microRNA-21 blockade in human triple negative breast cancer allografts stopped progression and reduced immune checkpoint proteins

Yuan-Yuan Jin[1], Vilas Desai[2], Miguel Castro[3], Edith Mitchell[4], and Eric Wickstrom[1]

[1]Bound Therapeutics LLC, Cherry Hill NJ 08003; [2]University of Pennsylvania, Philadelphia PA 19104;
[3]Bio-Synthesis, Inc., Lewisville TX 75067; [4]Thomas Jefferson University, Philadelphia PA 19107, USA

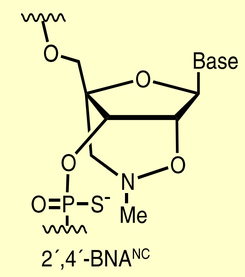
Introduction

Triple negative breast cancer (TNBC) is the most aggressive type of breast cancer, affecting >46,000 US women every year. TNBC recurs after standard-of-care surgery, chemotherapy and radiation, killing half its victims within 4 years. Poly(ADP-ribose) polymerase (PARP) inhibitors, topoisomerase inhibitors, immune checkpoint inhibitors, and antibody-drug conjugates extended the survival of a subset of TNBC patients by a few months. Thus, TNBC shows a critical need for molecularly-targeted therapies. Most TNBC cells show high microRNA 21 (miR-21), identical in humans and mice, which decreases tumor suppressor proteins that keep cell growth in check [1]. Most TNBC cells show strong insulin-like growth factor 1 receptor (IGF1R) expression and signaling activation, correlating with poor survival [2]. We hypothesized that short anti-miR-21 RNA analogs conjugated to an IGF1R ligand will direct TNBC cell uptake and slow the growth of TNBC orthotopic allografts with immune activation and minimal toxicity.

RNA-Peptide Therapeutic Design

- Eliminate passenger strand-mimicking side-effects of conventional anti-microRNA [3]
- Soluble in saline, no formulation necessary, intravenous administration
- Next generation RNA backbone (NC-BNA) [4] elevates efficacy, potency, and safety.

Next-Generation RNA Analog



Latest generation 2'-NC-bridged nucleic acid (NC-BNA) [4] backbone modification offers greater nuclease resistance, increased bio-availability, and improved solubility. Anti-miR-21 BND5412 is a 5-5-5 gapmer.

RNA-Peptide Analog Platform

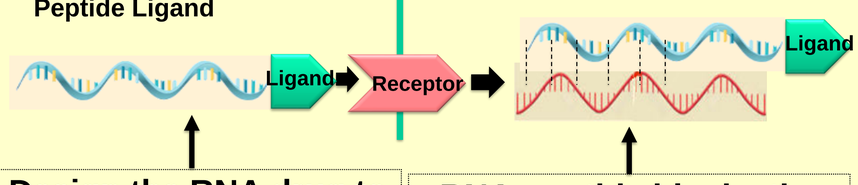
Outside Cancer Cell

Cancer cells overexpress receptors that bind to abundant growth factors in the blood, promoting accelerated cancer cell growth.

Inside Cancer Cell

Our platform utilizes a receptor-targeting peptide ligand to deliver a drug cargo, comprising a conjugated RNA analog, that blocks a cancer-driving RNA in the cytoplasm.

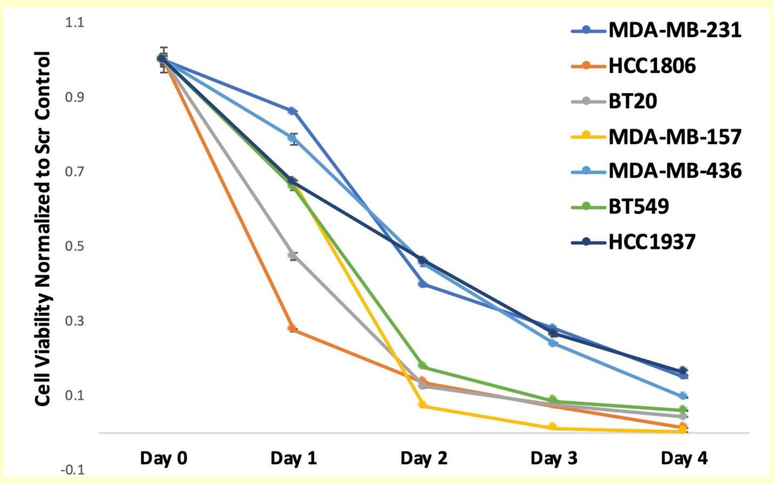
Cancer-Blocking RNA Drug with Receptor-Targeting Peptide Ligand



Design the RNA drug to target any cancer-promoting RNA.

