Nano-immunotherapy: Efficacy of nanoconjugate QN-247 in a Triple Negative Breast Cancer (TNBC) mouse model

Tariq Arshad[2], Stephen Fait[1], Guy Gammon[1], Andrew Hertig[2] and Mark Sarno[1]

[1]Vision Clinical Research, San Marcos CA, [2]Qualigen Therapeutics, Inc., San Diego CA.

Abstract #2283

Introduction

Triple-negative breast cancer (TNBC) accounts for 12% of breast cancers (BC) in the US and 15% worldwide. It lacks expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Compared to other types of BC, it is more aggressive and has higher risks of recurrence. In the metastatic setting, survival outcomes are much worse than other BC subtypes, with a 5-years survival rate of 8–16%. There is an urgent need to develop better treatment options for TNBC patients.

We have developed a novel therapeutic, QN-247, in a scalable production process by conjugating an anti-proliferative DNA aptamer to 10 nm diameter gold nanospheres. The aptamer-gold nanoparticle construct shows increased half-life and cytotoxic effects relative to the aptamer alone. QN-247 inhibits nucleolin, a key regulatory protein overexpressed in triple-negative breast cancer (TNBC) and other cancer cells, thereby reducing their proliferation.

Formulation development included a review of the construct's critical parameters which indicated that particle size and oligonucleotide loading were key to both manufacturability and efficacy. To ensure a hydrodynamic radius large enough to avoid glomerular filtration, and to facilitate processing, particles sizes of 8 and 10 nm diameter were selected. And based upon the observation that a higher oligonucleotide loading density and inclusion of a polyethylene glycol (PEG) layer can help protect DNA against serum degradation, we also prepared constructs with various oligonucleotide and PEG loading.

The resulting production process was found manufacturable, scalable, and capable of producing colloidally stable QN-247 formulations of various particle sizes, oligonucleotide loadings, and PEGylation suitable for in vitro and in vivo anti-proliferation studies.

Results from an in vitro potency assay utilizing DU-145 (Prostate Cancer) and a clonogenic assay using the MDA-MB-231 (TNBC) cells demonstrate the effectiveness of QN-247 to inhibit cancer cell growth. A dramatic reduction in the number of viable cells after treatment with QN-247 is seen relative to cells in growth media only, as well as cells Free Aptamer AS1411.

Results from an in vivo study in the MDA-MB-231 xenograft mouse model (TNBC) was performed with 12 daily doses (1 mg/kg) of test article. This efficacy study showed statistically significant reductions in mean tumor volumes for all QN-247 formulations compared to baseline (Day -1). All QN-247 formulations also significantly inhibited tumor growth compared to vehicle control.

Funding provided by Qualigen Therapeutics, Inc.

Covered by PCT Patent Application No. PCT/US2012/040577, "Anti Nucleolin Agent-Conjugated Nanoparticles"; PCT Patent Application No. PCT/US20/050261, "Anti-Nucleolin Agent-PEG Conjugated Nanoparticles" licensed from the University of Louisville to Qualigen Therapeutics, Inc.

Discussion

QN-247 is a colloidally stable and manufacturable drug combining the known nucleolin binding, anti-proliferative aptamer AS1411 with a gold nanoparticle support to increase its effectiveness. It builds on the qualities of aptamer AS1411 that showed promise in Phase 2 clinical trials, while exhibiting excellent in vitro and in vivo anti-proliferative effects on multiple cancer cell types, especially MDA-MB-231 cells (a triple negative breast cancer (TNBC) acell line), with no evidence of toxicity observed.

Development of QN-247 continues with additional formulations and targeted indications.

Results

Preparation of QN-247

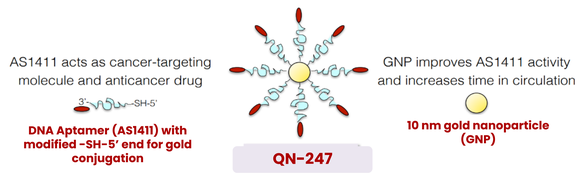


Figure 1: Schematic Representation of QN-247

QN-247 is a novel drug combining the anticancer DNA aptamer AS1411 with a gold nanoparticle (GNP) support. The sequence of AS1411 (5'-GGTGGTGGTGGTTGTGGTGGTGGTGG) is capable of G-quadruplex formation, and functions as an aptamer to nucleolin. The DNA Aptamer is modified at its 5' end with a sulfhydryl (SH) group to effect conjugation to the gold nanoparticle.

