RNA NanoBiotics

Executive Summary

Development of RNA Nanoparticles for Targeted Cancer Therapy

Overview

RNA NanoBiotics is at the forefront of oncology innovation, transforming the groundbreaking RNA nanoparticle technology developed by Dr. Peixuan Guo at The Ohio State University into a versatile, next-generation therapeutic platform. Our proprietary RNA Nanotechnology Platform is engineered to deliver RNA interference (RNAi), microRNAs, nucleoside analogs, chemotherapeutics, and radioisotopes directly to cancer cells, enhancing treatment efficacy while minimizing systemic toxicity. Crucially, these embedded molecules morph into active therapeutic drugs only after being internalized into target cells, ensuring high specificity and controlled release precisely to the tumor.

Lead Clinical Candidate

We are pioneering a transformative RNA nanotechnology platform that overcomes the limitations of traditional cancer therapies. Our patented RNA four-way junction (4WJ) nanoparticles deliver an unprecedented combination of high drug payload, precise tumor targeting, and exceptional safety.

Preclinical data demonstrate robust tumor regression and metastasis suppression in multiple cancer models with no toxicity, weight loss, or off-target effects. The 4WJ platform is modular, enabling rapid functionalization with additional drugs, RNAi agents, or targeting ligands, making it ideally suited for multi-drug resistant cancers.

With scalable CMC-ready production and compelling proof-of-concept efficacy and safety data, this RNA nanoparticle platform represents a new generation of precision therapeutics poised for first-in-human trials. We invite visionary investors to join us at the forefront of oncology innovation - delivering safer, smarter, and more effective treatments to patients who need them most.

Key Innovations

- RNA Nanocarrier Design: Utilizes a proprietary design **4WJ RNA scaffold** that enables precise, programmable assembly, modular drug loading, and rapid renal clearance to minimize off-target effects.
- Conjugation: The platform enables **high-payload** delivery while maintaining nanoparticle stability and tumor targeting capability.
- Tumor-Specific Targeting: Aptamer- and radio-targeting ensurs **selective accumulation** in tumor tissues while sparing normal cells.

Lead Clinical Candidate – 4WJ-SN38-EpCAMapt

Targeting Metastatic Colon Cancer to the Lung and Liver which is the leading cause of cancer death in men <50 years.

Preclinical Efficacy

- Tumor Volume Reduction: Up to **90% reduction** in tumor size in colorectal cancer xenograft models at **2 mg/kg** SN38 dosing.
- Targeting Advantage: EpCAM-targeted nanoparticles showed a **20.4% greater tumor weight reduction** than non-targeted controls.
- Apoptotic Activity: Induced **31.6% apoptosis** in HT29 colorectal cancer cells in vitro, comparable to free SN38.

Safety and Tolerability

- **No observable toxicity** in mice at therapeutic doses: no weight loss, no organ histopathology changes, and no elevated cytokine levels.
- **Hemocompatibility** confirmed: <5% hemolysis, no complement activation, and no coagulopathy.
- Immunogenicity: **No significant TNF-α or IL-6 elevation** in treated PBMCs or mouse serum.
- Renal clearance and fast systemic elimination mitigate long-term tissue accumulation.

Preclinical Proof of Concept Achieved

RNA NanoBiotics' data meets or exceeds all preclinical benchmarks for efficacy, targeting specificity, and systemic safety, thus establishing **clear proof-of-concept** for the SN38-RNA nanoparticle system. These findings justify advancement toward formal IND-enabling studies.

Lead Potential Improved Clinical Candidate – 4WJ-SN38-GEM-EpCAMapt

Data shows broader application of the drug with the incorporation of Gemcitibine (GEM) into the nanoparticle structure. The structure of the drug would not be altered and the GEM would be incorporated into the synthesis of the nanoparticles. We would also have the potential to incorporate an RNAi drug such as BCCL2/BCL-XL, Survivin (BIRC5), or TOP1.

Second Clinical Candidate - 4WJ-EGFRapta-Paclitaxel-anti-miR21

Targeting Triple Negative Breast Cancer which is notably the most aggressive breast cancer subtype with a low survival rate.

