EnCore-LNP mediated tumor delivery of MYC and CTNNB1 Dicer substrate RNAs (DsiRNAs)

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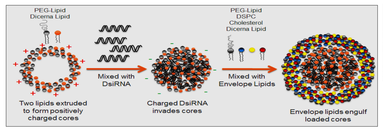
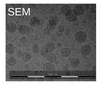
358

Abstract

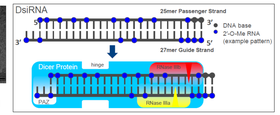
MYC and CTNNB1 (β-catenin) are well-characterized drivers of numerous tumor types. Since the proteins encoded by these genes are challenging to target via conventional modalities, progress in new therapeutic agents has been slow despite decades of research. RNA interference technology has enabled the inhibition of previously-undruggable genetic targets at the mRNA level, and has advanced to clinical development for several indications. DCR-MYC is a Phase I lipid nanoparticle (LNP)-formulated Dicer substrate siRNA (DsiRNA), representing a potent class of RNAi triggers being developed by Dicerna Pharmaceuticals. Dicerna's unique EnCore LNP technology enables tumor delivery of oligonucleotides using a GMP-compatible and scalable formulation. Here we describe new preclinical data that increase our understanding of the parameters that impact tumor delivery and activity of DsiRNA. We optimized the PK properties of EnCore LNPs to minimize hepatic extraction and thus favor tumor bioavailability. In addition to demonstrating MYC mRNA silencing activity in orthotopic liver tumors, we also are reporting results from a quantitative RNAi target engagement assay which showed that >90% of MYC mRNA fragments in the tumor match the DsiRNA target site. Finally, we are reporting efficient targeting of CTNNB1 and efficacy in a colorectal tumor model, and data from ongoing efforts further optimize both the LNP lipid composition and DsiRNA payload for distal tumor sites. These data support clinical development of tumor-selective RNAi therapeutics for multiple oncology indications.

Background: EnCore Lipid Nanoparticles (LNPs) for delivery of potent RNA interference (RNAi) triggers





Mean diameter (DLS): 80-100nM

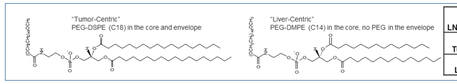


EnCore platform is designed to fulfill multiple delivery functions
- Compositional tuning enables preferential accumulation in liver or tumors
- High encapsulation efficiency (>90%) and high yield (>95%), scalable and GMP compatible
- Significantly different in composition and functionality from so-called SNALP-class and other LNPs that progressed to the clinic

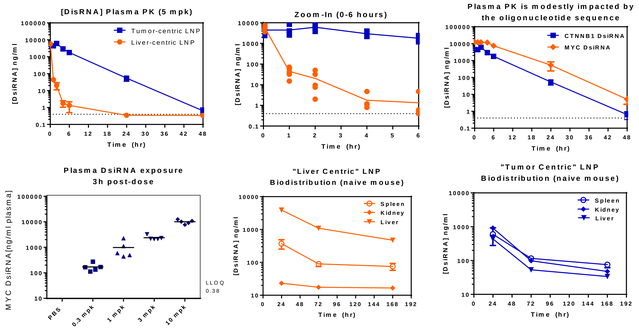
Dicer-substrate siRNAs (DsiRNAs)
- High fM to low pM EC50s for mRNA silencing *in vitro*
- Processing by the Dicer enzyme at the RNAse III sites (as shown) yields the 21-22mer active siRNA, which then incorporates into the RISC machinery. The activated guide (antisense) strand enables Ago2-mediated cleavage of the target mRNA
- Chemically-modified, including 2'-O-Me substitutions, to increase oligonucleotide stability and evade immunostimulation