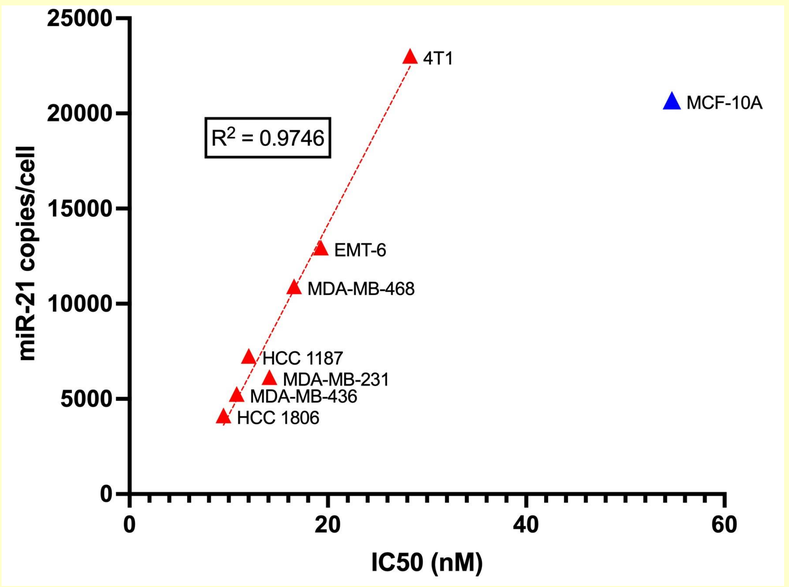
RNA-peptide blocks the cancer-driving miR-21 inside the cancer cell.

Anti-miR-21 TNBC Cell Inhibition



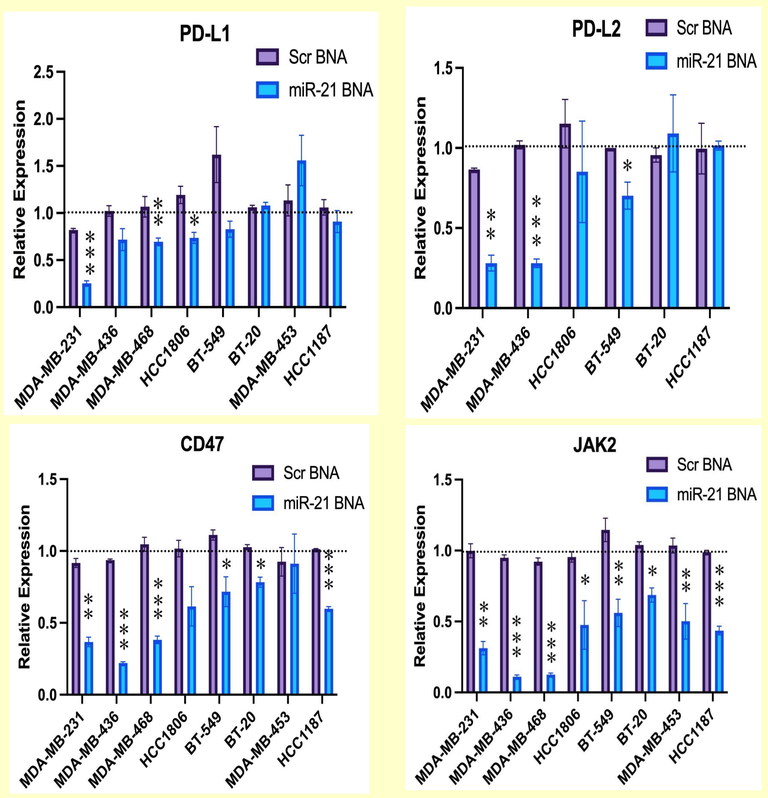
Cell Titer Glo assay ± s.d. was performed following transfection of TNBC cells with 50 nM anti-miR-21 BND5412 on day 0. BND5412 resulted in a greater than 8-fold decrease in cell proliferation over 4 days.

