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

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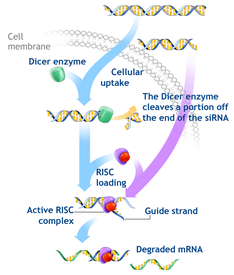
Abstract

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 mitotic progression and inhibition of apoptosis, and over-expression of survivin is associated with cancer progression and resistance to chemotherapy. Inhibition of protein expression via RNAi requires a highly efficacious siRNA and efficient delivery system. MDRNA has developed a novel siRNA construct (UsiRNA) targeting survivin that contains unlocked nucleobase analogs and possesses high potency with greater drug-like properties. UsiRNAs are delivered to the target tissues using novel Di-alkylated Amino Acid (DiLA^2) liposomes. In an orthotopic liver model, systemic administration of UsiRNA/DiLA^2 liposomes resulted in significant decrease in survivin expression in human cell-derived tumors and reduced tumor volume. A survivin UsiRNA/DiLA^2 liposome formulation delivered locally via intravesical instillation in an orthotopic bladder cancer model resulted in 90% knockdown of survivin mRNA and substantial decrease in tumor volume. This response in bladder tumor was dose dependent and sustained over at least a three week period. Our future work includes screening additional genes that exploit multiple pathways in cancer to target critical cancer phenotypes for enhanced efficacy and prolonged therapeutic effect against a variety of cancers.

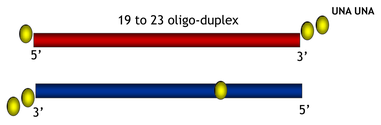
SiRNA Therapeutics: Mechanism of Action

- siRNA compounds silence or down regulate genes and viruses via an endogenous, catalytic mechanism



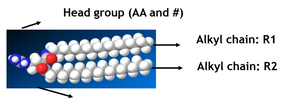
Unlocked Nucleobase Analogs and UsiRNAs

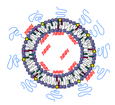
- UNAs are non-nucleotide, acyclic monomers that provide greater structural flexibility in the RNA backbone
- UsiRNA – Blunt-ended double-stranded siRNAs that are modified with strategically placed non-nucleotide entities – termed Unlocked Nucleobase Analogs (UNA)
  - Retains full activity
  - Reduces nuclease sensitivity
  - Eliminates microRNA-like effects
  - Decreases cytokine response



DiLA^2 (Di-Alkylated Amino Acids) Delivery Platform

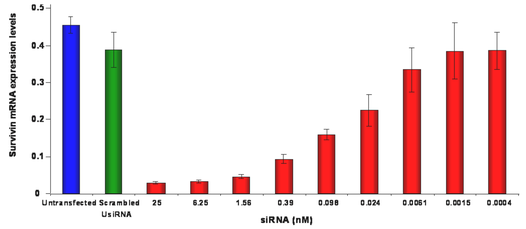
- Synthetic compounds composed of unique combinations of head groups, linkers and alkyl chains
- Self-assembly into liposomes with siRNA and other components
  - Unilamellar, 100-130 nm particle size and > 80% siRNA encapsulation
- siRNA Delivery with DiLA^2 liposomes
  - Effective delivery to hepatocytes and solid tumors
  - Well tolerated with single dose and repeat dose





Freeze-Fracture

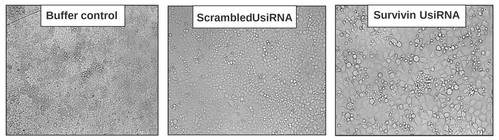
Survivin UsiRNA : Efficacy and Potency in vitro



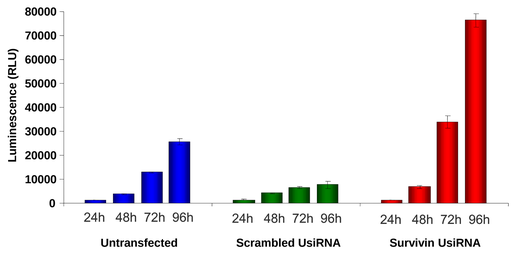
KU-7 cells, Transfection reagent, 24 h transfection

