Antisense Oligonucleotide Therapeutics with Receptor-Targeted Delivery in Triple Negative Breast Cancer Cells via MicroRNA Blockade without Passenger Strand Side Effects

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Introduction

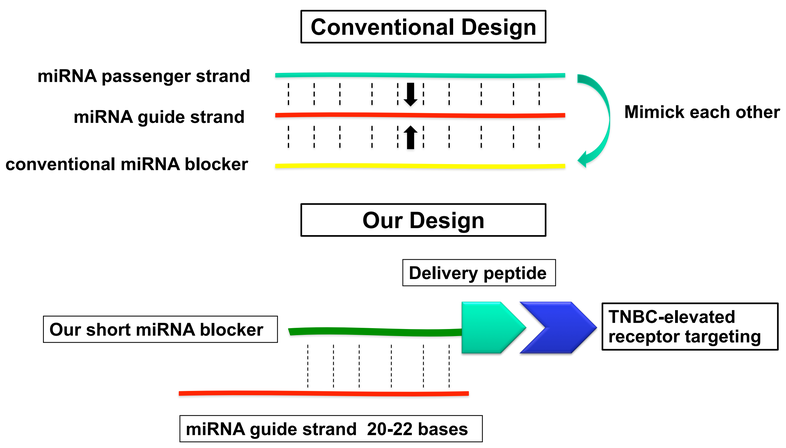
Triple negative breast cancer (TNBC) attacks >40,000 young women annually in the US, and has no molecular targeted therapies. Highly expressed microRNAs, such as miR-17-5p and miR-21-5p, are distinctive in TNBC and contribute to cancer cell survival. We hypothesized that reduction of oncogenic miRNA activity by delivering oligonucleotide-based antagomiRs specifically to breast cancer cells via receptor-mediated endocytosis would inhibit metastatic behavior of TNBC cells.

We designed guide strand specific blockers of miR-17-5p or miR-21-5p composed of gapmers with anionic backbone derivatives 2'-fluoro-arabino nucleic acid (FANA), and 2'-aminomethyl-bridged nucleic acid (NC-BNA). Since most breast cancer cells overexpress insulin-like growth factor receptor (IGF1R), a peptide derivative of the IGF1 was conjugated to anti-miR-17-5p antagomiRs to achieve breast cancer cell specific delivery.

Our results showed that in TNBC cells, the NC-BNA gapmers displayed high efficacy with sub-nM activity for miRNA blockade and significant inhibition of cell growth. Anti-miR-17-5p-IGF1 peptide conjugate successfully inhibited miR-17-5p activity in the luciferase reporter system. In addition, a newly designed NC-BNA gapmer against *MYCC* oncogene was able to reduce c-myc protein in TNBC cells, and lowered the expression of immune checkpoint inhibitor PD-L1.

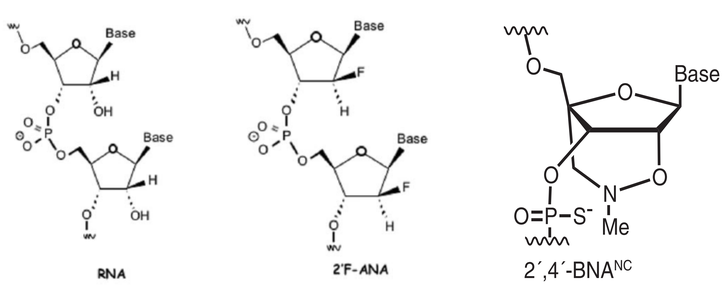
MicroRNA Therapeutics Platform Design

- **Eliminate passenger strand-mimicking side-effects of conventional microRNA blockers**
- **No complicated formulation, soluble in saline, intravenous route**
- **Next generation RNA backbones (FANA & NC-BNA vs. PNA & LNA) will elevate efficacy and potency**



Next-Generation Oligonucleotide Chemistry

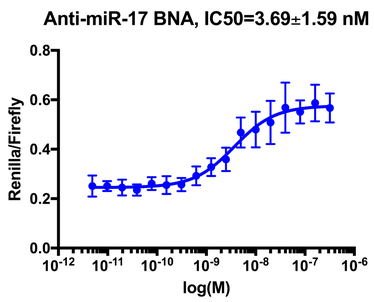
Latest generation of oligonucleotide backbone modifications offers greater resistance against protease and nuclease, increased bio-availability, and improved solubility.

