Delivery of Dicer-substrate siRNAs (DsiRNAs) to patient-derived xenograft tumors

Girish R. Chopda, Shanthi Ganesh, Martin Koser, Wendy Cyr, Bo Ying, Dongyu Chen, Serena Shui, Natalie Pursell, Nicole Avitahl-Curtis, Hank Dudek, Cheng Lai, Weimin Wang, Bob D. Brown, Marc Abrams *Dicerna Pharmaceuticals, Inc., Cambridge, MA USA*

Abstract

RNAi-based therapeutics represents a promising class of drugs for achieving pharmacological intervention of classically-"undruggable" targets, and are proceeding through clinical trials for numerous indications. Dicer substrate siRNAs (DsiRNAs) are a potent class of RNAi triggers that exploit the interactions between the endogenous Dicer enzyme and the RNA-induced silencing complex (RISC) to achieve gene silencing. β-catenin, encoded by the CTNNB1 gene, is a well-studied oncology target that is validated by human genetic and functional evidence for tumors of diverse origin, including colorectal and hepatocellular carcinomas. Lipid Nanoparticle (LNP) technology is an elegant solution for delivery of RNAi triggers, since it enables both bioavailability to target organs as well as the ability to transfect target cells. However, while LNPs are well-characterized for delivery of RNA oligonucleotides to the normal liver, much remains to be explored regarding the mechanisms of LNP-mediated delivery to tumors. In this study, we investigated the ability of Dicerna's unique LNP platform, termed EnCore, to deliver Dicer-substrate siRNAs (DsiRNAs) to xenograft tumors of diverse origin. After evaluating patient-derived xenografts (PDX) representing 6 different tumor types, as well conventional cell line-derived xenografts (CLDX), we observed a strong correlation between gross tumor exposure of DsiRNA and knockdown of CTNNB1 mRNA. Additionally, we achieved efficient DsiRNA delivery to tumors implanted subcutaneously as well as orthotopically. Structure-function analyses lead to improvements in the critical LNP components that drive tumor biodistribution, cell internalization and cytosolic partitioning. Taken together, these studies led to deeper understanding of those parameters that drive EnCore-mediated tumor delivery and will result in an improved clinical development strategy.

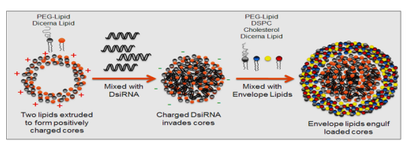
EnCore LNPs for delivery of DsiRNAs to tumors

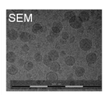
EnCore LNP platform is a fit-for-purpose technology designed for oncology

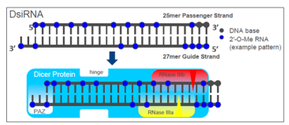
- Components were optimized using *in vivo* tumor models (not normal liver)
- Significantly different in composition, manufacture process, and functionality from so-called SNALP-class LNPs designed primarily for liver targets
- High encapsulation efficiency (>90%) and high yield (>95%), rigorous analytical release criteria, scalable and GMP compatible
- Advanced CMC capabilities due to clinical experience with DCR-MYC

Dicer-substrate siRNAs (DsiRNAs) as the active pharmaceutical ingredient

- Potency: High fM to low pM EC50s for mRNA silencing *in vitro*
- Processing by the Dicer enzyme at the RNAse III sites (as shown) yields the 21-22mer active siRNA, which then incorporates into the RISC machinery. The activated guide (antisense) strand enables Ago2-mediated cleavage of the target mRNA
- DsiRNAs are rendered immunosilent via 2'-ribose and other modifications







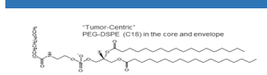
Cationic lipids

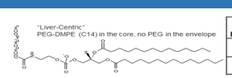
- Structure of the ionizable head group and acyl chains affect cell transfection, cytosolic delivery and lipid clearance
- pKa can be manipulated to survive the acidic microenvironment

PEG-lipids

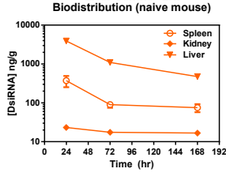
- The acyl chain anchor length is a primary determinant of plasma half-life, hepatic extraction and tumor bioavailability