Anti-miR-21 IC50 vs. miR-21 Copy Number in Multiple TNBC Cell Lines



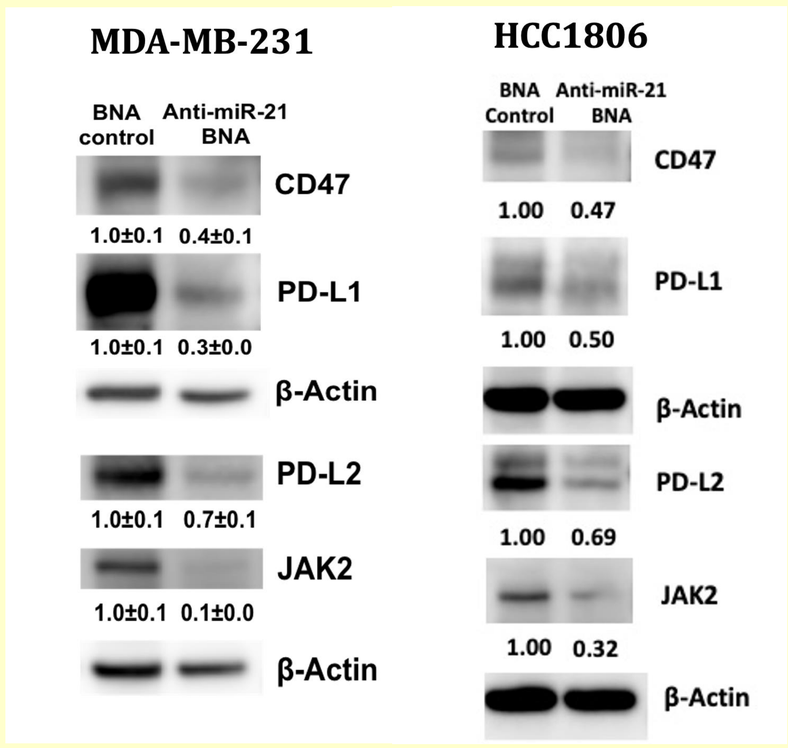
Significant correlation between cell proliferation IC50 and miR-21 copies/cell in 7 TNBC cell lines vs. non-tumorigenic breast epithelial MCF-10A cells transfected with concentration gradients of anti-miR-21 gapmer BND5412.

Anti-miR-21 Significantly Reduced Immune Checkpoint mRNAs in TNBC Cells



Transfecting 8 human TNBC cell lines with 50 nM of anti-miR-21 BND5412 for 24 hours resulted in a significant reduction in immune checkpoint mRNAs, including PD-L1, PD-L2, CD47, and JAK2. Error bars, s.e.m.