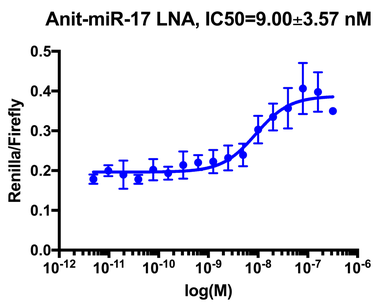


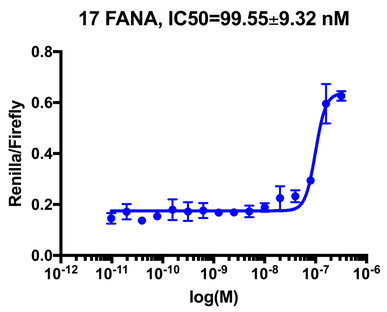
IC50 Comparison of Anti-miR-17-5p Oligonucleotides in TNBC Cells

We used a luciferase reporter system harboring a miR-17-5p binding site in the 3' UTR of renilla luciferase gene to characterize IC50s of miR-17 blockers with various oligonucleotide backbones in MDA-MB-231 cells.

NC-BNA gapmer showed higher efficacy than LNA and FANA.





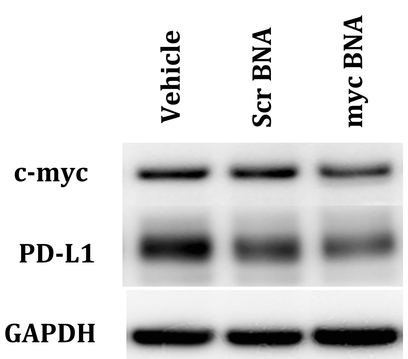


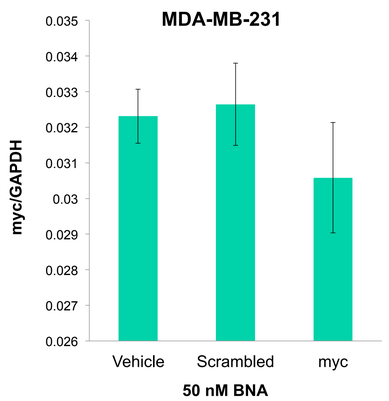
Oligonucleotide	IC50 (nM)
Anti-miR-17 BNA	3.69±1.59
Anti-miR-17 LNA	9.00±3.57
Anti-miR-17 FANA	99.55±9.32

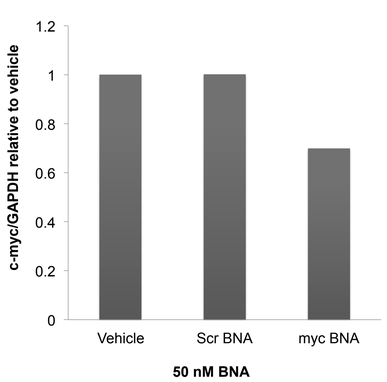
Dose dependent anti-miR-17-5p oligonucleotides were co-transfected with miR-17 luciferase reporter vector for 24 hours in MDA-MB-231 cells. Error bars, s.e.m.

