Turning Cancer Against Itself:
Usurping oncogenic pathways to drive cancer-activated cytokine expression

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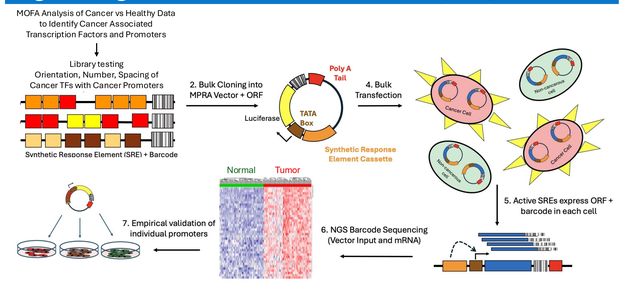
Background and Rationale

Background: Earli is developing an orthogonal genetic approach to cancer treatment that uses synthetic cancer-activated promoters (CAPs) to selectively drive expression of therapeutic payloads in malignant cells while remaining transcriptionally inert in normal adjacent tissues and benign lesions. This focuses therapeutic activity selectively within tumors and avoids on-target, off-tumor activity. Here we describe validation of double stranded (ds) DNA constructs, comprised of CAPs driving cytokine expression, delivered via lipid nanoparticles (LNPs) in preclinical models. Our data demonstrate robust therapeutic impact via both 1) activation of Innate Immune Pattern Recognition Receptors by dsDNA and 2) specific secretion of therapeutic cytokines (IL-2 and IL-12) by CAPs which are uniquely produced in cancer cells, but not normal adjacent tissues or benign lesions.
Methods: dsDNA Nanoplasmids were designed using CAPs to drive cancer-activated expression of IL-2 or IL-12. The activity of each construct was validated *in vitro* for cytokine expression in tumor cell lines and for cytokine function in reporter cells and primary immune cells. In vivo activity was tested following formulation into LNPs [2,3] in MB49 or B16F10 syngeneic tumor models or in human tumor xenograft studies.
Results: Our cancer-activated genetic constructs expressed well in tumor cell lines and produced cytokines with bioactivity similar to purified wildtype cytokines. Whole body and tissue analysis of treated mice demonstrated tumor specificity for our cancer-activated genetic constructs. Robust and durable responses such as dose dependent reductions in tumor growth were observed along with concomitant changes in Teff/Treg ratios. Control DNA LNPs exhibited partial and non-durable anti-tumor activity which was abrogated in STING-dependent manner. In humanized H358 and Huh7 xenograft models, CAP-IL-2 demonstrated substantial anti-tumor efficacy and increased CD8 memory T cells. In MB49 tumors, CAP-IL-2 controlled tumor growth and increased memory T cells. In MB49 and/or B16F10 tumors, CAP-IL-12 induced myeloid and T cell activation, complete regressions and abscopal tumor efficacy.

Screening Libraries of Cancer TF/Promoter Combos Allows Engineering of Cancer-Activated Promoters (CAP)

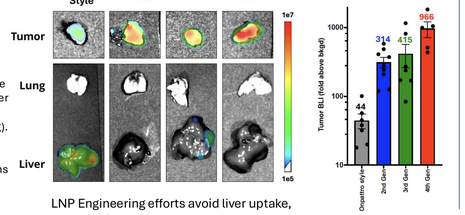


Earli's engineered LNPs have enhanced tumor uptake

Standard LNP formulations, such as Onpattro, dosed IV induce rapid hepatic uptake via ApoE binding/opsonization, limiting their systemic utility.

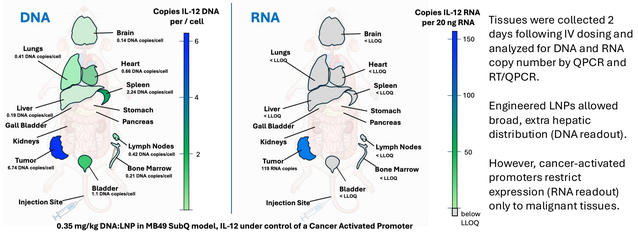
Earli's LNPs use unique surface chemistry to avoid hepatic uptake and allow longer exposure to other tissues such as tumors (without needing tumor-specific targeting).

Right: Distribution data for IV dosed Earli LNP/DNA formulations (DNA encodes CMV promoter driving luciferase reporter)



LNP Engineering efforts avoid liver uptake, improve tumor uptake.

