A Nucleic Acid Sensing System for Selective Therapeutic Protein Expression in Breast Cancer

Alinés Lebrón-Torres[3], Nolan Priedigkeit[1,2,3], Jeremy Koob[3,4], Janny Liao[4], Yixue Wang[3,4], Jordan Chinai[1,2,3], Melissa E. Hughes[1], Daniel L. Abravanel[1], Sara M. Tolaney[1], Nancy U. Lin[1], Steffi Oesterreich[5], Adrian V. Lee[5], Fei Chen[3], Todd R. Golub[1,2,3]

[1]Dana-Farber Cancer Institute, Boston, MA, [2]Harvard Medical School, Boston, MA, [3]The Broad Institute of MIT & Harvard, Cambridge, MA., [4]Harvard University, Cambridge, MA, [5]University of Pittsburgh, Pittsburgh, PA

Supported by: Lubin Family Foundation, Susan G. Komen, AACR American Association for Cancer Research, Conquer Cancer The ASCO Foundation

INTRODUCTION

Somatic structural variation (SV) is a hallmark of breast cancer, producing cancer-specific nucleotides including both driver and truncal fusion RNAs that can be **highly expressed**, representing a promising target in cancer

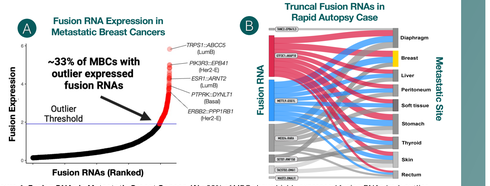


Figure 1. Fusion RNAs in Metastatic Breast Cancer. **(A)** ~33% of MBCs have highly expressed fusion RNAs (red; outlier threshold in blue). **(B)** Rapid autopsy case with 2 truncal fusions (*GTF3C1::AKAP10*, red;*METTL9::GSG1L*, blue) detected in all metastases as well as archived primary breast cancer (yellow)— not detected in normal tissue.

We **hypothesize** that fusion RNAs arising from truncal SVs or therapy-driven selection may be underrecognized cancer-specific targets, exploitable by recent advances in nucleic-acid technologies

We aim to optimize an RNA-editing approach, **Reprogrammable ADAR sensors (RADARS),** to drive the **selective expression of cytotoxic, immune-stimulatory, and other therapeutic protein payloads in cancer cells**

METHODS

Fusion RNAs as Molecular Switches for Selective Protein Expression via RNA Sensors

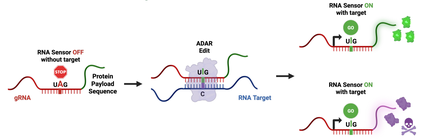


Figure 2. Mechanism of Action of Reprogrammable ADAR sensors (RADARS). Engineered nucleic acid "sensors" selectively bind target RNAs, forming a dsRNA duplex that recruits ADAR to edit an intentional A:C mismatch, triggering translation of a linked protein cargo only when the target RNA is present.

Designing Fusion Targeting RNA Sensors

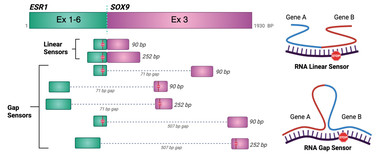
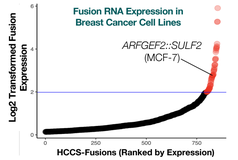


Figure 3. Design Architectures of Fusion-targeting RNA Sensors. We designed and tested 91 sensors against cancer fusion RNA targets with two broad designs—*linear* sensors that hybridize directly across the fusion RNA breakpoint sequence and *gap* sensors that target distinct regions of the 5' and 3' fusion partners on the same guide. *ESR1::SOX9* representative fusion is shown.

Validating Endogenous Fusion RNA Sensor Targets in BrCa Models



Sample	Fusion	Split Reads	Spanning Reads	FFPM
MCF-7	*ARFGEF2::SULF2*	603	162	8.6

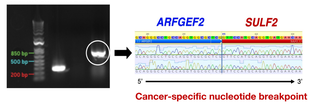


Figure 4. Validation of Outlier Expressed Fusion RNAs in BrCa Cell Line Models. Outlier-expressed fusion RNAs are prevalent across luminal, HER2+, and basal-like cell models, mirroring human tumor data. We selected the *ARFGEF2::SULF2* fusion as a candidate RNA target for sensor design and confirmed its cancer-specific nucleotide breakpoint by RT-PCR using primers spanning the fusion breakpoint in MCF-7. An ~850 bp product spanning the breakpoint was detected *exclusively* in MCF-7, and sequencing confirmed the fusion junction.

General Experimental Workflow

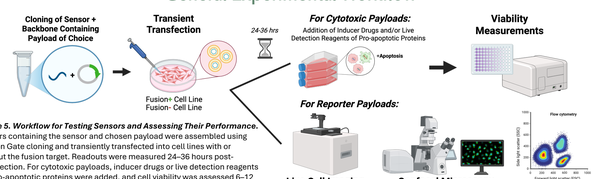


Figure 5. Workflow for Testing Sensors and Assessing Their Performance. Vectors containing the sensor and chosen payload were assembled using Golden Gate cloning and transiently transfected into cell lines with or without the fusion target. Readouts were measured 24–36 hours post-transfection. For cytotoxic payloads, inducer drugs or live detection reagents for pro-apoptotic proteins were added, and cell viability was assessed 6–12 hours later using CellTiter-Glo. For reporter payloads, fluorescence was measured via live-cell imaging, confocal microscopy, or flow cytometry.