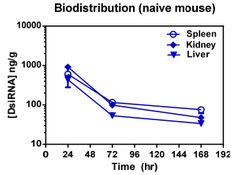
Tumor-centric LNPs avoid rapid hepatic extraction

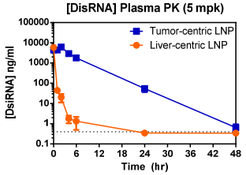




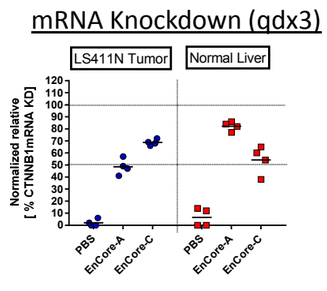
LNP Formulation	Cmax (ng/ul)	AUC (hr*ng/ul)
Tumor-Centric	6800	41000
Liver-Centric	5900	3100

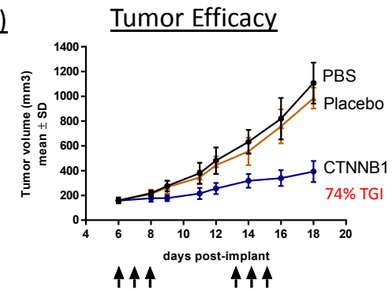




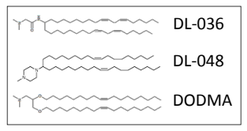


Silencing of CTNNB1 (β-catenin) mRNA yields growth inhibition in Wnt-activated CRC cell line-derived tumors



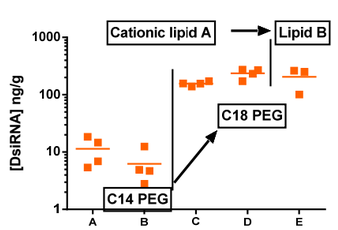


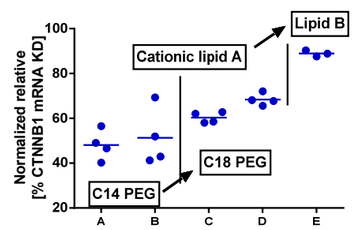
	Core Cationic Lipid	Core PEG Lipid	Env Cationic Lipid	Env PEG lipid	[DsiRNA] tumor exposure (qdx3, 3mpk) ng/g
EnCore-A	DODMA	DMPE (C_{14})	DL-036	DSPE (C_{18})	11.4
EnCore-C	DL-048	DSPE (C_{18})	DL-036	DSPE (C_{18})	237

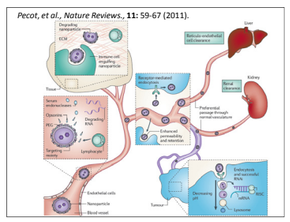


LS411N colorectal carcinoma cells are APC+/- and Wnt-pathway activated. Tumors were implanted subcutaneously in nude mice. When tumors reached >150mm^3 EnCore LNPs were administered i.v. at 3mg/kg/dose. EnCore-D, containing PEG-DSPE in the core and envelope, demonstrates a favorable tumor selectivity profile and delivery efficiency sufficient to drive antitumor efficacy.

Efficient mRNA silencing requires engineering tumor bioavailability and cationic lipid transfection efficiency







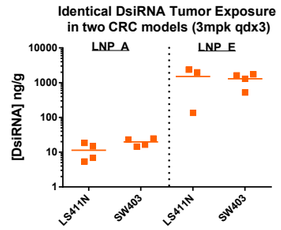
Formidable microenvironment barriers to tumor delivery; biodistribution is only the first hurdle

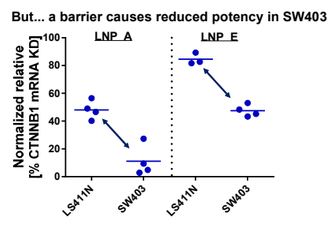
LS411N subcutaneous xenografts, q3dx3 dosing

