment with ionizing radiation (IR) and non-targeted oligo (MIX). (**C**) Gene Set Enrichment Analysis (GSEA) identified expression of transcription factor NF-κB targets as significantly enriched upon TFO treatment, suggesting a potential activation of NF-κB signaling pathway.

Triplex-induced DNA damage triggers NF-κB nuclear translocation and creation of micronuclei in SKBR3 breast cancer cells



(**A**, **B**) Confocal immunofluorescence microscopy revealed that TFO-induced DNA damage (γH2A.X) and DNA damage response (p-ATM Ser1981) lead to translocation of NF-κB p65 to cell nucleus and formation of extra-nuclear DNA structures (micronuclei). (**C**, **D**, **E**, **F**) NF-κB p65 nuclear localization as well DNA damage and response are comparable with other anticancer agents, however micronuclei formation was highly elevated in cells treated with TFOs.
Scale bars: A = 20μm; B = 10μm.

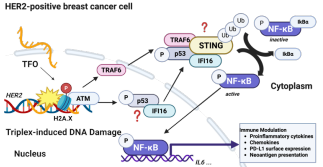
HER2-targeted TFOs and anticancer agents induce similar levels of NF-κB activation and expression of NF-κB target genes



(**A**) DNA damage (p-H2A.X S139) induced by HER2-targeted TFOs primarily triggers NF-κB activation (p-NF-κB Ser536), reflecting the non-canonical STING signaling response induced by nuclear DNA damage as analyzed by Western blot compared to canonical STING signaling (p-IRF3 Ser396). HER2-targeted TFOs also induced apoptosis as determined by cleaved PARP and cleaved caspase 3. (**B**, **C**) Levels of NF-κB activation induced by TFOs (205 and 5922 in low (LD) and high (HD) dose) are comparable to NF-κB activation induced by classical anticancer agents: cisplatin (CIS), etoposide (ETO), and ionizing radiation (IR). (**D**, **E**) TFOs treatment leads to increased expression of NF-κB target gene *Il6* (interleukin-6) but has no effect on expression of interferon β (*IFNB1*) as analyzed by qPCR.

Summary and future directions

We have validated that HER2-targeted TFOs can induce activation of the innate immune system signaling in BC cells through activation of NF-κB, however the exact mechanism of this activation remains to be explored. Our work contributes to understanding of the crosstalk between DNA repair, apoptosis, and innate immune signaling. This will aid in the design of novel combination therapeutic approaches to increase responsiveness of BCs to immune-targeted treatments through activation of DNA damage response and ultimately combating therapy resistance mechanisms.



References

[1] Kaushik Tiwari, M. *et al.*, Direct targeting of amplified gene loci for proapoptotic anticancer therapy. *Nat Biotechnol.* 40, 325–334 (2022).

[2] DepMap, Broad DepMap Public 25Q3. Dataset. depmap.org (2025).

Graphics created in BioRender (biorender.com)

Acknowledgments

This work was supported by a Postdoctoral Fellowship Grant PF-23-1077635-01-CDP from the American Cancer Society to A.K..