- Low IC_{50} values ~ 28.5 pM with UsiRNAs targeting Survivin in KU-7 bladder cancer cells
  - Similar response in Hep3B liver cancer cells

Phenotypic Response to UsiRNA in vitro



KU-7 cells, 200x magnification, 96 h post-transfection

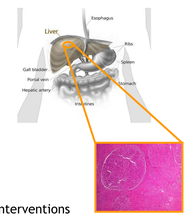


KU-7 cells; Transfection reagent; Caspase-Glo 3/7 Assay System

- Induction of Apoptosis with Survivin UsiRNA results in cell growth arrest

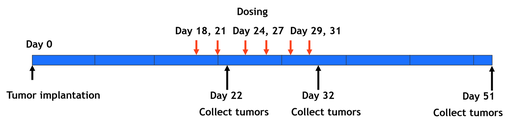
Primary Hepatocellular Carcinoma (HCC)

- Primary HCC arises from hepatocytes
- Leading causes are viral infection (hepatitis), toxins, and insult to the liver that results in cirrhosis
- Worldwide >500,000 new cases/year
  - ~20,000 in the US
- Five year survival rate ~7%
  - "First line" therapy (Sorafenib) extends survival ~3 months



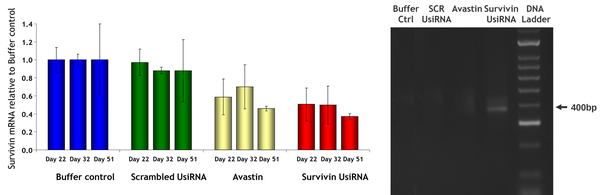
- Treatment indication for a RNAi-based therapeutic
  - Primary disease when surgery is not an option
  - Recurrence following surgical intervention
  - In conjunction with chemotherapy and mechanical interventions

Orthotopic Liver Cancer Model Dosing Scheme



- Model
  - Hep3B (human) liver cancer cells injected orthotopically in the right lateral liver lobe in SCID Beige mice
- Treatment
  - DiLA^2 liposome formulation for intravenous dosing
  - Dosing schedule: 2mg/kg; q3d x 6 doses (Mean tumor volume ~50 mm^3 at day 22 based on AFP levels in serum)
  - Positive control: Avastin (5 mg/kg; q3d x 6; i.p.)
- Evaluation
  - RNAi biology
    - Inhibition of target mRNA and protein, 5'-RACE PCR and sequencing
  - Tumor growth/distribution
    - Tumor cell volume/weight and histopathology

UsiRNAs targeting Survivin in Orthotopic Liver Tumors

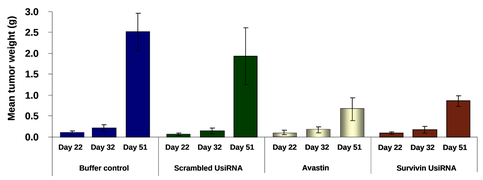


2 mg/kg; q3d x 2 or 6; i.v.; DiLA^2; n=5/group; Standard Error

5'RACE-PCR, 2% Agarose gel; Nested PCR product at day 22

- UsiRNAs demonstrated ~60% knockdown of Survivin mRNA in orthotopic tumors
  - No inhibition with scrambled control
  - Identified 5'RACE-PCR product of predicted molecular size (~400 bp) confirming RISC-mediated RNAi activity in orthotopic tumors

Tumor Weights in Orthotopic Liver Cancer Model

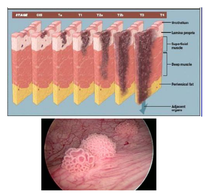


2 mg/kg; q3d x 6; i.v.; DiLA^2; n=5/group; Standard Error

- UsiRNAs elicited ~60% reduction in mean tumor weight at day 51 post tumor implantation

