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Targeting HIF2α with an RNAi Therapeutic for the Treatment of Clear Cell Renal Cell Carcinoma

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BACKGROUND

Approximately 80%-90% of clear cell renal cell carcinoma (ccRCC) tumors express an inactive mutant form of the von Hippel-Lindau protein (pVHL). This functional loss of pVHL leads to the accumulation of hypoxia-inducible factor. An overabundance of HIF2α transcription factor is thought to act as a tumorigenic driver of ccRCC. Arrowhead has developed a RNA interference therapeutic (HIF2 RNAi) to selectively target and silence HIF2α, using a proprietary targeted-RNAi molecule (TRiM™) platform. The TRiM™ platform comprises a highly potent RNAi trigger using sequence specific stabilization chemistries, high affinity targeting ligands to facilitate delivery, and structures to enhance pharmacokinetics. Utilizing HIF2 RNAi in tumor xenograft models, we demonstrate deep HIF2α mRNA knockdown with tumor growth inhibition and increased overall survival.

METHODS

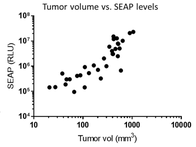
Cellular uptake of Cy3-labeled, tumor targeted HIF2 RNAi was evaluated in A498 ccRCC cells *in vitro* and orthotopic tumor bearing mice *in vivo*. A498-SEAP cells stably express the Secreted Embryonic Alkaline Phosphatase transgene as a biomarker for tumor growth. In a tumor growth inhibition study, orthotopic A498-SEAP tumor bearing mice received weekly doses of HIF2 RNAi via IV injection for 5 weeks with SEAP plasma levels monitored weekly. Tumors were collected for histopathology, tumor weight, and HIF2α mRNA expression. For overall survival evaluation, orthotopic patient derived xenograft (PDX) bearing mice were treated with HIF2 RNAi or D5W via IV injection 3 times per week. For exploratory toxicology, HIF2 RNAi was administered via IV injection 3 times per week for 5 weeks in rats. Body weights, clinical chemistry, hematology, and limited histopathology were evaluated.

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

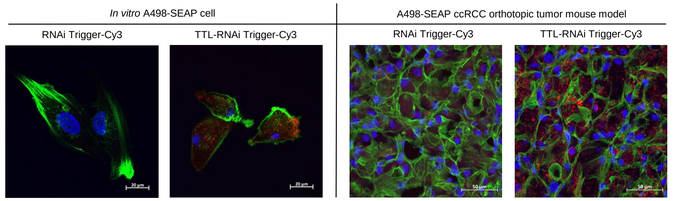
A498 orthotopic kidney xenograft mouse model

- 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

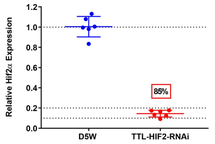


- Tumor cell uptake is greatly enhanced with TTL functionalized HIF2 RNAi
- A498 cell uptake *in vitro* (Left panel), 2 ug/mL of trigger incubated overnight at 37ºC
- A498-SEAP tumor bearing mice *in vivo* (Right panel). Mice were administered with 2 mg/kg (IV) of RNAi trigger and euthanized 4 hours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

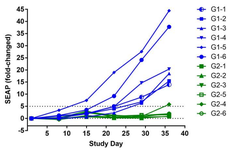
Tumor targeted HIF2 RNAi inhibits tumor growth in A498 ccRCC orthotopic mouse model

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

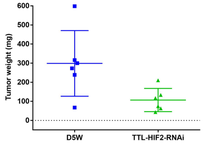
HIF2α mRNA Expression: Day 36



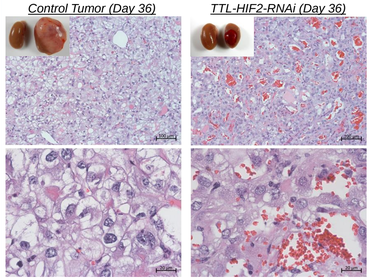
SEAP: G1 (Control) vs G2 (TTL-HIF2 RNAi)



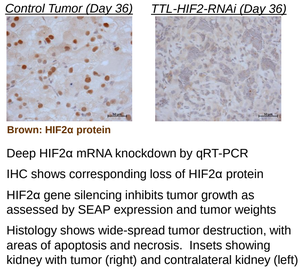
Tumor Weights: Day 36



Control Tumor (Day 36) | *TTL-HIF2-RNAi (Day 36)*



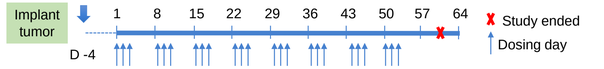
Control Tumor (Day 36) | *TTL-HIF2-RNAi (Day 36)*