IV delivered Earli DNA/LNP encoding IL-12 provides extra-hepatic LNP distribution with precise cancer specificity



0.35 mg/kg DNA:LNP in MB49 SubQ model, IL-12 under control of a Cancer Activated Promoter

Tissues were collected 2 days following IV dosing and analyzed for DNA and RNA copy number by QPCR and RT/QPCR.

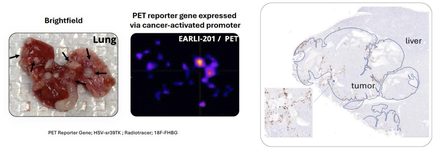
Engineered LNPs allowed broad, extra hepatic distribution (DNA readout).

However, cancer-activated promoters restrict expression (RNA readout) only to malignant tissues.

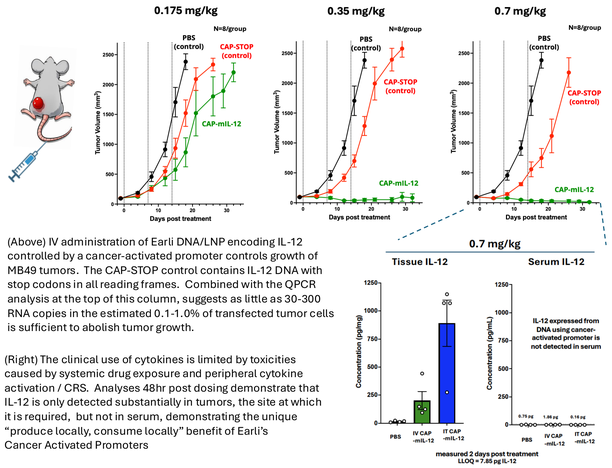
IV injection with cancer-activated genetic constructs demonstrate tumor specific PET and IHC signals

Reporter genes encoded by cancer-activated promoters demonstrate tumor specificity: PET reporter genes and imaging tracers (left), Immuno-histochemistry (right).

Although the percentage of transfected/expressing cells is low, the specificity is exquisite i.e. only occurs within the diseased cells.



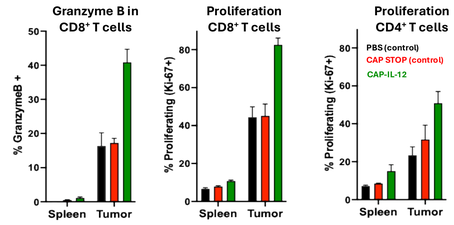
IV administration of DNA using cancer-activated promoter to express IL-12 completely ablates MB49 SubQ tumor growth



(Above) IV administration of Earli DNA/LNP encoding IL-12 controlled by a cancer-activated promoter controls growth of MB49 tumors. The CAP-STOP control contains IL-12 DNA with stop codons in all reading frames. Combined with the QPCR analysis at the top of this column, suggests as little as 30-300 RNA copies in the estimated 0.1-1.0% of transfected tumor cells is sufficient to abolish tumor growth.

(Right) The clinical use of cytokines is limited by toxicities caused by systemic drug exposure and peripheral cytokine activation / CRS. Analyses 48hr post dosing demonstrate that IL-12 is only detected substantially in tumors, the site at which it is required, but not in serum, demonstrating the unique "produce locally, consume locally" benefit of Earli's Cancer Activated Promoters

CAP IL-12 induces CD8 T activation in Tumors



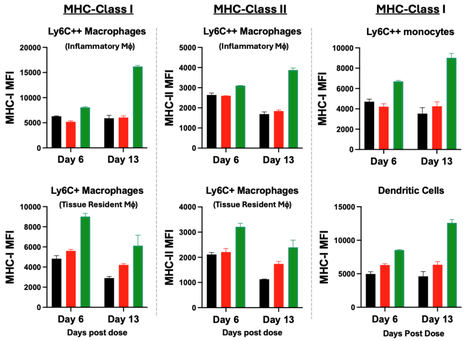
LNPs carrying DNA encoding IL-12 under the control of a Cancer Activated Promoter:

Activate Tumor CD4 and CD8 T cells with muted effects on peripheral T cells (IV administration).

