Systemic nanoplasmid gene therapy with engineered cancer-activated promoters selectively programs tumor cells to express inflammatory interleukins and drive antitumor immunity

Evan Bishop, Priyanka Balasubrahmanyam, Ajda Rojc, Trupti Patil, Moataz Reda, Lingyun Li, Dang Dang, Xiaobin Wu, Robby Chandra, Nikki Kimura, Kim Tran, Blaine McCarthy, Sushil Lathwal, Sathyapriya Rajagopal, Jayalakshmi Ramani, Badriprasad Ananthanarayanan, Nadege Morisot, David Rosen, and David Suhy

Earli Inc., Redwood City, CA, USA

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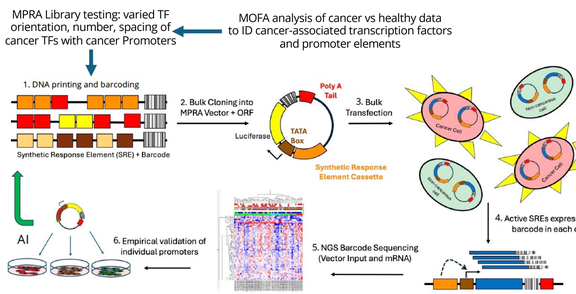
Background and Rationale

Background: Earli is developing an orthogonal genetic approach to cancer treatment with systemically delivered DNA nanoplasmids engineered with synthetic cancer-activated promoters (CAPs) to selectively express therapeutic payloads in malignant cells while remaining transcriptionally inert in healthy tissues. This selectivity concentrates encoded therapeutic activity within the tumor and concomitantly avoids systemic on-target, off-tumor toxicity. Here we describe the anti-tumor effects of CAPs driving expression of specific cytokines (i.e. IL-12), delivered IV using lipid nanoparticles (LNPs). Our data demonstrate tumor selective IL-12 expression with complete ablation of syngeneic tumor growth and minimal systemic IL-12 expression or IL-12 exposure in the serum.

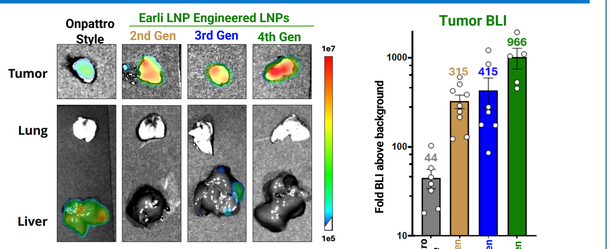
Methods: DNA Nanoplasmids were engineered for exquisite CAP specificity and validated for cytokine expression in tumor cell lines and for cytokine function in reporter cells and primary immune cells. LNP-formulated constructs were tested for in vivo distribution, efficacy, immune phenotyping and expression profiling in MB49 or B16F10 syngeneic tumor models.

Results: CAP-cytokine constructs were expressed in tumor cell lines and produced cytokines with wild type activity. When administered IV, CAP-IL-12 LNPs but not control DNA-LNPs, induced dose-dependent, robust, durable anti-tumor activity. While tissue profiling demonstrated broad extra-hepatic biodistribution of the DNA template, IL-12 mRNA expression was exquisitely restricted to tumor tissue (<LLOQ in 12 other tissues tested). Similarly, although IL-12 protein was present in tumor tissues, serum IL-12 was often below LLOQ. CAP-IL-12 treated mice demonstrated robust TIL CD4 and CD8 activation via robust upregulation of Ki67 and Granzyme B. Furthermore, CAP-IL-12 also upregulated MHC-I/II antigen presentation machinery in multiple myeloid and DC subsets, and, in contrast with IL-12 protein treatment, only gradually induced low levels of systemic IFNg without IL-12 induced bodyweight loss or acute toxicity-associated NK cell hyperactivation.