In vitro Proliferation Assay, MDA-MB-231 (TNBC) cells

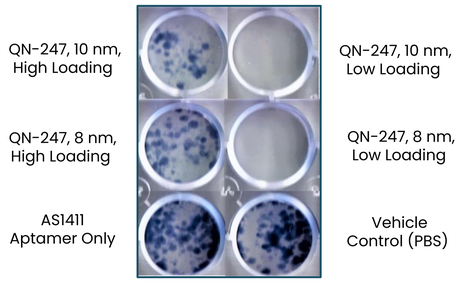
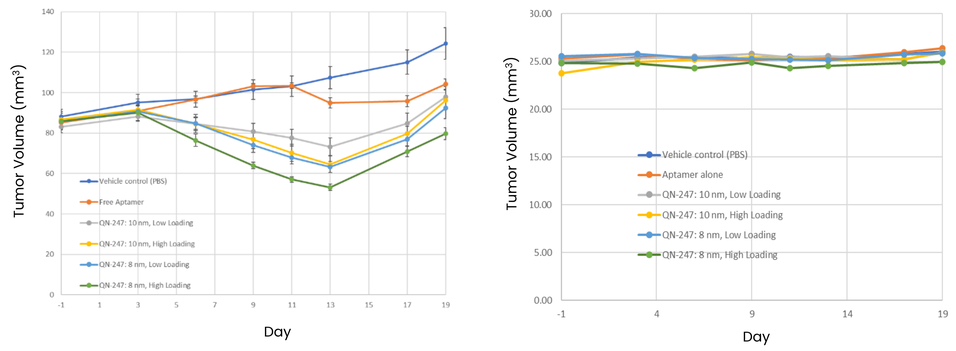


Figure 3: Stained Colonies of MDA-MB-231 (TNBC Cells) treated with QN-247

Treatment of MDA-MB-231 cells with 125 nM QN-247 or AS1411 Aptamer. All four QN-247 preparations are more anti-proliferative than AS1411 Aptamer only which has negligible effect at 125 nM relative to Vehicle Control (PBS).

Note that proliferating cells stain purple.

In Vivo Efficacy Study, Mouse TNBC Xenograft Model



Summary of Results	*TNBC MDA-MB-231 Tumor volume (mm^3)*			
Treatment	**Initial Tumor Volume ± SEM (N=9)**	**Day 13 Tumor Volume ± SEM (N=9)**	**p-value (Paired t-test)**	**Interpretation**
Vehicle Control	88.12 ± 3.65	107.45 ± 5.52	0.0011	Significant Tumor Growth
Free Aptamer	85.13 ± 3.05	94.98 ± 2.52	0.02	Significant Tumor Growth
QN-247: 10 nm, Low Loading	83.02 ± 2.93	73.24 ± 4.35	0.0216	Significant Tumor Reduction
QN-247: 10 nm, High Loading	86.76 ± 3.97	64.57 ± 4.15	<0.0001	Highly Significant Tumor Reduction
QN-247: 8 nm, Low Loading	85.76 ± 2.86	63.21 ± 2.74	<0.0001	Highly Significant Tumor Reduction
QN-247: 8 nm, High Loading	85.81 ± 4.08	53.18 ± 1.58	<0.0008	Highly Significant Tumor Reduction

Figure 2: In Vivo Results from MDA-MB-231 (TNBC), Mouse Xenograft Model of Tumor Volumes (mm^3), Paired t-test, and Body Weights (g) during treatment with QN-247

Treatment with Vehicle Control or Free Aptamer shows significant growth in tumor volumes at Day 13. Treatment with any QN-247 formulation shows significant to highly significant reductions in tumor volumes. 1 mg/kg dose (n=9 mice, error bars= SEM). Average body weights for each group of treated mice vs. Day of study. Body weights remain stable for duration of the study. TNBC tumors treated with the four QN-247 conjugates show a reduction in tumor volume during treatment phase (treatment administered from Day 0 to Day 11) and are more effective than Free Aptamer.

Fluorescent Images and Response Curve of DU-145 (Prostate Cancer) cells

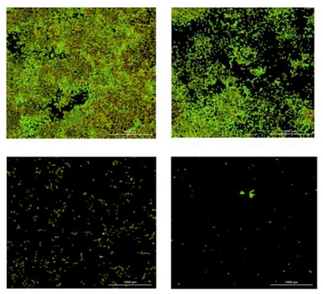


Figure 4A: In vitro Potency Assay against DU-145 cells.

Top Right: 10 nm PEGylated Gold (Nanocomposix, Cat # AUGB10) Treated Cells (Negative Control) Bottom Left: 300 µM Free Aptamer (AS1411) alone Treated Cells Bottom Right: QN-247: 10 nm gold particles, Low Oligonucleotide Loading with PEG Treated Cells.

Note that viable cells fluoresce green.

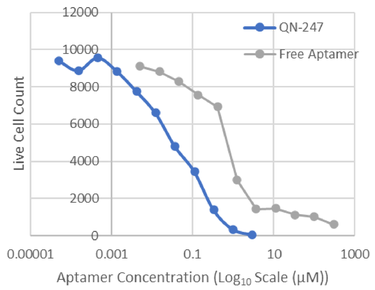


Figure 4B: In vitro Potency Assay Response against DU-145 cells

Response plot of DU-145 cell viability vs. Log10 scale concentration of QN-247 (10 nm, Low loading) or Free Aptamer. The difference in response indicates QN-247 is 15 times more potent than the Free Aptamer.

Conclusion

1. We have prepared QN-247 conjugates that build upon the legacy of the anti-proliferative DNA aptamer AS1411.
2. The process developed to produce QN-247 conjugates results in colloidally stable material that is manufacturable and scalable.
3. QN-247 is active in vitro against a variety of cancers including Prostate Cancer and Triple Negative Breast Cancer (TNBC), and more potent than the anti-proliferative aptamer AS1411 itself.
4. QN-247 is effective in vivo against a xenograft, mouse TNBC model showing highly significant reductions in tumor volumes.
5. There is no evidence of adverse effects.