PK and biodistribution of DsiRNA in mice

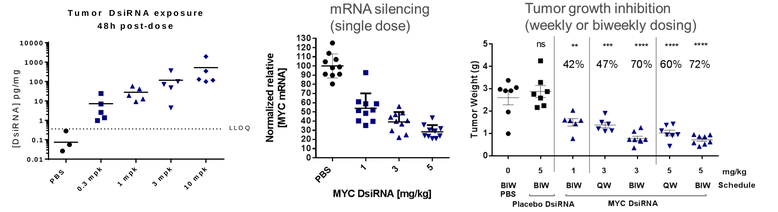
As a class, LNPs are well-characterized for delivery of RNAi triggers to the liver. LNP delivery to tumors is not as efficient and still poorly understood. In EnCore LNPs, the PEG-lipids are a key determinant of LNP biodistribution after systemic administration. For example, a longer acyl chain anchor on the PEG-lipid may increase the plasma T1/2 by preventing immediate extraction by normal hepatocytes. The presence of a PEG-lipid in the LNP envelope as well as the core may also impact PK and favor tumor biodistribution.



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

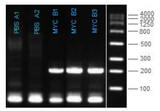


Delivery of MYC DsiRNA, mRNA silencing, and tumor growth inhibition in a model of HCC



Demonstration of target engagement and mechanism of action of MYC DsiRNA *in vivo*

Isolate total RNA
↓
5'RACE adapter ligation
↓
RT cDNA synthesis (oligo dT)
↓
TouchDown PCR
↓
Secondary PCR for NGS barcode addition
↓
QC and Illumina MiSeq



Predicted PCR product: 191bp

Test Article	Mouse	Total counts	Sequences with RACE Adapter	Sequences with RACE adapter and MYC cDNA
MYC DsiRNA	B1	617,220	167,302	161,967
MYC DsiRNA	B2	597,142	178,221	173,776
MYC DsiRNA	B3	558,750	214,752	206,318

In the 5'RACE assay, uncapped mRNA fragments are ligated to a universal adapter, which is used along with MYC mRNA sequence to PCR-amplify the region spanning the RNAi cleavage site (left). Illumina next-generation sequencing then determines the nature and frequency of partial MYC mRNAs in the tumor in a highly quantitative manner (below).

The Ago2 cleavage site is determined by 5'RACE as the position on the passenger (sense) strand corresponding to the 5' terminus of the MYC mRNA. The location of Ago2 cleavage predicts the sequence of the mature siRNA (post-Dicer processing). The Ago2 site is known to be invariably fixed at position 10/11 of the sense strand.

5' Percent of sequence reads containing a 5' end each position in the MYC mRNA target site: 3'

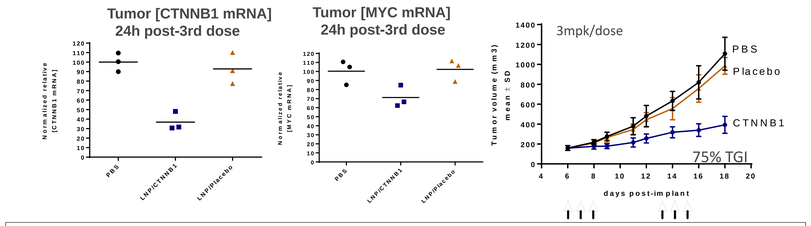
[DsiRNA]	ID	A	G	C	T	T	T	T	T	G	C	C	C	T	G	C	G	T	G	A	C	C	A	G	A
Control	A1	-	-	-	0.02	3.15	2.62	<0.01	2.03	1.92	-	3	<0.01	-	3.35	-	-	<0.01	-	-	-	-	-	-	-
Control	A2	-	-	0.50	<0.01	0.22	0.52	0.27	0.65	1.10	<0.01	2.6	<0.01	-	-	0.45	-	0.82	-	-	-	-	-	-	-
MYC	B1	<0.01	-	<0.01	<0.01	-	-	-	-	0.04	0.25	**93**	0.17	<0.01	<0.01	<0.01	-	<0.01	-	<0.01	<0.01	0.06	<0.01	-	<0.01	<0.01
MYC	B2	-	-	<0.01	-	-	-	-	-	-	0.04	0.30	**94**	0.18	<0.01	-	-	-	-	0.05	0.02	-	-	-	0.03
MYC	B3	-	-	-	-	-	-	-	-	0.19	**96**	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-

