Development of Novel RNAi-based Therapeutics Targeting Survivin for Treatment of Liver and Bladder Cancer

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Abstract

Harnessing RNA interference (RNAi) to silence aberrant gene expression is an emerging approach for cancer therapy, and could provide much-needed treatment for highly refractory cancers such as liver and bladder. Survivin is involved in estrogen production and promotes cell survival by inhibiting apoptosis. RNAi-based therapies using single stranded RNAi molecules (siRNAs) and Liposome formulation requires a highly efficacious and efficient delivery system. MDselect™ (MDRNA) is a novel non-viral delivery system that provides a unique and robust platform for RNAi-based therapy that is optimized for efficacious and sustained delivery. Amino nucleobase analogs (UNA) are designed to target Survivin mRNA in KU81 cells with 90% inhibition and a non-cytotoxic effect that can be monitored by cell viability assay. Inhibition of survivin mRNA and protein, 5'-RACE PCR and sequencing, Tumor growth/behavior, and Tumor cell volume/weight and histopathology for enhanced efficacy and prolonged therapeutic effects against a variety of cancers.

Survivin UsiRNA: Efficacy and Potency in vitro

- Low IC₅₀ values (~28.5 pM with UsiRNAs targeting Survivin in KU81 liver cancer cells)
- Similar response in Hep3B liver cancer cells

Phenotypic Response to UsiRNA in vitro

- CI50 cells, 21 days post transfection
- Induction of Apoptosis with Survivin UsiRNA results in cell growth arrest

Unlocked Nucleobase Analogs and UsiRNAs

- UNAs are non-nucleotidic, acyclic moieties that provide greater structural flexibility in the RNA database
- DiLA-alkylated double-stranded UNAs that are modified with strategically placed non-nucleotidic entity to termed Unlocked Nucleobase Analogue (UNA)
- Metabolite full activity
- Reduced nuclease sensitivity
- Eliminates RNAi tolerability effects
- Decrease cytokine response

Primary Hepatocellular Carcinoma (HCC)

- Primary HCC arises from hepatocytes
- Leading causes are infection (hepatitis), toxins, and result to the liver that results in cirrhosis
- Worldwide ~500,000 new cases/year
- 100,000 in the US
- Five-year survival rate ~75%
- "First line" therapy (Sorafenib) extends survival ~3 months
- Treatment indication for a RNAi-based therapeutics
- Primary disease when surgery is not an option
- Recurrence following surgical intervention
- In conjunction with chemotherapeutics and mechanical interventions

UsiRNAs targeting Survivin in Orthotopic Liver Tumors

- UsiRNAs demonstrated ~40% reduction of Survivin mRNA in orthotopic liver tumors
- No inhibition with scrambled control
- Identified 80% for RISC-mediated RNAi activity in orthotopic liver tumors

Tumor Weights in Orthotopic Liver Cancer Model

- UsiRNAs elicited ~40% reduction in mean tumor weight by day 51 post tumor implantation

DiLA² (Di-alkylated Amino Acids) Delivery Platform

- Synthetic compounds composed of unique combination of head groups, linkers and alkyl chains
- Self-assemble into liposomes with UNAs and other components
- Unaltered 100% particle rise and ~ 80% UNA encapsulation
- UNA delivery with DiLA² liposomes
- Effective delivery to hepatocytes and solid tumors
- Well tolerated with single dose and repeat dose

SIRNA Therapeutics: Mechanism of Action

- SIRNA comprises a sense or an antisense strand of RNA
- Each strand contains 21 to 23 oligo dinucleotides
- 5' and 3' ends are modified to enhance stability and specificity
- Alkyl chain: R2
- Alkyl chain: R3

Survivin UsiRNA: Tumor Model and Dosing Scheme

- Dosing scheme: 2 mg/kg; q.d.; 3 days/week liver tumor volume ~50 mm³ at day 22 based on day 0
- Control: 0.5 mg/kg; q.d.; day 4
- Positive control: Javine (0.5 mg/kg; q.d.; 4 l.p.)

Evaluation

- RNA Biology
- Confirmation of target mRNA and protein; 5'-RACE PCR and sequencing
- Tumor growth/behavior
- Tumor cell volume/weight and histopathology

Orthotopic Liver Cancer Model Dosing Scheme

- Model: KU81 (human) liver cancer cells injected orthotopically in the right lateral liver lobe in SCD Beige mice
- Treatment: DiLA² liposome formulation for intravenous dosing
- Dosing schedule: 2 mg/kg; q.d.; 3 days/week liver tumor volume ~50 mm³ at day 22 based on day 0
- Control: 0.5 mg/kg; q.d.; day 4
- Positive control: Javine (0.5 mg/kg; q.d.; 4 l.p.)

Evaluation

- RNA Biology
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Tumor Growth in Orthotopic Bladder Cancer Model

- Dose-dependent decrease in tumor weight in orthotopic bladder tumors
- No decrease in scrambled control group

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Unlocked Nucleobase Analogue

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Summary

- Hepatocellular Carcinoma
- Established systemic delivery of Survivin UsiRNAs to tumors in liver and subcutaneous space using DiLA²-based liposomes
- Demonstrated UsiRNA-mediated inhibition of Survivin mRNA in orthotopic and subcutaneous tumors
- Confirmed RNAi-mediated mechanism of action in orthotopic and subcutaneous tumors
- Decreased liver tumor weights in survivin transgenic animals with orthotopic liver tumors

- Bladder Cancer
- Confirmed local delivery to tumors in the bladder urothelium using DiLA²-based liposomes
- Confirmed UsiRNA-mediated inhibition of Survivin mRNA in orthotopic bladder cancer
- UsiRNA demonstrated dose-dependent reduction in bladder tumor growth
- Duration of RNAi effect sustained over a period of 11 days post dose