Nanoparticle delivery of cancer-activated DNA constructs for the diagnosis of liver tumors

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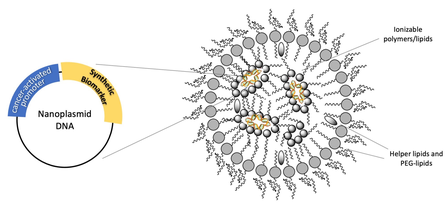
ABSTRACT #: 7513

Background and Rationale

Early detection of liver cancer has the potential to substantially improve survival rates. Yet, diagnosis of hepatocellular carcinoma (HCC) is hampered by poor sensitivity of traditional imaging techniques - such as ultrasound, CT, and MRI - which often miss small or atypical tumors, particularly in cirrhotic livers with a substantially altered anatomy. As a result, more than a third of early-stage HCCs are missed. Biomarkers offer one potential solution for early detection, yet many cancers do not have such unique biomarkers, and, even among those that do, the biomarkers are typically only present in some patients with each cancer type.

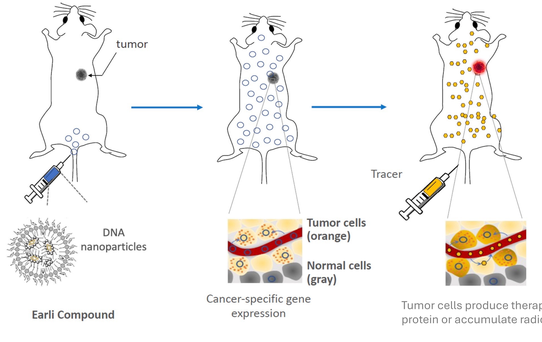
Earli is developing a highly sensitive platform that uses systemically administered cancer-activated nucleic acid constructs with engineered synthetic promoters to usurp dysregulated cancer pathways and force the tumor to produce a synthetic biomarker to detect and localize the malignancy. Alternatively these DNA molecules can be programmed to produce immunotherapies such as cytokines that can be secreted directly into the tumor microenvironment to overcome the mechanisms that tumors use to evade immune detection.

Although nucleic acid therapies have been utilized over the last few decades, many of the delivery vehicles currently used in oncology, such as lipid nanoparticles (LNP) and viral vectors, provide little in the way of selectively targeting pathogenic cancer tissues (viral vectors do have tissue tropism but the selective targeting generally relates to the healthy tissue type, e.g., nerve cells, skeletal muscle, or lungs). Non-viral LNPs were selected for delivery of the genetic constructs because they are relatively inexpensive to produce and allow for repeat dosing to achieve sustained levels of expression. Although LNPs have proven effective for RNA delivery, DNA delivery to tumor cells remains challenging. With both LNPs and viral vectors, off-target or off-tissue expression has been a major limitation that contributes towards a narrow therapeutic window.



Instead, to achieve specificity, Earli pairs up the development of novel LNPs with the presence of novel promoters embedded into DNA nanoplasmids that are engineered to activate significant levels of expression only in the presence of cancer-specific dysregulation, not in healthy cells or cells with other comorbidities. These promoters can "force" the cancer cells—and only the cancer cells—to express an encoded protein or peptide of choice.

Cancer-activated expression for cancer Dx and Tx

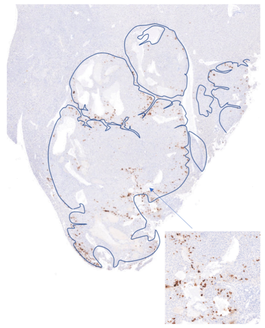


2nd GEN LNPs produce modest levels of expression in liver tumors

Lipid nanoparticles (LNPs) are effective transporters of nucleic acids to the liver, primarily due to their association with serum proteins like apolipoprotein E (ApoE), which targets liver cells by binding to low-density lipoprotein (LDL) receptors on hepatocytes.

