Abstract # 4775

Optimizing the potency and dosing design for ARO-HIF2: An RNAi therapeutic for clear cell renal cell carcinoma

So Wong, Anthony Nicholas, Jeff Carlson, Dongxu Shu, Che Liu, Rui Zhu , Amanda Frankiewicz, Holly Hamilton, Casi Schienebeck, Aaron Andersen, Matthew Fowler-Watters, Stephanie Bertin, Xiaokai Li, Bo Chen, Josh Schumacher, Julia Hegge, Bruce Given and Zhen Li

Arrowhead Pharmaceuticals Inc., Madison, WI, United States

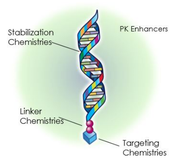
BACKGROUND

Clear cell renal cell carcinoma (ccRCC) frequently involves the inactivation of the von Hippel-Lindau (*VHL*) tumor suppressor. Loss of VHL functions lead to the accumulation of hypoxia-inducible factors (HIFs). HIF2α has been regarded as a key tumorigenic driver of ccRCC and an attractive therapeutic target. Arrowhead has developed a RNA interference therapeutic (HIF2 RNAi) to selectively target and silence *HIF2α* expression, using a proprietary targeted-RNAi molecule (TRiM™) delivery platform for the treatment of ccRCC. The TRiM™ based HIF2 construct comprises a highly potent RNAi trigger using stabilization chemistries, targeting ligands to facilitate delivery, and structures to enhance pharmacokinetics (PK). The optimization of HIF2 RNAi to enhance the potency and safety profile to maximize the potential clinical success is described.

METHODS

Functional optimization of HIF2 RNAi was evaluated in an orthotopic ccRCC tumor xenograft model established with A498 ccRCC cells that stably expresses the reporter gene SEAP (secreted embryonic alkaline phosphatase) as a serum biomarker for monitoring tumor growth. HIF2 RNAi was delivered by intravenous injections. *HIF2α* gene silencing was evaluated by isolating tumor RNA and measuring relative gene expression by qRT-PCR.

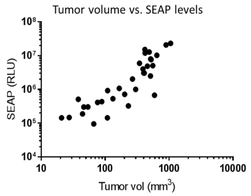
TRiM™ Platform



- Rules and algorithms allow selection of optimized RNAi trigger sequences
- Limit cross-reactivity with off-target genes
- Maximize innate stability
- Rational use and placement of modifying chemistries
- Targeting moiety facilitates tumor uptake and endocytosis of triggers
- Active endosomal escape chemistries not required

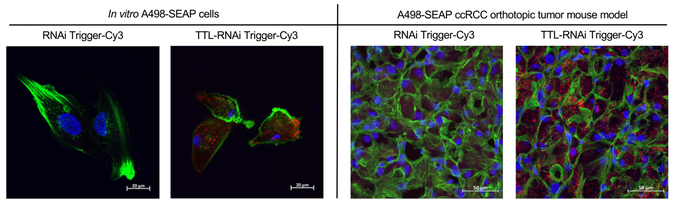
A498 orthotopic kidney xenograft mouse model

- A498 is an established ccRCC cancer cell line
- Stably express SEAP (secreted embryonic alkaline phosphatase)
- Good correlation between SEAP levels and tumor volumes
- Used as sensitive serum biomarker to monitor tumor growth



RESULTS

Tumor targeting ligand (TTL) facilitates receptor-mediated tumor uptake of a HIF2α RNAi trigger

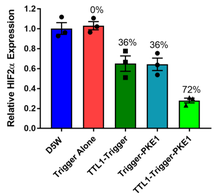


- Tumor cell uptake is greatly enhanced with TTL functionalized HIF2 RNAi
- TTL selectively binds to integrin receptors αvβ3 and αvβ5 with high affinity
- A498 cell uptake *in vitro* (Left panel), 2 ug/mL of trigger incubated overnight at 37ºC
- A498-SEAP tumor bearing mice *in vivo* (Right panel). Mice were administered 2 mg/kg (IV) of RNAi trigger and euthanized 4 hours later.