CONCLUSION

Fusion RNAs are prevalent cancer-specific nucleotide alterations in breast cancer–representing promising therapeutic targets

An RNA-editing approach enables precise and selective expression of therapeutic proteins; triggered by cancer-defining nucleic acids

Fusion RNA-targeting sensors armed with cytotoxic payloads can selectively kill breast cancer cells *in vitro*

More broadly, nucleic acid sensing systems may offer a scalable, modular, and precise strategy to selectively rewire protein-based vulnerabilities into cancer cells

Looking Ahead

Design

What design rules are most essential for cancer-killing sensors to increase specificity, decrease toxicity?

1

Delivery

How to deliver effectively in a patient with widely metastatic disease?

2

Death

What are the key protein payload / vulnerabilities to rewire selectively into cancer cells?

3



Large-scale screen of modular sensors to establish design rules and *in-vivo* testing of sensors



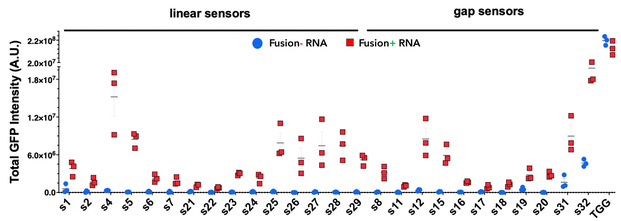
Figure 9. RNA Sensor Pooled Screening Strategy and In-Vivo Testing. **(A)** Schematic of pooled library workflow including cloning, lentiviral transduction, FACS sorting, and barcode sequencing. Representative output plot on right showing on-target vs off-target scores, with optimal sensors highlighted (red). **(B)** Testing of reporter, apoptotic, and immune stimulatory payload armed sensors in PDOs and PDX with AAV delivery.

Acknowledgements

We would like to thank patients who selflessly contribute to translational research efforts and the collaborative research groups / resources of the Broad Institute and DFCI. **Funding:** Breast Cancer Research Foundation, Terri Brodeur Breast Cancer Foundation, Benderson Family Fund, AACR/ASCO YIA, Richard K. Lubin Family Foundation through the Lubin Family Foundation Scholar Award, and Susan G. Komen.

RESULTS

Selective Expression of a Reporter Protein Payload in Presence of Cancer-Specific RNA



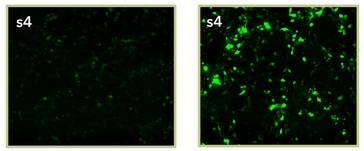
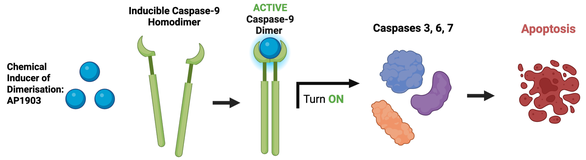
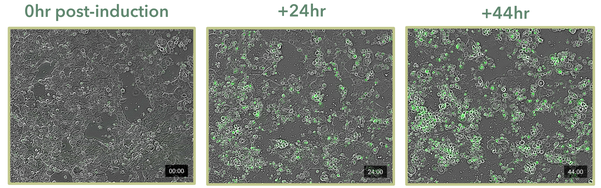


Figure 6. Selective Expression of mNeon in Prescence of Cancer-Specific RNA Target. Sensors targeting the *ESR1::SOX9* fusion RNA were tested in HEK293T cells. Co-transfection with the fusion RNA resulted in significantly elevated mNeon expression, while cells lacking the fusion showed minimal background fluorescence (mean 25-fold increase vs. no-target controls, $p < 0.0001$), demonstrating sensor specificity. Fusion-positive cells red, fusion-negative blue.

Exogenous iCaspase-9, a Human Tested Apoptotic Protein Payload, kills MCF-7 Cells

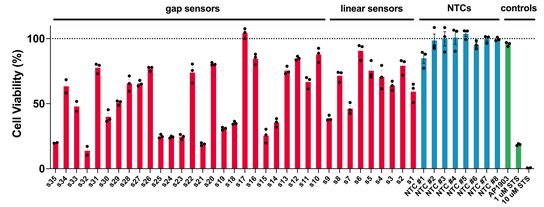


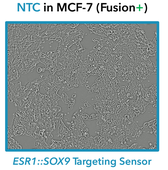


Live Cleaved Caspase 3/7 Detection Assay

Figure 7. Exogenous Expression of iCaspase-9 in MCF-7 cells stained with Cleaved Caspase 3/7 Detection. Live-cell imaging of transiently transfected MCF-7 cells expressing constitutive iCaspase-9 shows pronounced induction of apoptosis following AP1903 treatment, evidenced by a time-dependent accumulation of green fluorescent apoptotic cells detected using CellEvent™ Live Cleaved Caspase-3/7 Green Reagent, up to 44 hours post-induction.

Selective Killing of MCF-7 Cells with Endogenous Fusion RNA Targeting Sensors







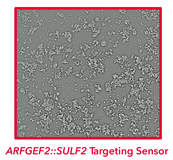


Figure 8. Cell Viability of MCF-7 Expressing ARFGEF2::SULF2 Targeting Sensors Relative to Controls. Transfection of both linear and gap fusion-targeting sensors into MCF-7 cells, followed by treatment with the iCaspase-9 inducer AP1903, led to a significant reduction in cell viability (up to 84% at 36 hours post-transfection compared to non-targeting controls, $p < 0.0001$). Live-cell imaging confirmed robust sensor-induced cell death in MCF-7 cells, whereas fusion-negative cells showed no toxicity when treated with the same sensors, demonstrating high target specificity. Fusion-targeting sensors are *red*, nontargeting sensor controls are blue, empty vector and apoptosis positive controls (STS, staurosporine) are green.

This presentation is the intellectual property of the author/presenter. Contact them at alebront@broadinstitute.org for their permission to reprint and/or distribute.