Preclinical Efficacy

- **Cellular activity (MDA-MB-231, EGFR+):** Targeted 4WJ-X-24PTX-EGFRapt bound cells strongly and was more cytotoxic than controls; apoptosis at 24 h was **45.1%** (vs 37.3% non-targeted 4WJ-X-24PTX, 24.6% free PTX, 8.3% aptamer alone). Growth inhibition notable at ≥250 nM.
- **Biodistribution:** After i.v. dosing, targeted RNA nanoparticles **preferentially accumulated in tumor** with low signal in liver/other organs (8 h post-injection).
- **Tumor growth inhibition (orthotopic TNBC xenograft):** Dosed at **8 mg/kg PTX-equivalent** every 2 days ×5, the 4WJ-X-24PTX nanoparticles (±EGFR aptamer) **significantly slowed tumor growth** vs PBS and free PTX (Cremophor/EtOH). Representative tumor images and stats are shown, with stronger inhibition for the targeted version.

Safety and Tolerability

- **General tolerability:** Treatments were **well tolerated** over two weeks; **no notable body-weight loss** and **no histologic organ damage** on post-treatment pathology.
- **Immunostimulation:** At **5 mg/kg PTX-equivalent i.v.**, 4WJ-X-24PTX induced **negligible TNF-α, IL-6, IFN-α, IFN-γ** compared with elevated cytokines from Cremophor-formulated PTX; a 25-chemokine panel showed no significant broad elevations (a few chemokines mildly increased vs PBS).
- **Acute toxicity margin:** Compared against the **LD50 of free PTX (~12 mg/kg)**, mice receiving 4WJ-X-24PTX at study doses had **no fatalities**, consistent with improved tolerability when PTX is carried by RNA nanoparticles

Systemic delivery of anti-miR-21:

Preclinical Efficacy

- **Tumor targeting:** Anti-miR-21 3WJ nanoparticles conjugated with a folate aptamer preferentially accumulated in TNBC tumors after i.v. injection, with minimal uptake in normal tissues.
- **Gene silencing:** Significant downregulation of miR-21 in tumor tissue after treatment, confirmed by qPCR.
- **Molecular effects:** Increased expression of miR-21 target genes **PDCD4** and **PTEN**, consistent with successful miR-21 inhibition.
- **Tumor growth inhibition:** Repeated systemic dosing (5 mg/kg oligo-equivalent, twice weekly × 4 weeks) **slowed tumor growth** significantly compared to scrambled-miRNA controls.
- **In vitro apoptosis:** Treated MDA-MB-231 cells showed increased apoptosis and reduced proliferation.

Safety & Tolerability

- **No acute toxicity:** Mice tolerated the regimen well, with **no significant body-weight loss** during treatment.
- **Histopathology:** Major organs (heart, liver, spleen, lung, kidney) appeared normal by H&E staining after treatment.
- **Cytokine profile:** Anti-miR-21 nanoparticles caused **negligible induction of inflammatory cytokines** (IL-6, TNF-α, IFN-α, IFN-γ) compared to PBS controls.
- **No hematological toxicity:** Blood counts remained within normal ranges after repeated dosing.

Preclinical Proof of Concept Achieved

RNA NanoBiotics' data meets or exceeds all preclinical benchmarks for efficacy, targeting specificity, and systemic safety, thus establishing **clear proof-of-concept** for the EGFRapt nanoparticle system. These findings justify advancement toward formal IND-enabling studies.

Additional Drug Candidates with Animal Data

- **Third Clinical Candidate – 4WJ-GalNex-PTX-miR122-HtLs**

  Targeting Liver Cancer

- **Fourth Clinical Candidate -4WJ-anti-mRNA21-LNA-PSMAapt**

  Targeting Prostate Cancer

RNA Nanoparicles for use inTheranostics/Targeted Radiation Therapy

Recent work from *RNA NanoMed* (Aug 2025) provides a compelling **proof-of-concept** for using modular SF5 three-way junction (3WJ) RNA nanoparticles as **targeted radiotherapeutic agents**. The 3WJ Nanoparticles can be quicly converted to 4WJ and chemical and localization behavior is the same. These nanoparticles demonstrate **rapid tumor accumulation** within 1–4 hours, minimal off-target retention, and a "plug-and-play" design where the targeting ligand and chelator strand can be swapped without altering the core architecture. This makes it possible to seamlessly substitute therapeutic alpha or beta emitters (e.g., ^{212}Pb, ^{225}Ac, ^{177}Lu) for the diagnostic ^{68}Ga used in the study, enabling a **true theranostic pipeline** across multiple cancer indications.