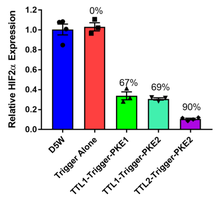
Targeted ligand and pharmacokinetic enhancer improves gene silencing

- A498 tumor bearing mice were administered a single 10 mg/kg IV dose of HIF2 RNAi on study Day 1
- Tumor *HIF2α* gene silencing was evaluated on study Day 8

Prototype HIF2 RNAi



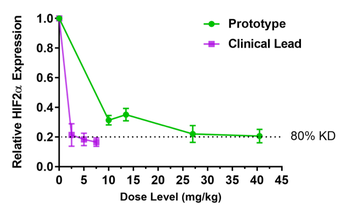
Clinical lead HIF2 RNAi



- Prototype HIF2 RNAi demonstrated that *HIF2α* gene silencing is dependent on the presence of targeting ligand (TTL1) and pharmacokinetic enhancer (PKE1)
- Combining these components appears to be additive
- Improving overall properties by replacing PKE1 with PKE2 without effect on potency
- Optimization of targeting ligand design in additional studies further improve HIF2 RNAi potency in mice

HIF2α gene silencing dose response: clinical lead vs prototype HIF2 RNAi

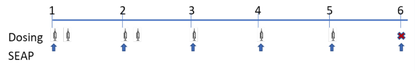
- A498 tumor bearing mice were administered a single dose of HIF2 RNAi on study Day 1
- Tumor *HIF2α* gene silencing was evaluated on study Day 8



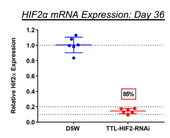
- **Prototype** = TTL1-Trigger-PKE1
- **Clinical Lead** = TTL2-Trigger-PKE2
- Clinical lead HIF2 RNAi is ~10-fold more potent than the prototype conjugate (at 80% KD

Clinical Lead HIF2 RNAi inhibits tumor growth in A498 ccRCC orthotopic mouse model: Study 1

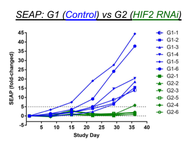
- Mice received weekly 5 mg/kg/dose HIF2 RNAi or D5W via IV injection as indicated
- SEAP levels monitored weekly



HIF2α mRNA Expression: Day 36

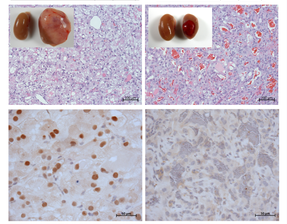


SEAP: G1 (Control) vs G2 (HIF2 RNAi)



- Deep *HIF2α* mRNA knockdown by qRT-PCR
- IHC shows corresponding loss of HIF2α protein
- HIF2α gene silencing inhibits tumor growth as assessed by SEAP expression and tumor weights
- Histology shows wide-spread tumor destruction, with areas of apoptosis and necrosis. Insets showing kidneys with tumor (right) and contralateral kidney (left)

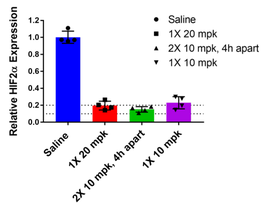
Control Tumor (Day 36) *HIF2 RNAi (Day 36)*



Brown: HIF2α protein by IHC

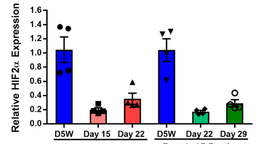
Optimizing dose levels and dosing frequency

Optimizing dose levels



- A498 tumor bearing mice were given a single dose or two IV doses (4h apart) of clinical lead HIF2 RNAi
- Two doses of 10 mg/kg (4h apart) appears to have a slight advantage in gene KD over a single injection of 10 or 20 mg/kg dose

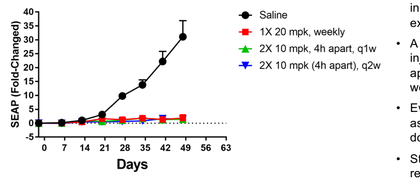
Optimizing dosing frequency



- A498 tumor bearing mice were given two single 5 mg/kg IV dose of clinical lead HIF2 RNAi either one or two week(s) apart
- Gene silencing was evaluated one and two week(s) after the final dose was given
- Data suggest weekly dosing is likely not required for optimal efficacy