Brown: HIF2α protein

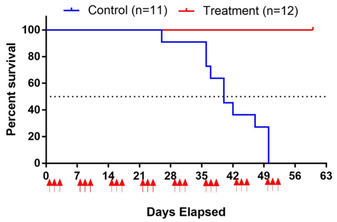
- Deep HIF2α mRNA knockdown by qRT-PCR
- IHC shows corresponding loss of HIF2α protein
- HIF2α gene silencing inhibits tumor growth as assessed by SEAP expression and tumor weights
- Histology shows wide-spread tumor destruction, with areas of apoptosis and necrosis. Insets showing kidney with tumor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

TTL-HIF2 RNAi increases overall survival in patient derived xenograft (PDX) tumor bearing mice using an early generation of TTL-HIF2 RNAi trigger

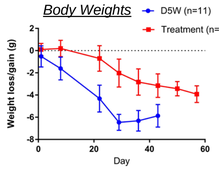


- Using an early generation of TTL-HIF2 RNAi which is less potent, PDX tumor bearing mice were administered 15 mg/kg (IV) TTL-HIF2 RNAi 3 days per week

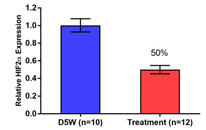
Kaplan-Meier Survival Analysis



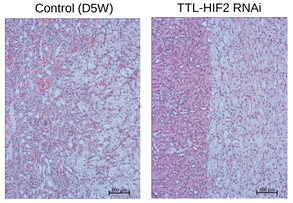
Body Weights



HIF2α mRNA Expression



Histology of Tumor/Kidney Interface



- TTL-HIF2 RNAi treated mice showed 100% survival on day 60, end of study; all mice from the control group died between days 26 and 50
- Overall improvement in survival correlates with 50% HIF2α mRNA reduction and less weight loss
- Less invasiveness at tumor/kidney interface with clear demarcation between tumor and kidney

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

- Developed immunohistochemistry method to identify TTR positive samples from primary and metastatic tumors in patients
- Percentage of TTR positive patients ranges from 50% - 68.5% in 3 out of 4 different microarrays
- Higher TTR expression frequency in metastatic tumors

Table. Tumor Targeting Receptor (TTR) Expression in Human ccRCC

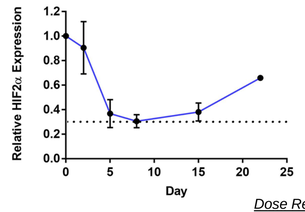
Company	Total ccRCC case #	% TTR positive					
		Overall	Tumor Grade				Metastatic
			1	2	3	4	
Vendor 1	77	31.2 (24/77)	29.5 (13/44)	29.6 (8/27)	50 (3/6)	n.a.	50 (1/2)
Vendor 2	20	50 (10/20)	n.a.	37.5 (3/8)	71.4 (5/7)	33.3 (1/3)	50 (2/4)
Vendor 3	34	64.7 (22/34)	n.a.				
Vendor 4	146	68.5 (100/146)	66.7 (30/45)	62.7 (32/51)	62.5 (10/16)	61.1 (11/18)	80 (12/15)

TTL-HIF2 RNAi is well tolerated in an exploratory toxicity study

- In naïve rats, latest generation of TTL-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

Duration and dose response of TTL-HIF2 RNAi in A498 ccRCC orthotopic mouse model

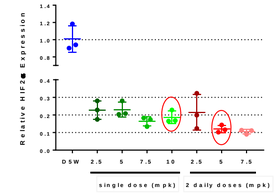
Duration of HIF2α mRNA KD after a single dose



- A498 tumor bearing mice were administered a single 5 mg/kg IV dose of TTL-HIF2 RNAi on study Day 1
- Nadir observed around Day 8, with robust knockdown between Days 5-15

Dose Response; Single vs Two Daily Doses

- A498 tumor bearing mice were given a single dose or two daily IV doses (24h apart) of TTL-HIF2 RNAi at various dose levels
- Dose response more shallow with single dose regimens
- Two daily doses of 5 mg/kg can achieve deeper KD than a single dose of 10 mg/kg
- Suggests receptor saturation at higher dose levels



CONCLUSIONS

Tumor targeted HIF2 RNAi molecules facilitate receptor-mediated uptake in ccRCC xenograft mouse models. In these models, HIF2α gene silencing resulted in tumor growth inhibition or improvement in overall survival. Importantly, exploratory toxicity studies in rats predict a wide safety margin. Collectively, this demonstrates 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.

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