The commercial potential is substantial: the global targeted radiotherapy market is projected to exceed **$15 billion by 2030**, driven by expanding indications, earlier-stage treatment, and strong reimbursement for high-value precision oncology drugs. By extending this platform from diagnostics into therapy, each new ligand–isotope pairing becomes a differentiated asset with billion-dollar addressable markets, leveraging the same GMP manufacturing backbone and regulatory dossier.

- **^{212}Pb → TCMC on the 4WJ chelator strand**
  Swap the current NOTA strand for **TCMC** (Pb^{2+} chelator) and keep everything else (ligand strand, core) the same. ^{212}Pb's ~10.6 h half-life aligns with the NP's **1–4 h tumor uptake window** and rapid background washout, so you'll get useful tumor dose with low normal-organ dose. This is the cleanest drop-in.
  (The paper explicitly contemplates ^{212}Pb among intended therapy nuclides.)
- **^{225}Ac → DOTA (or macropa) on the 4WJ chelator strand**
  Also a straight chelator-strand substitution. Practically, you'll plan for **daughter recoil** and may want multivalency/dendrimer variants to help with payload and potential daughter retention, but it's still architecturally a swap.
- Potential Theranostic Applications
  - Designs include dual-use payloads, such as:
    - Therapeutic isotopes (Astatine-211, Actinium-225)
    - Diagnostic isotopes (Gallium-68, Fluorine-18 for PET/CT imaging)

Cancer Type	Targetable Surface Markers	Applicable Radioisotopes	Why It's a Fit
Prostate Cancer	PSMA, EpCAM	Actinium-225, Lutetium-177	Already proven in radioligand therapies; RNA platform can improve targeting/safety
Neuroendocrine Tumors (NETs)	SSTR2 (somatostatin receptor)	Lutetium-177, Actinium-225	Alpha-emitters could overcome resistance to current Lutathera®-based therapies

Colorectal Cancer (CRC)	EpCAM	Astatine-211, Lutetium-177	EpCAM aptamers enable high-precision targeting of liver/lung metastases
Pancreatic Cancer	Mesothelin, EpCAM	Actinium-225, Astatine-211	Hard-to-penetrate tumor; small, stable RNA particles improve delivery
Ovarian Cancer	FRα, EpCAM	Astatine-211, Lutetium-177	Suitable for residual disease and intraperitoneal delivery
Glioblastoma (GBM)	CD133, EGFRvIII	Astatine-211, Actinium-225	Exosomes or small RNA carriers may cross the BBB better than antibodies
Multiple Myeloma	BCMA, CD38	Actinium-225, Lutetium-177	Novel route for alpha-emitting therapies targeting marrow-resident tumor cells
Breast Cancer (TNBC)	EpCAM, MUC1	Astatine-211	No hormone targets in TNBC—RNA-guided radiation offers a non-toxic alternative
Lung Cancer	EGFR, EpCAM	Astatine-211, Actinium-225	Can target both primary NSCLC and CRC lung metastases
Liver Cancer (HCC)	GPC3, EpCAM	Astatine-211, Lutetium-177	High unmet need; radioRNA may bypass resistance to kinase inhibitors

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Other Licensable or Parterable Technologies Within IP Portfolio

Strategic Platform Applications

1. **RNA-Ligand Displaying Exosomes for Targeted Therapeutics**

RNA nanoparticles are displayed on the surface of exosomes. Designed to target specific cell types (e.g., cancer cells) by combining an RNA targeting aptamer (or RNA nanoparticle) with an exosome vector that carries therapeutic or diagnostic payloads.