Ago2 DICER
5'–AGCTTTTTTGCCCTGCGTGACCAGA–3' Passenger Guide
CCTCGAAAAAACGGGACGCACTGGTCT
DICER

DCR-MYC Ongoing Phase I Clinical Trials

- Open label, dose-escalation trial
- Patients with advanced solid, tumors, lymphoma or multiple myeloma are eligible
- Study is being conducted in 2 sites in the US
- Starting dose was 0.1 mg/kg
- Drug is given by IV infusion once a week for 2-weeks, followed by a 1-week break (3-week cycle)
- Primary objective of the study is to evaluate safety, dose limiting toxicities (DLT), and Maximum Tolerated Dose (MTD)
- Efficacy is being evaluated using FDG-PET scans (to study effects on tumor metabolism), and CT/MRI scans (for RECIST measurements)
- Data from this ongoing study are to be presented at a medical meeting in 2015
- A second study of DCR-MYC in patients with advanced hepatocellular cancer is being initiated

Beyond HCC: Activity of EnCore-delivered CTNNB1 DsiRNA in a subcutaneous xenograft model of CRC



Knockdown of CTNNB1 mRNA in LS411N tumors caused >75% tumor growth inhibition after a two week, well-tolerated dosing regimen. In addition to measuring CTNNB1 mRNA 24h after the second dose, we also measured MYC mRNA as a downstream, transcriptional target of β-catenin. Since the response of MYC mRNA was subtle, other Wnt pathway effectors are likely to contribute to tumor growth inhibition.

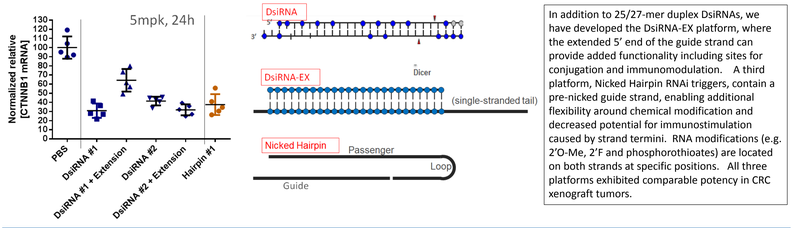
Further optimization of the envelope cationic lipid increases potency of CTNNB1 silencing in CRC tumors and shows tissue selectivity

Cationic Lipid	Structure (Dimethylglycines)	% CTNNB1 KD (3mpk, qdx3) Liver	Tumor
O-9462		21	62
O-9435		55	71
O-9472		46	66
O-9473		53	36

Cationic Lipid	Structure (Piperazines)	% CTNNB1 KD (3mpk, qdx3) Liver	Tumor
O-9412		-8	2
O-9434		55	47
O-9461		6	26

The envelope cationic lipid is the key driver of internalization and cytosolic delivery of RNAi triggers for the EnCore platform. In this lipid optimization screen, dimethyl glycine head groups were more effective than piperazine head groups. Within the dimethyl glycine class, differences in the ratio of mRNA KD between the liver and tumor were noted. Further lead optimization efforts are ongoing.

The API: Evaluation of different classes of RNAi triggers in EnCore LNPs using the LS411N xenograft model



In addition to 25/27-mer duplex DsiRNAs, we have developed the DsiRNA-EX platform, where the extended 5' end of the guide strand can provide added functionality including sites for conjugation and immunomodulation. A third platform, Nicked Hairpin RNAi triggers, contain a pre-nicked guide strand, enabling additional flexibility around chemical modification and decreased potential for immunostimulation caused by strand termini. RNA modifications (e.g. 2'O-Me, 2'F and phosphorothioates) are located on both strands at specific positions. All three platforms exhibited comparable potency in CRC xenograft tumors.

Summary

- The PK properties of EnCore LNPs can be tuned to favor tumor biodistribution
- EnCore LNPs exhibit robust delivery of MYC DsiRNAs to HCC xenograft tumors and MYC mRNA silencing
- Target engagement can be quantitatively measured by the 5'RACE assay and Illumina sequencing
- DCR-MYC is currently progressing through Phase 1 clinical trials
- CTNNB1/β-catenin can be robustly silenced in colorectal xenograft tumors, yielding robust tumor growth inhibition
- Both the formulation and the oligonucleotide payload (API) are being optimized for clinical development of CTNNB1 DsiRNAs