Anti-miR-21 Down-Regulated Immune Checkpoint Proteins in TNBC Cells

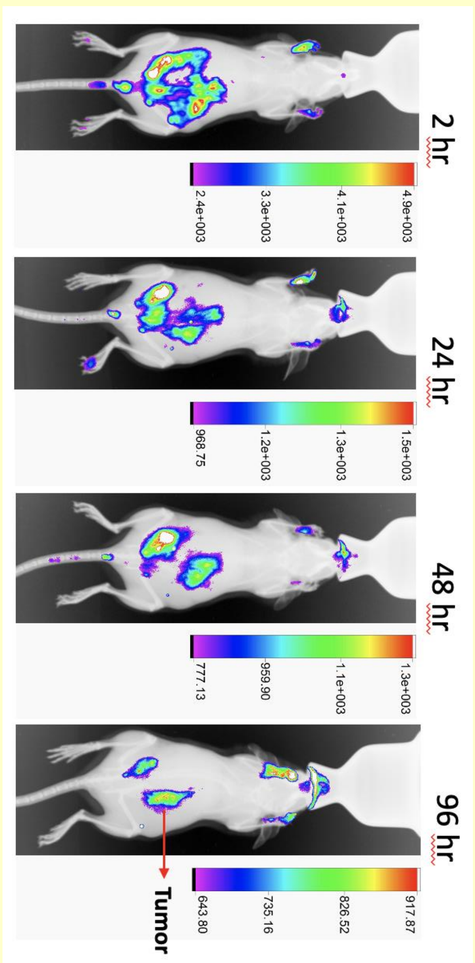


Transfecting MDA-MB-231 (left) or HCC1806 cells (right) with 50 nM of anti-miR-21 blocker BND5412 for 72 hours significantly decreased immune checkpoint protein levels.

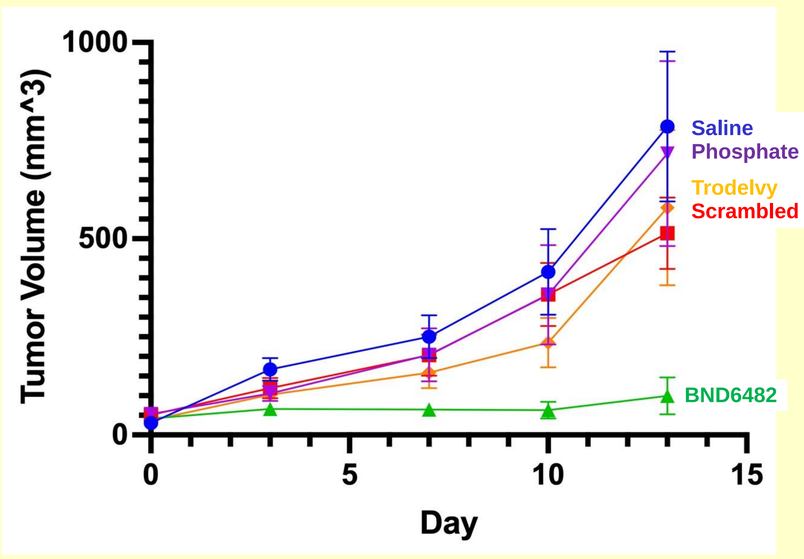
IGF1R-Targeting Peptide-RNA Conjugation Yields BND6482

Fluorescent BND6482 distributed to EMT6 TNBC allografts in syngeneic female immunocompetent Balb/c mice

AlexaFluor647-BND6482 distribution to EMT6 allografts in n=3 female immuno-competent female Balb/c mice 2 hr - 96 hr after a single 5 mg/kg intraperitoneal injection. Mice were imaged in a Bruker FX Pro In Vivo Imaging System, exc. 630 nm, emm. 700 nm.



BND6482 stopped TNBC tumor growth in a mouse model, but Trodelvy did not.



Tumor volumes of EMT6 orthotopic TNBC allografts in mammary fat pads ceased to progress upon twice weekly intraperitoneal administration of BND6482 at 5 mg/kg. Vehicle, scrambled drug, and anti-Trop-2-irinotecan (Trodelvy) showed continued growth. Error bars, s.e.m.

Conclusions

- BND5412 stopped TNBC cell growth in multiple TNBC cell lines.
- BND5412 reduced several immune checkpoints in TNBC cells, potentially enhancing susceptibility to immune cell attacks.
- BND5412 is safe for normal breast cells.
- The anti-miR-21 RNA-peptide conjugate BND6482 exhibited retention in TNBC tumor allografts in syngeneic mice.
- BND6482 stopped TNBC tumor allograft growth in syngeneic mice.
- BND6482 was more active than recently approved ADC Trodelvy.
- Blood chemistry showed no liver or kidney toxicity from BND6482 (data not shown).

References

1. Jin, et al. (2014) *Breast Cancer Res Treat* 146(1):41-50, PMID: 24863696.
2. Law, et al. (2008) *Cancer Res* 68(24):10238-46, PMID: 19074892.
3. Jin, et al. (2015) *PLoS One* 10(12):e0142574, PMID: 26629823.
4. Rahman, et al. (2008) *J Am Chem Soc* 130(14):4886-96. PMID:18341342.

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