EARLI-211: *In vivo* drug manufacturing with extrahepatic cancer LNPs and synthetic genetic switches enables safe, tumor-selective expression of IL-12 and robust anti-tumor immunity

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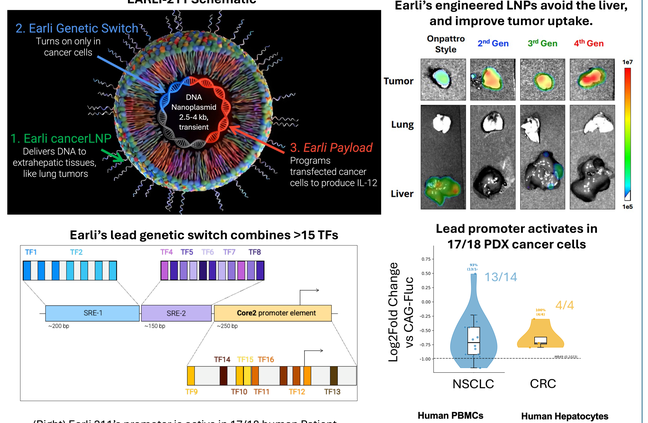
Background and Rationale

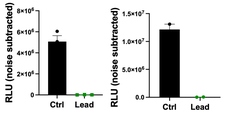
Background: IL-12 has potent anti-tumor activity but has been limited clinically by systemic toxicity. Earli has engineered extrahepatic cancer LNPs and synthetic genetic switches (Cancer-Activated Promoters, CAP) to selectively express therapeutic payloads only in malignant cells, concentrating therapeutic activity within the tumor thereby avoiding systemic on-target, off-tumor toxicity. Here we describe the exquisite tumor-specificity and preclinical anti-tumor efficacy of IV administered cancer LNPs containing CAP constructs encoding IL-12 (CAP-IL12) and the development of clinical candidate EARLI-211.

Methods: CAPs were engineered by computational identification of cancer-associated genes and transcription factors driving cancer hallmark pathways, followed by massive library screening of over 250,000 DNA sequences in cancer cell lines and PDX cells vs. normal hepatocytes, immune cells, and naïve animals. Cancer LNPs were built by systematic engineering of LNP composition using unique lipid components to de-target clearance by liver and immune cells while maximizing cancer cell transfection. LNP delivered CAP-IL12 candidates were tested for in vivo biodistribution, expression, safety, efficacy, and immune phenotyping in various subcutaneous and metastatic syngeneic mouse tumor models, and in healthy mice and NHPs.

Results: Library screening identified CAPs with strong expression across several solid tumor cell lines and PDX samples. Candidate CAPs were prioritized based on strong cancer expression and minimal off-target expression in normal PBMCs, hepatocytes, and healthy tissues in naïve mice. When administered IV, CAP-IL-12 LNPs induced dose-dependent, robust, durable anti-tumor efficacy in multiple syngeneic models. CAP-IL-12 produced sustained IL-12 protein levels in tumor tissues from 2-7 days after dosing, whereas matched serum, lung, and liver IL-12 levels were often below LLOQ. Similarly, CAP-IL-12 induced robust tumor but not serum IFNγ levels, from 2-7+ days after dosing, which were sustained upon repeat dosing, as well as myeloid/DC MHC upregulation and TIL CD8 activation (Ki67 and Granzyme B). CAP-IL-12 repeat-dosing was well-tolerated in NHPs with only mild and transient cytokine induction (IL-6, IFNβ) and acute phase inflammation (AST/CRP) resolving within 24-72h. Importantly, negligible IL-12 expression was detected in healthy mice and NHPs.

EARLI-211 uses extra-hepatic LNPs, and cancer cell-specific genetic switches to drive highly localized IL-12 expression

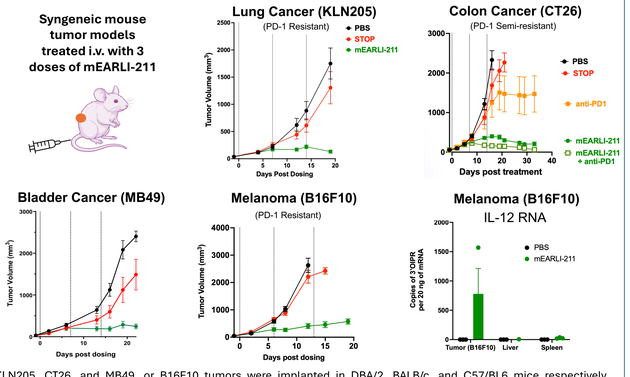




(Right) Earli 1's promoter is active in 17/18 human Patient Derived Xenograft (PDX) non-small cell lung cancer or colorectal cancer lines treated with an EARLI-211 reporter encoding luciferase delivered by the EARLI-211 LNP.