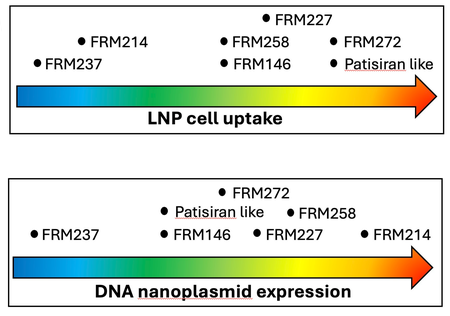
We assessed the feasibility of delivering DNA nanoplasmids to liver cancer lesions using second-generation LNPs. Mice with Hep3B orthotopic liver tumors, grown for 3-4 weeks, were intravenously administered LNP-formulated DNA nanoplasmids. These nanoplasmids contained a synthetic promoter sequence that exploits liver cancer-dysregulated transcription factors to express an HA-tagged reporter protein (sr39TK). After treatment, the livers were isolated and analyzed using immunohistochemistry to detect the HA-tagged biomarker. The results showed co-localization of anti-HA staining with Hep3B tumor lesions, confirming delivery and expression in liver tumors.

However, HA-positive cells were also detected in normal liver tissue, albeit to a lesser extent, highlighting the need for further engineering of the delivery agent and nanoplasmids to achieve higher cancer specificity.

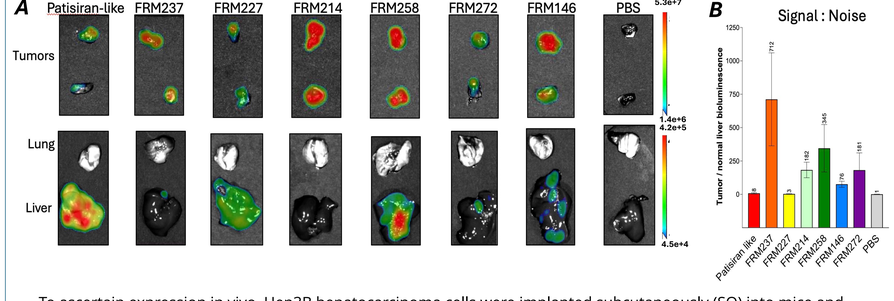


LNPs can be engineered for enhanced tumor uptake & expression

Comprised of ionizable lipids, sterols, helper lipids and differing surface chemistries, LNPs can be engineered to have different transfection properties depending on the identity of the components and their relative ratios. We designed series of LNPs with differential properties related to tumor cell transfection and nuclear delivery. Each iteration was used to formulate a DNA nanoplasmid comprised of a constitutively active promoter to drive the expression of a reporter gene (firefly luciferase or GFP). Uptake into cells was tested by labeling the DNA nanoplasmid with Cy3 fluorescent dye; Reporter expression was used to monitor transduction. Schematic representation of the formulations' (FRM) ranking based on (A) uptake and (B) expression levels.



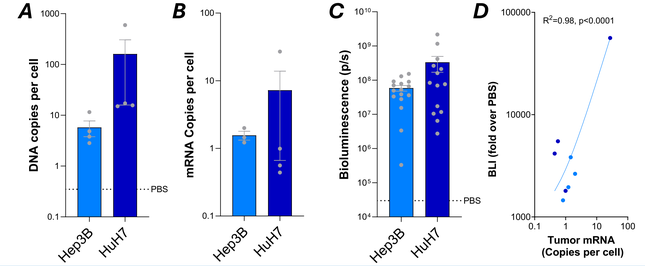
Earli LNPs have preferential expression in liver xenograft models



To ascertain expression in vivo, Hep3B hepatocarcinoma cells were implanted subcutaneously (SQ) into mice and allowed to grow to ~200 mm³. EARLI-206, a DNA nanoplasmid comprised of a constitutively active promoter to drive the expression of firefly luciferase, was packaged in different LNP formulations as described above. After IV dosing with the EARLI-206 formulation, the tumors were permitted to grow for two additional days and tissues were isolated for BLI imaging. Representative Bioluminescence Images (BLI) of the tissue (A) showed high tumor and low normal liver expression. The Ratio of BLI in tumor versus liver (Signal : Noise) showed strongest tumor tropism upon dosing with FRM237, with maximal signal to noise ratio (SNR) of 712.