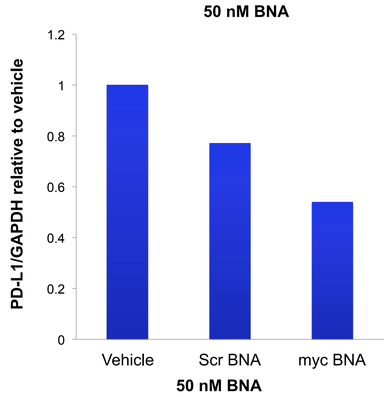
Down-regulation of Immune Checkpoint Inhibitor via *MYCC* mRNA Knockdown

Anti-*MYCC* NC-BNA gapmer reduced c-myc protein and mRNA, while decreasing the protein level of immune checkpoint inhibitor PD-L1 in MDA-MB-231 cells. Error bars, s.e.m.



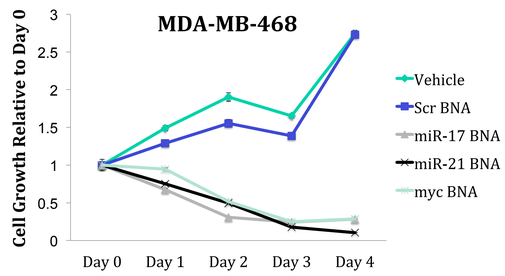






Growth Inhibition in TNBC and Lung Cancer Cells

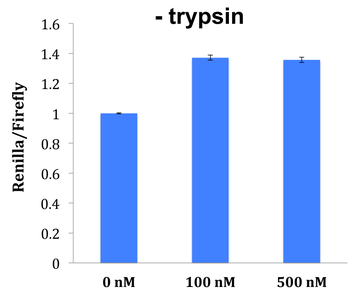
50 nM anti-miR-17-5p, anti-miR-21-5p, or anti-myc NC-BNA gapmers significantly regressed cell proliferation in multiple subtypes of TNBC cell lines. Error bars, sd.

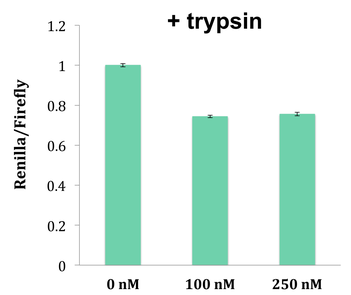


Fold Reduction in Cell Growth after 4 Days Treatment with 50 nM BNA				
Cell Line	**Subtype**	**miR-21 BNA**	**miR-17 BNA**	**MYC BNA**
MDA-MB-468	BL1	26.3	9.6	9.5
MDA-MB-231	MSL	9.5	2.4	8.6
MDA-MB-436	MSL	10.5	9.7	18.3
BT-549	M	16.7	2.4	5.0
HCC1937	BL1	6.2	1.5	4.1
HCC1806	BL2	73.9	22.0	83.8
BT-20	Unclassified	23.1	5.9	20.2
A549 (lung)	NSCLC	309.5	94.3	89.5

TNBC Cell Directed Delivery and Inhibition of miR-17-5p Activity

Anti-miR-17-5p NC-BNA gapmer conjugated with an IGF1R targeting peptide inhibited miR-17-5p in MDA-MB-231 cells without any transfection agent. Error bar, sd.





Conclusions & Future Direction

- **TNBC cells and NSCLC cells slowed proliferation dramatically upon transfection with 50 nM microRNA and *MYCC* mRNA BNA-DNA-BNA 15mer gapmers.**
- ***MYCC* BNA-DNA-BNA gapmers reduced PD-L1 expression in TNBC cells.**
- **The specificity of NC-BNA gapmers against target RNAs will be evaluated by RNA-seq.**
- **The effect of the NC-BNA-peptides on metastatic behavior of TNBC cells will also be tested.**
- **The expressions of other cancer immune surveillance marker will be evaluated as a result of *MYCC* inhibition.**

References

1. Jin, et al. (2014) *Breast Cancer Res Treat* **146**(1):41-50, PMID: 24863696.
2. Jin, et al. (2015) *PLoS One* **10**(12):e0142574, PMID: 26629823.

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