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



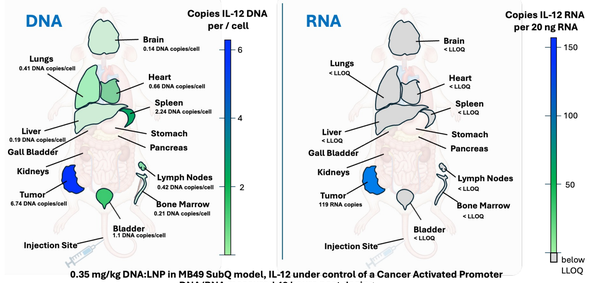
Earli's LNPs avoid liver, have enhanced tumor uptake



DNA uses a CMV promoter to encode a luciferase reporter

IV dosed standard LNP formulations, such as Onpattro, have rapid hepatic uptake via ApoE binding / opsonization thus limiting systemic utility. Earli's LNPs use unique surface chemistries to avoid hepatic uptake and permit longer exposure to other tissues such as tumors (without needing tumor- targeting agents).

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

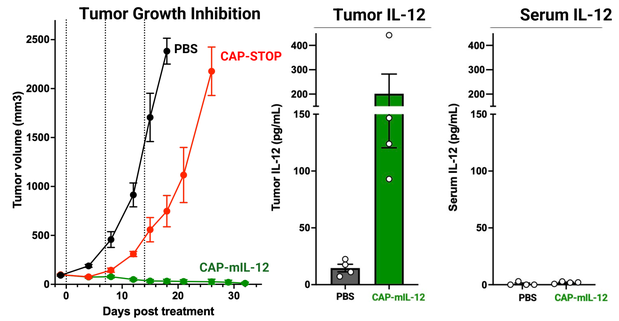


0.35 mg/kg DNA:LNP in MB49 SubQ model, IL-12 under control of a Cancer Activated Promoter
DNA/RNA measured 48 hours post dosing

Engineered LNPs permit **broad, extra hepatic** DNA biodistribution following IV **delivery.**

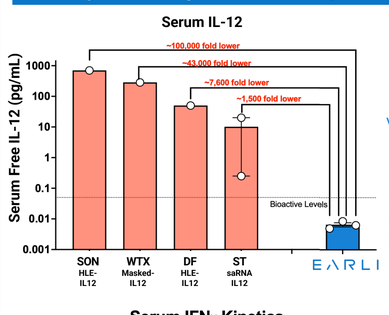
However, cancer-activated promoters show significant **RNA** expression **only in malignant tissues.**

IV administered DNA for cancer-activated IL-12 expression ablates tumors AND avoids high systemic IL-12 exposure



IV administration (q1w x 3 doses) of Earli DNA/LNP using a cancer-activated promoter to produce mIL-12 inhibits growth of subcutaneous (SQ) MB49 tumors. The CAP-STOP control is identical to CAP-mIL-12 except that it contains stop codons in all reading frames. Tumor or Serum IL-12 protein levels at 48 hr were measured by ELISA after the first dose. Note: doses vary somewhat across studies due to LNP and promoter improvements over time.

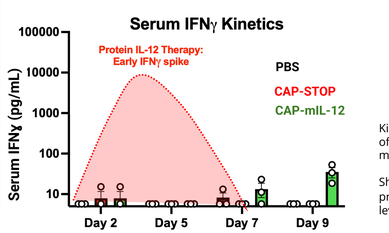
EARLI DNA-encoded IL-12 provides durable tumor expression yet limits systemic exposure and IFNγ vs other IL-12 approaches



Earli:

~2,600 to 180,000 times lower plasma mIL-12 levels vs. masking or RNA technologies (efficacious doses)

Comparison of maximum free (unmasked) IL-12 protein levels in serum within a week after efficacious doses of the Earli LNP/CAP-mIL-12 (0.2mg/kg in MC38 SQ tumor model) vs. alternate approaches for IL-12 therapeutics (data from publications / posters).