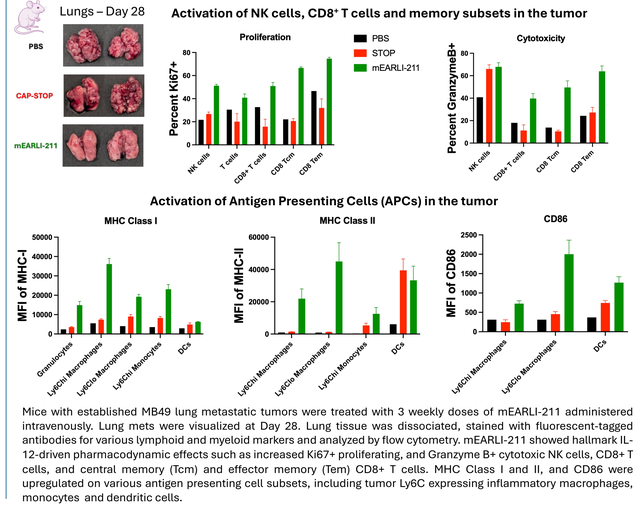
(Right) In healthy human PBMCs or hepatocytes, a globally active control promoter drives luciferase expression upon nucleofection, while EARLI-211's promoter is inactive and luciferase signal remains undetectable.

IV administration of mouse surrogate mEARLI-211 is safe and efficacious in multiple syngeneic murine tumor models



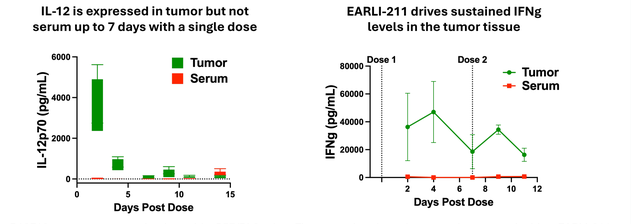
KLN205, CT26, and MB49, or B16F10 tumors were implanted in DBA/2, BALB/c, and C57/BL6 mice respectively. Established tumor-bearing mice were treated with 3 doses of mouse surrogate mEARLI-211, administered weekly IV. Tumor growth inhibition was observed in all four tumor models, including in combination with anti-PD-1 in CT26.
To detect transcripts, mRNA was extracted from tumor, spleen and liver, and IL-12 transcripts were measured by RT-qPCR using payload-specific probes at 48 hr post-dose.

mEARLI-211 controls lung metastases and inflames tumors by driving tumor lymphoid and myeloid activation



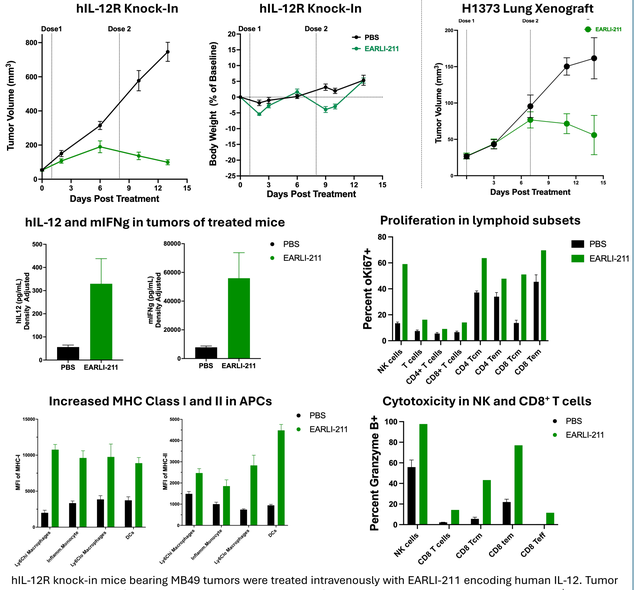
Mice with established MB49 lung metastatic tumors were treated with 3 weekly doses of mEARLI-211 administered intravenously. Lung mets were visualized at Day 28. Lung tissue was dissociated, stained with fluorescent-tagged antibodies for various lymphoid and myeloid markers and analyzed by flow cytometry. mEARLI-211 showed hallmark IL-12-driven pharmacodynamic effects such as increased Ki67+ proliferating, and Granzyme B+ cytotoxic NK cells, CD8+ T cells, and central memory (Tcm) and effector memory (Tem) CD8+ T cells. MHC Class I and II, and CD86 were upregulated on various antigen presenting cell subsets, including tumor Ly6C expressing inflammatory macrophages, monocytes and dendritic cells.

mEARLI-211 drives a slow drip of IL-12 and sustained IFNg in the tumor and still avoids systemic cytokine exposure



B16F10 s.c. tumors were implanted in C57/BL6 mice. The tumor-bearing mice were then treated with mEARLI-211 administered intravenously. Tumor tissue and serum were collected at multiple time points after dosing. Lysates prepared from tumor and serum were analyzed by ELISA to measure IL-12 and IFNg. Tumor cytokine levels were normalized to tissue weight and adjusted (using a g/mL density of water conversion) to obtain pg/mL values.