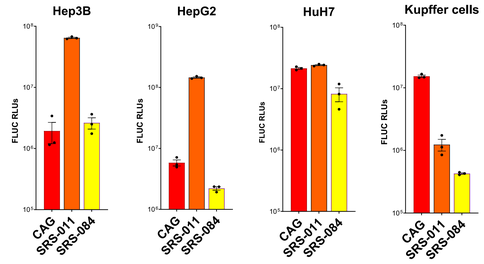
Tumor expression of BLI correlates with uptake of DNA and mRNA

EARLI-206 was formulated in FRM146 and dosed intravenously into NSG mice bearing Hep3B or HuH7 subcutaneous (SQ) tumors that were ~200 mm³. Two days later, tissues were isolated for BLI imaging as well as DNA and mRNA content analysis. Uptake of DNA (A) as well as corresponding transcript level (B) were similar in both xenografts. Protein expression was confirmed by BLI (C) that positively correlated (D) with mRNA amount from (B).

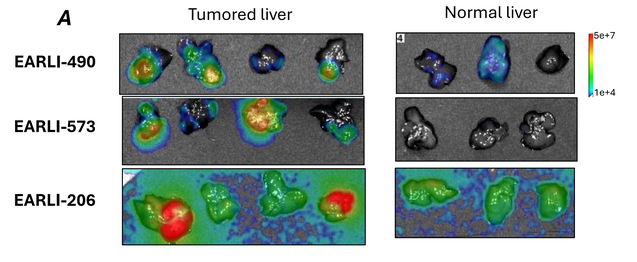


Synthetic promoters drives strong cancer-activated gene expression

We next added a synthetic promoter sequence, engineered to leverage aberrant transcription factor levels present in cancer to drive the expression of a firefly luciferase reporter gene and are designed to be highly active in cancer cells while remaining transcriptionally silent in normal adjacent tissues and benign lesions. LNP-encapsulated nanoplasmids engineered with cancer-specific synthetic regulatory sensors (SRS-011 or SRS-084) were administered in vitro to tumor (Hep3B, HepG2 and HuH7) or immortalized mouse Kupffer cells. SRS-011 and SRS-084 enable cancer expression at similar or higher level than the constitutive promoter (CAG) while limiting Kupffer cells expression.



Cancer-activated promoters boost specificity in orthotopic HCC models



EARLI-490 and EARLI-573 are products that have been packaged with FRM146 and contain DNA nanoplasmids that are comprised of a firefly luciferase gene driven, respectively, by the cancer-specific synthetic promoter sequences SRS-011 and SRS-084. EARLI-206 was used as benchmark. Formulations were administered intravenously into mice implanted with Hep3B orthotopic liver tumors. Non-tumor bearing animals served as negative controls. Two days after vector administration, the mice were sacrificed and the liver isolated for (A) BLI imaging. Quantitative measurements demonstrate high signal from orthotopic liver tumors (B) versus liver BLI in non-tumored animals (C). Earli-573 had superior cancer specific expression with a signal to noise ratio of 321 (D).

Conclusions and Next Steps

- LNPs were engineered for systemic administration of DNA nanoplasmids to increase tumor cell transfection and nuclear delivery. FRM237, resulted in highest tumor tropism in a liver xenograft model and is being advanced as our lead liver candidate.
- Adding cancer-activated synthetic promoters to our DNA nanoplasmids significantly enhances specificity of transgene expression in tumors. The promoter SRS-084 in Earli drug product EARLI-573 produced >300x SNR in orthotopic liver tumors vs normal liver tissues.
- The present data collectively points to successful delivery of DNA to hepatic tumors via engineered LNP formulations following intravenous administration. When combined with payloads such as PET reporter genes or therapeutic molecules with the potential to treat the tumor such as cytokines and further validation in preclinical mouse models, this represents a promising synthetic biomarker platform for liver cancer diagnostics and therapeutics.