LNP	Core: Cationic lipid	Core: PEG lipid	Envelope: Cationic lipid	Envelope: PEG lipid
A	DODMA	C14	DL-036	C-18, DSPE
B	DL-48	C14	DL-036	C-18, DSPE
C	DL-48	C18	DL-036	C-18, DSPE
D	DL-48	C18	DL-036	C-18, DSG
E	DL-48	C18	DL-075	C18, DSPE

- Adding 4 carbons to the core PEG-lipid increases tumor exposure >10x (EnCore-B vs. EnCore-C)
- Increasing tumor exposure >10x has only a modest effect on mRNA KD
- However, replacing the envelope cationic lipid with a closely-related compound results in significantly-improved mRNA KD (EnCore-D vs. EnCore E)

Post-exposure delivery barriers vary amongst different CRC cell line-derived xenograft models

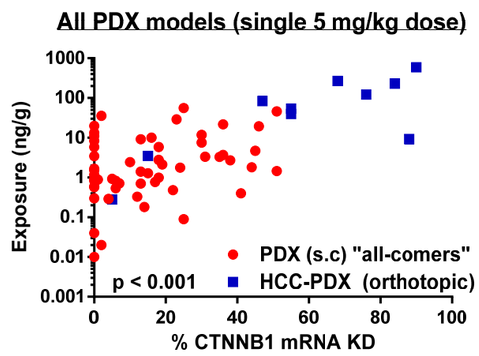


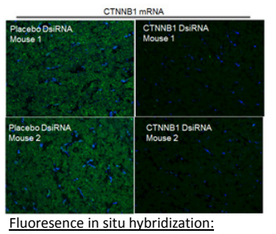


Patient-derived xenografts (PDX): A single-dose PK/PD survey

This correlation plot is a meta-analysis of mRNA KD and tumor exposure of data for 11 subcutaneously-implanted and 2 orthotopic HCC models dosed with LNP-E at 5 mg/kg.

- A single 5 mg/kg dose of CTNNB1 DsiRNA in LNP-E yields up to 55% mRNA KD in s.c. PDX models, and 90% KD in orthotopic PDX models
- LNP-E delivery more efficiently to orthotopic PDX tumors than subcutaneous PDX tumors
- Good overall correlation observed between exposure and KD in this data set





Fluoresence in situ hybridization:
Orthotopic HCC PDX model (model L10050): homogenous delivery throughout the section (green, CTNNB1 mRNA; blue, CD31 mRNA to stain the vasculature)

Model	Tumor status	Cancer type	Histology	Harvest site	Disease stage	% CTNNB1 mRNA KD (LNP-E, relative to placebo)	[DsiRNA] exposure (ng/g)
CTG-0033	Metastatic	Breast	Adenocarcinoma	Lung	IV	11	0.5
CTG-0158	Primary	NSCLC	Squamous cell carcinoma	Lung	III	-4	1.26
CTG-0252	Metastatic	Ovarian	Papillary serous adenocarcinoma	Omentum	N/A	29	8.96
CTG-0253	Primary	Ovarian	Papillary serous adenocarcinoma	Ovary	N/A	37	2.46
CTG-0256	Primary	Ovarian	Papillary serous adenocarcinoma	Ovary	III	ND	3.34
CTG-0828	Metastatic	NSCLC	Large cell adenocarcinoma	Lymph node	II	38	2.62
CTG-1017	Primary	Breast	Invasive ductal carcinoma	Breast	III	10	1.33
CTG-1064	Metastatic	Osteosarcoma	Not available	Scapula	N/A	-8	0.02
CTG-0243	Not available	Osteosarcoma	Osteosarcoma	Bone	III	42	4.55
CTG-0067	Metastatic	Colorectal	Adenocarcinoma	Liver	IV	10	0.87
CTG-0992	Metastatic	Ovarian	Adenocarcinoma	Liver	III	-37	0.7

Summary

- Next generation EnCore LNPs have favorable PK and biodistribution properties for tumor delivery
- Delivery of CTNNB1 DsiRNA yields robust mRNA KD and tumor growth inhibition in Wnt-activated cell line-derived xenograft tumors
- Tuning the core PEG-lipid anchor resulted in higher payload tumor exposure, and modifying the envelope cationic lipid increased transfection efficiency
- A single dose of LNP-delivered DsiRNA was sufficient to reduce CTNNB1 mRNA in multiple patient-derived xenograft models