Clinical Lead HIF2 RNAi inhibits tumor growth in A498 ccRCC orthotopic mouse model: Study 2

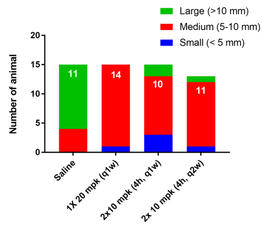
- Mice (n=10) received weekly 20 mg/kg, 2X 10 mg/kg 4 hours apart either q1w or q2w via IV injection as indicated. A control group received saline
- SEAP levels monitored weekly



- All treatment groups show HIF2 RNAi inhibits tumor growth as assessed by SEAP expression
- A weekly 20 mg/kg dosed as a single injection or as 2X 10 mg/kg doses 4 hours apart appears to have similar efficacy at 7 weeks after dosing initiated
- Every other week dosing has similar efficacy as weekly dosing, supporting a less frequent dosing regimen
- Study on-going to monitor long-term response

Clinical Lead HIF2 RNAi inhibits tumor growth in PDX mouse model

- Orthotopic tumor bearing mice (n=15) received weekly 20 mg/kg, 2X 10 mg/kg 4 hours apart either q1w or q2w via IV injection as indicated. A control group received saline
- Body weight and tumor growth monitored by palpation and caliper estimate weekly



- Effects on tumor growth up to study Day 34 is shown
- Study on-going to monitor overall survival
- A weekly 20 mg/kg dosed as a single injection or as 2X 10 mg/kg doses 4 hours apart appears to have similar tumor growth inhibition effects
- Every other week dosing has similar efficacy as weekly dosing

Clinical lead HIF2 RNAi is well tolerated in an exploratory toxicity study

- In naïve rats, clinical lead HIF2 RNAi was dosed at 30 mg/kg (IV) 3 times per week for 5 weeks
  - Dosing regimen was more frequent than anticipated clinically and at a dose level 4-6 times higher than anticipated therapeutic dose
- No abnormal clinical observations or changes in body weight during in-life evaluations
- Minor increases in cholesterol and creatinine, and minor decreases in triglyceride and albumin were noted at Day 32 when compared to vehicle control
- No other notable clinical pathology findings
- Microscopic changes noted in the liver and kidney were consistent with the alterations typically found in rats administered with RNAi therapeutics

Tumor targeting receptor (αvβ3) expression in human ccRCC tumor microarray

- Developed immunohistochemistry method to identify αvβ3 positive samples from primary and metastatic tumors in patients
- Percentage of αvβ3 positive patients ranges from 50% - 69% in 3 out of 4 different microarrays
- Higher αvβ3 expression frequency in metastatic tumors

Integrin αvβ3 Expression in Human ccRCC TMA

Company	Total ccRCC case #	% αvβ3-Positive					
		Overall	Tumor Grade				Metastatic
			1	2	3	4	
Vendor 1	77	31 (24/77)	30 (13/44)	30 (8/27)	50 (3/6)	n.a.	50 (1/2)
Vendor 2	20	50 (10/20)	n.a.	38 (3/8)	71 (5/7)	33 (1/3)	50 (2/4)
Vendor 3	34	65 (22/34)	n.a.				
Vendor 4	146	69 (100/146)	67 (30/45)	63 (32/51)	63 (10/16)	61 (11/18)	80 (12/15)

CONCLUSIONS

We demonstrate that the TRiM™ delivery platform can be utilized to deliver a RNAi therapeutic selectively targeting HIF2α for the treatment of ccRCC. This represents a novel therapeutic approach either as a monotherapy or in combination with other therapies in seeking better tolerated and/or more effective treatment for ccRCC

ACKNOWLEDGEMENTS

We thank Vladimir Subbotin, Maria Afrazi and Anna Rowe for their contributions in histopathology evaluations; the Arrowhead Laboratory Animal Research team for their excellent animal care and surgical techniques