- Surface-displayed RNA nanoparticles: Exosomes are engineered so that the RNA nanoparticle (including targeting moieties) is presented on their membrane, facilitating specific cell binding and uptake.
- Therapeutic or diagnostic function inside exosome: The exosome itself can include a "functional moiety" e.g. therapeutic cargo such as siRNA, miRNA, or imaging compound.
- Multifunctional platform: Supports treating cancer, infections, and diagnostic imaging using a unified exosome-RNA nanoparticle delivery system.

This application extends the RNA nanotechnology IP into the realm of extracellular vesicle-based delivery, combining the modularity and targeting of RNA nanostructures with the natural delivery advantages of exosomes. Key benefits:

- Hybrid platform potential: Merges two validated delivery modalities—engineered exosomes and RNA nanoparticles—for added targeting, stability, and payload versatility.
- Differentiation: Distinct from current ADCs or lipid nanoparticles, leveraging exosome immune stealth and cell compatibility.
- Extension opportunities: Can be licensed or co-developed in areas such as cancer, infectious disease, and diagnostic imaging.

Cancer Type	**Known Aptamer Targets**	**Platform Cargo Potential**
Colorectal (mCRC)	EpCAM	SN38, miRNA, siRNA, radiotherapeutics
Breast (TNBC)	EpCAM, MUC1	SN38, siRNA, checkpoint inhibitor RNA
Ovarian	EpCAM, FRα	siRRM2, SN38, miRNA
Liver (HCC)	ASGR1, EpCAM	miR34, siRNA
Prostate	PSMA	siRNA, radiotherapeutics
Lung	EGFR, EpCAM	Chemotherapeutics, siRNA
Brain (GBM)	EGFRvIII, CD133	siRNA, radiotherapeutics
Pancreatic	Mesothelin, EpCAM	miRNA, chemo
Multiple Myeloma	BCMA, CD38, SLAMF7	siRNA, miRNA, immunomodulatory RNA, BiTE RNA

2. **Cell Therapy Applications**

RNA nanoparticles functionalized with aptamers that bind to immune cells (e.g., CD4, CD8, CD28, 4-1BB).These particles can:

- Deliver activation signals to T cells or NK cells (immune stimulation)
- Deliver gene-modulating RNAs (e.g., siRNA, miRNA) to reprogram immune responses
- Target CAR-T cells post-infusion to regulate their activity or persistence

Exosome-RNA Nanoparticle Hybrids

1. The RNA-decorated exosome platform (US20190024085A1) can be adapted to:
    - Deliver co-stimulatory signals to immune cells in vivo
    - Package and direct immunoregulatory RNAs to myeloid or lymphoid cells
    - Potentially carry CAR constructs or guide RNAs for transient gene editing or expression

Cell-Specific Aptamer Targeting

- RNA aptamers can be engineered to bind:

    - T cell markers (CD3, CD4, CD8)
    - Myeloid markers (CD11b, CD14)
    - Stem cell markers (CD133, Sca-1)
- This enables RNA-particle–guided programming of immune cells in vivo—without viral vectors or electroporation.

Cancer Type	Immune Targets / Pathways	RNA Platform Application
Acute Lymphoblastic Leukemia (ALL)	CD19, CD22 (CAR-T targets)	In vivo CAR mRNA delivery or checkpoint knockdown (PD-1, CTLA-4)
Diffuse Large B-Cell Lymphoma (DLBCL)	CD19, CD20	RNA nanoparticle support for CAR-T enhancement or immune evasion
Multiple Myeloma	BCMA, SLAMF7, CD38	In vivo delivery of anti-BCMA CAR constructs or immune modulators
Non–Small Cell Lung Cancer (NSCLC)	PD-L1, EGFR	siRNA delivery to tumor or TME; checkpoint modulation in T cells
Triple-Negative Breast Cancer (TNBC)	EpCAM, MUC1	Combination of RNA-targeted drug + immune checkpoint knockdown
Ovarian Cancer	FRα, EpCAM	Dendritic cell RNA vaccines or T cell modulator delivery
Colorectal Cancer (mCRC)	EpCAM, CEACAM5	Tumor-associated antigen delivery to DCs for vaccination; TAM reprogramming
Prostate Cancer	PSMA	NK cell activation or T cell stimulation via RNA payloads
Glioblastoma (GBM)	EGFRvIII, CD133	Delivery to tumor-infiltrating lymphocytes (TILs); checkpoint silencing
Melanoma	gp100, MART-1, PD-1/PD-L1 axis	mRNA vaccines or immune checkpoint RNA delivery to T/NK cells
Pancreatic Cancer	Mesothelin, CD40, PD-L1	Macrophage/monocyte reprogramming or RNA-activated DC therapy