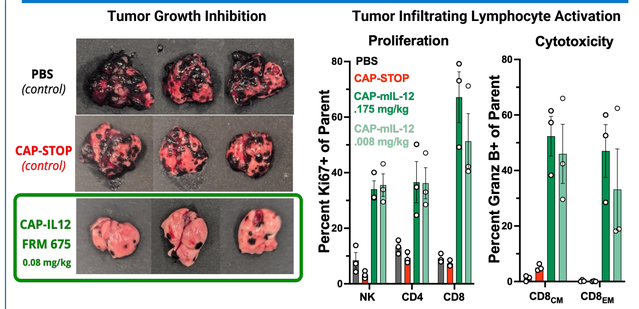
Earli:

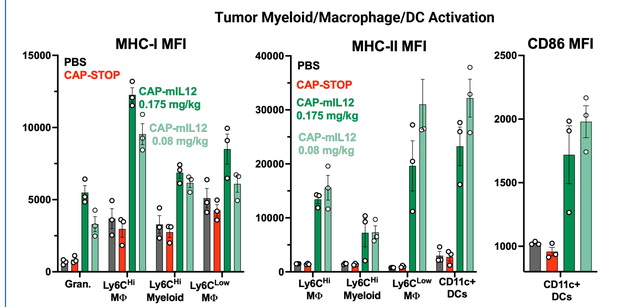
Avoids early IFNγ spikes

Kinetics of serum IFNγ levels after a single dose of LNP/CAP-mIL-12 (0.35 mg/kg; Days 5-9; or 0.7 mg/kg; Day 2) in a MB49-SQ tumor mouse model.

Shown in shading is the typical range for IL-12 protein therapies with rapid high IFNγ serum levels within 3-4 days.

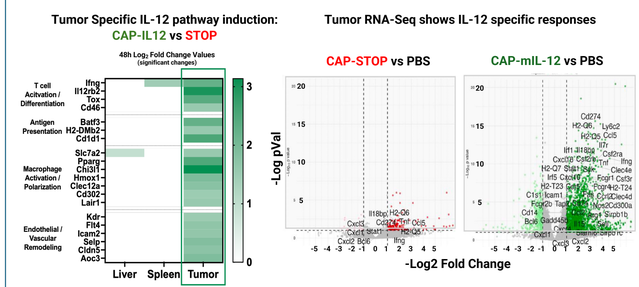
CAP-mIL-12 controls B16F10 mouse lung metastases model





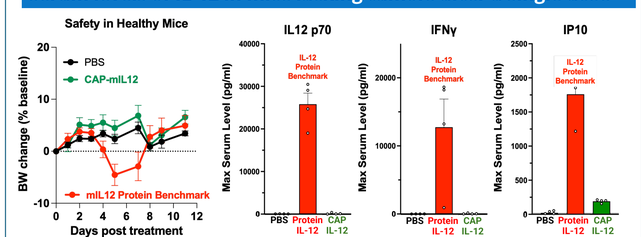
Top Left: Day 15 lungs from mice inoculated IV with B16F10 melanoma cells, treated every 4-5 days with indicated doses of CAP-mIL-12 or Control LNPs. Top Right (Lymphoid) and Bottom (Myeloid) Tumor Immunophenotyping.

IL-12 expressed from cancer activated promoters selectively upregulates immunostimulatory pathways in tumor tissue



Left: significant Log2Fold Changes (RNA-Seq) at 48 hours in a MB49-SQ mouse tumor model treated weekly with 0.7 mg/kg CAP-mIL-12 vs. CAP-STOP control LNP. Right: Day 9 RNA-Seq volcano plots of tumor tissue gene expression for CAP-mIL-12 or CAP-STOP vs. PBS (48 post second dose).