Human drug candidate EARLI-211 is safe and efficacious in hIL-12R knock-in mice and humanized xenograft models

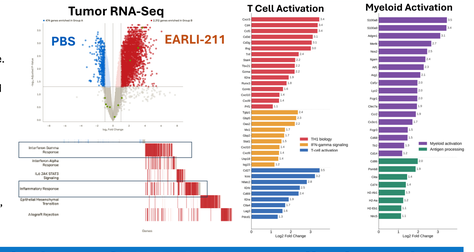


hIL-12R knock-in mice bearing MB49 tumors were treated intravenously with EARLI-211 encoding human IL-12. Tumor volumes and body weight were measured over time. Tumor tissue was collected and lysed at 48 hr post 2nd dose, and human IL-12 and mouse IFNg were measured by ELISA. Peripheral blood was collected, processed, stained with fluorophore-tagged antibodies for various lymphoid and myeloid markers, and analyzed by flow cytometry. As a humanized mouse model, NCG mice implanted with established H1373 human lung tumors and engrafted with human PBMC were treated with 2 weekly IV doses of EARLI-211. Tumor growth was measured over time.

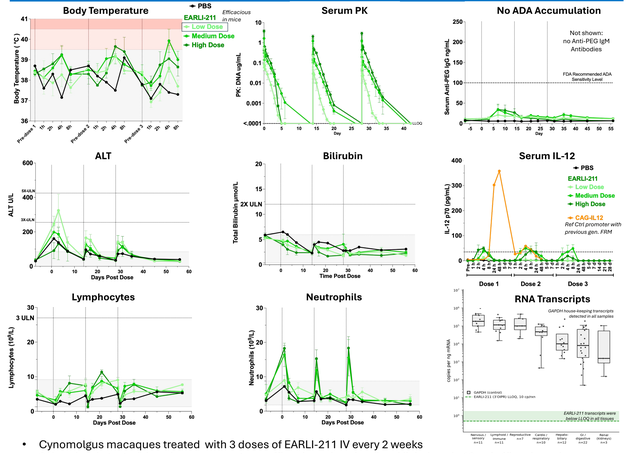
EARLI-211 shows IL-12 driven T cell and APC activation signature at the gene expression level in tumors

MB49 tumors from hIL-12R knock-in mice treated with EARLI-211 were analyzed by RNA-seq at 48h post 2nd dose. Volcano plot shows >2000 genes upregulated compared to PBS controls.

EARLI-211 treated tumors were enriched in pathways like IFNg response and inflammation and had increased expression of multiple genes related to Th1, antigen presentation, T cell and myeloid cell activation



In NHPs, multiple doses of EARLI-211 were well-tolerated with only transient self-resolving cytokine elevations and no IL-12 expression or treatment-related histology



- Cynomolgus macaques treated with 3 doses of EARLI-211 IV every 2 weeks
- 3 dose levels tested; low dose represents an efficacious dose in mice. High dose is 10x of efficacious dose
- Consistent post-dose serum DNA PK with minimal anti-PEG IgG/IgM antibodies even with repeat dosing
- No liver toxicity: Transient self-resolving post-dose elevations in ALT but not bilirubin
- Minimal variations in counts of lymphocytes and neutrophils, and barely detectable serum IL-12
- Comprehensive list of tissues tested by RT-qPCR showed IL-12 payload transcript levels below LLOQ
- *No treatment-related microscopic or macroscopic histopathology findings 28 days post 3rd dose* (data not shown)

Conclusions:

Conclusions: Historically systemic/masked IL-12 modalities have failed because of on-target, off-tumor toxicity while locally-delivered IL-12 has been limited to injectable lesions. By programming only cancer cells to produce and secrete IL-12 directly into the tumor microenvironment, EARLI-211 minimizes systemic exposure and associated on-target, off-tumor effects, and induces sustained local immune stimulation with intravenous systemic delivery. Based on these encouraging preclinical efficacy and safety data, EARLI-211 is currently in IND-enabling studies.