3. **Bispecific Applications**

Multi-arm RNA nanoparticles (e.g., 3WJ, 4WJ, 6WJ structures) can be engineered to simultaneously:

- Display a tumor-targeting aptamer (e.g., EpCAM, PSMA)
- Display an immune cell–binding aptamer (e.g., CD3 for T cells, CD28, 4-1BB, CD16 for NK cells)

This creates a **bispecific bridge** between cancer cells and immune effectors—mimicking the mechanism of bispecific antibodies or BiTEs (bispecific T-cell engagers).

Cancer Type	Tumor Target (Aptamer)	Immune Target (Aptamer)	Clinical Opportunity
Colorectal Cancer (mCRC)	EpCAM	CD3, CD28, 4-1BB	Recruit T cells to liver/lung mets; improve response to immunologically "cold" tumors
Ovarian Cancer	FRα, EpCAM	CD3, CD28	Target chemoresistant cells in peritoneum; engage immune effectors
Triple-Negative Breast Cancer (TNBC)	EpCAM, MUC1	CD3, CD16	No hormone targets—BiTE-style RNA can recruit T or NK cells
Multiple Myeloma	BCMA, CD38, SLAMF7	CD3, CD16	Novel immune engagement strategy vs marrow-resident tumors
Prostate Cancer	PSMA	CD3, CD28, 4-1BB	Use in metastatic CRPC with T cell or NK cell recruitment
Lung Cancer (NSCLC)	EGFR, CEACAM5	CD3, 4-1BB	Combine with immune checkpoint silencing (siPD-1, siTGF-β)
Glioblastoma (GBM)	EGFRvIII, CD133	CD3	Cross blood–brain barrier; engage T cells at tumor site
Pancreatic Cancer	Mesothelin, EpCAM	CD3, CD28	Target dense TME and stimulate immune infiltration
Melanoma	gp100, MART-1	CD3, PD-1	Combine antigen targeting with immune checkpoint interference
Liver Cancer (HCC)	ASGR1, GPC3, EpCAM	CD3, NKp46	Use in immunotherapy-refractory or virus-associated HCC
Non-Hodgkin Lymphoma (NHL)	CD19, CD20	CD3	RNA BiTEs mimic approved therapies with simpler manufacturing
Acute Myeloid Leukemia (AML)	CD33, CD123	CD3	Experimental—engage T cells directly via RNA bispecific scaffold

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Conclusion

Multiple nanoparticle platforms pioneered by RNA NanoBiotics represents a transformative approach to targeted cancer chemotherapy. With a strong body of preclinical data supporting

safety, specificity, and efficacy, the platform is well-positioned for clinical translation as a first-in-class RNA-based therapeutic delivery system for solid tumors.

Select References

1. Binzel DW, **Guo P**. Synergistic RNA particles for spontaneous and specific cancer targeting but low toxicity due to motility and deformation. Nanomedicine, 2025 Apr

2. Bohmer M, Binzel DW, Zhang W, **Guo P**. Constructing an active chimeric pRNA ring with a stoichiometry of six and identifying 12 domains of the pRNA ring binding to the 12-subunit channel of phi29 DNA packaging motor. RNA, 2025 Apr

3. Li X, Jin K, Cheng TC, Liao YC, Lee WJ, Bhullar A, Chen LC, Rychahou P, Phelps M, Ho YS, **Guo P**. RNA four-way junction (4WJ) for spontaneous cancer-targeting, effective tumor-regression, metastasis suppression, fast renal excretion and undetectable toxicity. Biomaterials, 2024 Mar

Full List of Dr. Peixuan Guo Publications:
https://rnanano.osu.edu/Guo/publications.html