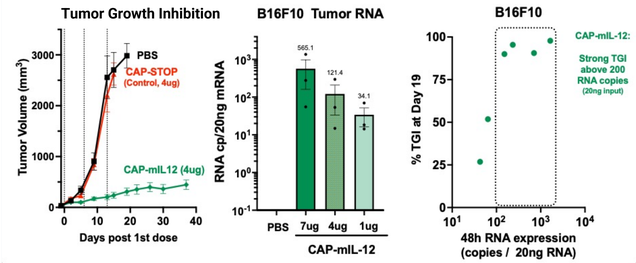
Safety: Cancer-activated promoters do not produce significant serum levels of IL-12 in mice lacking tumors after a single dose



Not shown: D7 FACS show IL-12 protein but not LNP/CAP-mIL-12 induces NK activation (Ki67/GrzB)

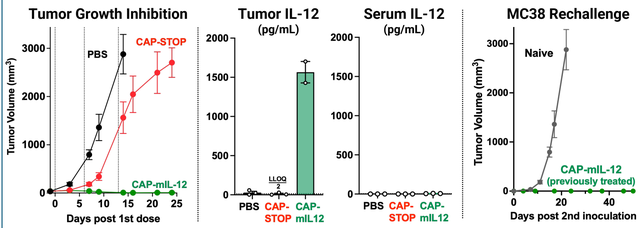
Left: Body Weight Change from treatment with QW IV LNP/CAP-mIL-12 or 3x/week IL-12 protein. Right: Maximum serum IL-12, IFNγ, IP10 levels measured at 48 hr, 96 hr, or 168 hr post first dose.

Efficacy Titrates with IL-12 Levels: B16F10 SQ Tumors



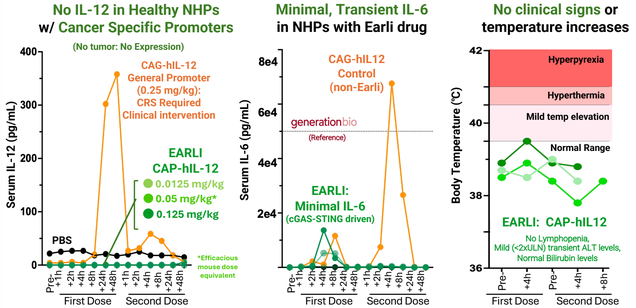
Left: Tumor Volumes in B16F10 SQ mouse model treated IV (q1W x 3 doses) with LNP-CAP-mIL-12 or CAP-STOP. Middle: 48h Tumor encoded IL-12 mRNA levels from mice treated with 0.35, 0.2, 0.5 mg/kg of LNP/CAP-mIL-12. Right: Correlation of Tumor IL12 mRNA expression vs. Tumor Growth Inhibition.

CAP-mIL-12 demonstrates immune memory: MC38 SQ Tumors



Left: MC38 SQ Tumor Volume in mice treated weekly IV with PBS, 0.2mg/kg LNP-CAP-mIL-12 or CAP-STOP. Middle: Corresponding to Left Panel, Serum and Tumor IL-12 protein levels at 48h post dose. Right: MC38 rechallenge of previous MC38 Complete Responders vs naive, non treated animals.

CAP-hIL-12 show an excellent safety profile with no clinical symptoms noted in healthy NHPs (weekly dosing)



NHPs were pre-dosed with Dexamethasone, NSAIDs, & H1/H2 blockers (in accordance with current clinical practice), then received the indicated doses of LNPs with CAP-hIL12 or CAP-control or CAG-hIL12 control and were monitored for serum IL-12 levels (left) serum IL-6 levels (middle) or temperature changes (right).

Conclusions: Cancer Activated Promoters (CAP) Increase Therapeutic Window

IV CAP-IL-12 LNP treatment controls tumor growth in multiple subcutaneous and lung tumor models and demonstrates tumor-selective IL-12 expression with substantially low serum IL-12 exposure or acute spikes in inflammatory cytokines (CRS) typical with other IL-12-based therapies.

Importantly, CAP IL-12 LNP treatment demonstrated cancer specificity, with minimal systemic IL-12 in healthy animals, and was well tolerated in NHPs.

These data show that systemically delivered Cancer-Activated Promoter constructs can selectively reprogram cancer cells to locally produce encoded IL-12 maintaining efficacy and safety and form the basis for pursuing CAP-IL12 as a clinical product.

Beyond IL-12, future work includes expressing different classes of payloads from the same genetic platform.