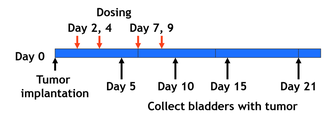
Bladder Cancer

- 5th most common cancer in the US
  - >67,000 new cases/year
  - >13,000 deaths/year
  - >80% are "Non-muscle invasive"
- Surgical removal is first-line therapy for non-muscle invasive disease
- Surgery is not curative
  - 50-70% for recurrence
  - 10-50% for progression



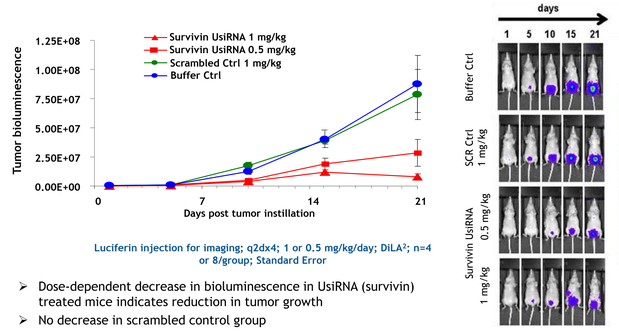
- Treatment indication for a RNAi-based therapeutic in non-muscle invasive bladder cancer
  - In conjunction with surgical removal of tumor tissue
  - Follow-up treatment post surgery
- Direct instillation (intravesical) via catheter is a common route for treatment of bladder cancer
  - Localized application for DiLA^2 Liposome formulation

Orthotopic Bladder Cancer Model Dosing Scheme



- Model
  - KU-7 (human) bladder cancer cells expressing luciferase implanted into the urothelium of Nude mice
- Treatment
  - DiLA^2 liposome formulation
  - Intravesical dosing
  - 50 µL dose volume (~0.5 or 1.0 mg/kg/day dose level)
- Evaluation
  - RNAi Biology
    - Inhibition of target mRNA and protein
    - 5'-RACE and sequencing
  - Tumor growth kinetics
    - Tumor imaging
    - Tumor histopathology

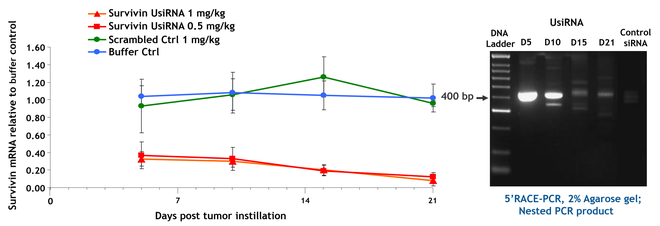
Tumor Growth in Orthotopic Bladder Cancer Model



Luciferin injection for imaging; q2dx4; 1 or 0.5 mg/kg/day; DiLA^2; n=4 or 8/group; Standard Error

- Dose-dependent decrease in bioluminescence in UsiRNA (survivin) treated mice indicates reduction in tumor growth
- No decrease in scrambled control group

UsiRNAs targeting Survivin in Orthotopic Bladder Tumors



DiLA^2 formulations; Human-specific PCR primers; bladder tissue; n=4/group; Standard Error

5'RACE-PCR, 2% Agarose gel; Nested PCR product

- UsiRNAs demonstrated 90% inhibition in Survivin mRNA in KU-7 derived orthotopic bladder tumors with knockdown persistent over 11 days post last dose (2 or 4 mg/kg total dose)
- Identified 5'RACE-PCR product of predicted molecular size (~400 bp) confirming RISC-mediated RNAi activity in bladder tumors

Summary

- **Hepatocellular Carcinoma**
  - Established systemic delivery of Survivin UsiRNAs to tumors in liver and subcutaneous space using DiLA^2-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-treated animals with orthotopic liver tumors
- **Bladder Cancer**
  - Confirmed local delivery to tumors in the bladder urothelium using DiLA^2-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 last dose