Day 13 (6 Days Post 2nd dose) data from B16F10 tumor bearing mice treated weekly IV with 0.35mg/kg CAP LNPs

Not Shown: No IL-12 induced Body Weight Loss was Observed

CAP IL-12 induces robust Myeloid activation



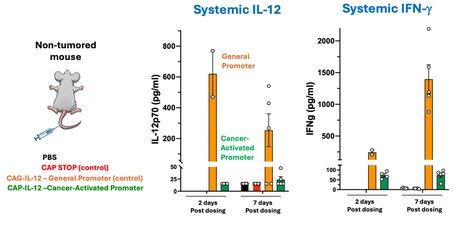
PBS (control)
CAP STOP (control)
CAP-IL-12

LNPs carrying DNA encoding IL-12 under the control of a Cancer Activated Promoter:

Upregulate MHC-I and MHC-II (Antigen Presentation) in Monocytes, Macrophages and Dendritic Cells (APCs) following IV administration in tumor bearing mice.

Day 6 or 13 splenocyte data from B16F10 tumor bearing mice treated weekly IV with 0.35mg/kg CAP LNPs

Robust safety: Cancer activated promoters do not produce any IL-12 in mice lacking tumors

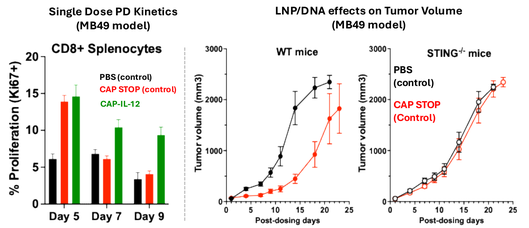


No tumors, No IL-12 expression

Systemic delivery of LNPs with cancer-activated promoters controlling IL-12 expression produced minimal IL-12 or downstream biomarker IFN-γ in healthy mice

Conversely systemic delivery of constructs that use a strong, constitutive promoter produce robust levels following IV injection

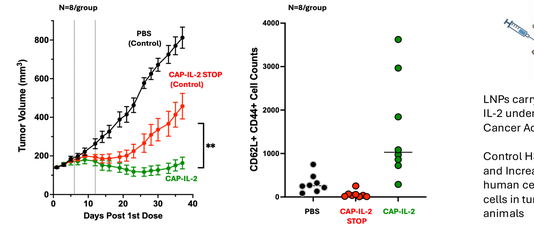
LNP/DNA induces transient Immune Activation & efficacy (STING-dependent), IL-12 potentiates immune activation.



(Left) Control DNA induces transient lymphocyte activation, IL-12 potentiates lymphocyte activation.

(Right) Control DNA induces transient TGI in WT but not STING$^{-/-}$ mice.

Expression of alternate cytokines, including IT delivered IL-2, controls tumor growth and expands memory T cells

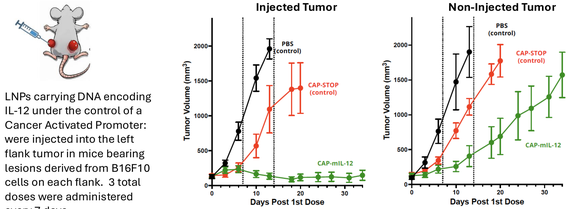


LNPs carrying DNA encoding IL-2 under the control of a Cancer Activated Promoter:

Control H358 tumor growth and Increase numbers of human central memory T cells in tumors of treated animals

Data from NCG mice co-engrafted with H358 tumor cells and human PBMCs with IT (0.75mg/kg) treatment on days 0, 4, 8.

Intratumoral CAP IL-12 DNA provides modest abscopal effect



LNPs carrying DNA encoding IL-12 under the control of a Cancer Activated Promoter: were injected into the left flank tumor in mice bearing lesions derived from B16F10 cells on each flank. 3 total doses were administered every 7 days.

B16F10 tumor bearing mice treated weekly IT x 3 (0.25mg/kg)

Conclusions:

Conclusions: Here we describe a novel genetic approach to engage the immune system directly in the tumor microenvironment by reprogramming cancer cells to produce therapeutic cytokines directly into the TME. Additionally, tumor-expressed cytokines tested here act in concert with anti-tumor DNA-platform driven innate immune agonism. Additional studies are warranted to understand the requirement for platform driven agonism in efficacy responses. Altogether, these efforts aim to reduce on-target, off-tumor effects and increase therapeutic window compared to systemically administered protein immune agonists. Future work includes expressing different classes of payloads from the same genetic platform such as bispecific engagers.