Targeting Pin1 in cholangiocarcinoma tumor and stromal cells using siRNA therapeutics

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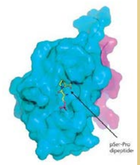
Introduction

Pin1 as a therapeutic target

- Pin1 (peptidyl-prolyl cis-trans isomerase NIMA-interacting 1) overexpression is seen in many tumor types and has been correlated with poor prognosis[1]
- Previous studies in pancreatic cancer have associated higher Pin1 expression with an immunosuppressive tumor microenvironment (TME)[2]
- Inhibition of Pin1 in animal models renders pancreatic cancer eradicable by chemoimmunotherapy[2-3]
- Like pancreatic cancer, cholangiocarcinoma (CCA) carries a poor prognosis and is characterized by desmoplastic stroma[4]
- We hypothesized that targeting Pin1 in CCA could be a well-tolerated approach with therapeutic benefit, in part by enhancing anti-tumor immune response to CCA



Pin1 structure[5]

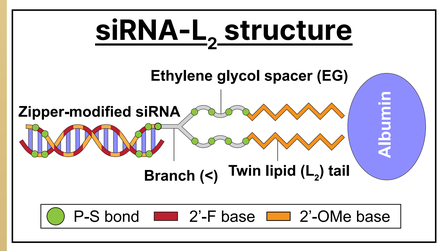
Rationale for targeting Pin1 using siRNA therapeutics

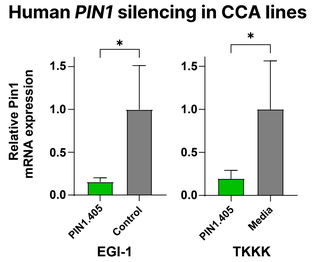
- Small interfering RNA (siRNA) can be designed to be specific for Pin1 and employ delivery methods that are tumor-tropic
- Other methods of targeting PIN1 exist, but have shortcomings:
  - All-trans retinoic acid (ATRA) +/- arsenic trioxide (ATO) are not molecularly specific for Pin1 and can cause various side effects such as cardiotoxicity or cytopenias (for ATO) and hypertriglyceridemia or teratogenicity (for ATRA)
  - A small molecule Pin1 inhibitor, sulfopin, is also available but does not have tumor tropism/selectivity

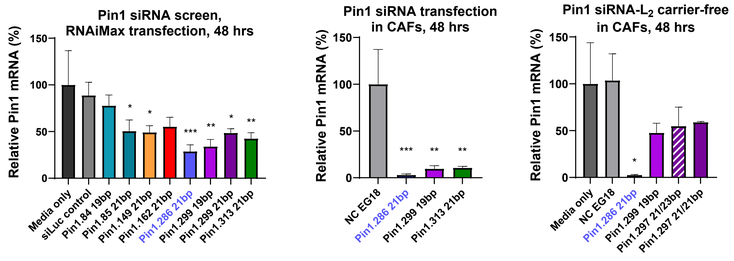
Pin1 siRNA Screening

Design of siRNA-L_2

- siRNA-L_2 conjugates have twin lipid tails that non-covalently bind albumin, improving circulation time and tumor-tropic delivery[6]
- Reversible albumin binding prevents scavenger-mediated recycling
- Entirely solid-phase synthesis



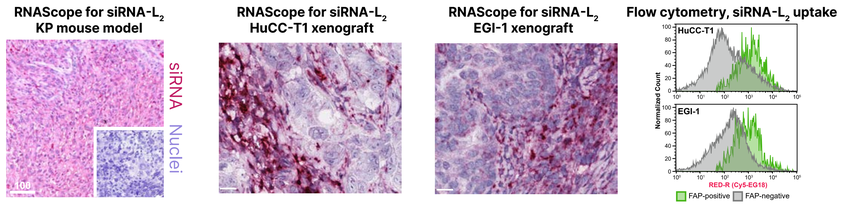




For knockdown of murine Pin1, the Pin1.286 21bp sequence results in the best knockdown, both as transfected siRNA and as carrier-free siRNA-L_2.

Biodistribution and Safety

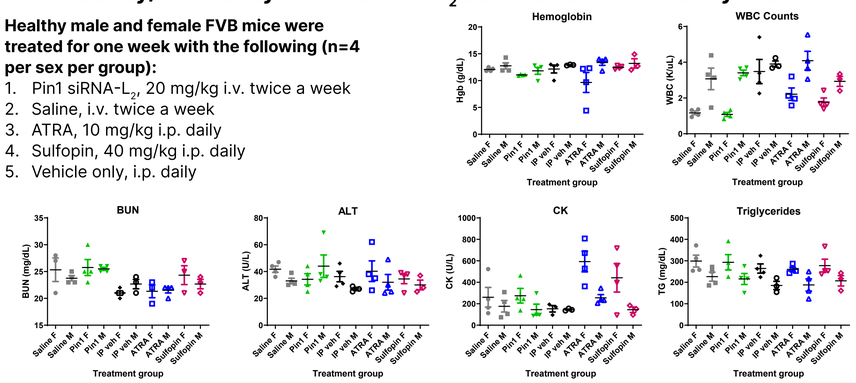
siRNA-L_2 accumulation in mouse and human tumors



Safety/tolerability of *Pin1* siRNA-L_2 administration in healthy mice

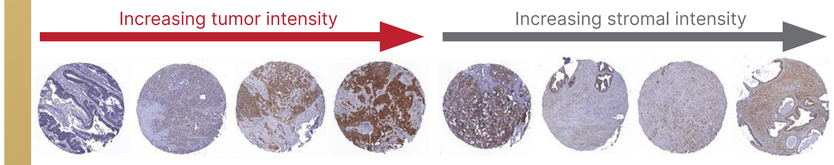
Healthy male and female FVB mice were treated for one week with the following (n=4 per sex per group):

1. Pin1 siRNA-L_2, 20 mg/kg i.v. twice a week
2. Saline, i.v. twice a week
3. ATRA, 10 mg/kg i.p. daily
4. Sulfopin, 40 mg/kg i.p. daily
5. Vehicle only, i.p. daily

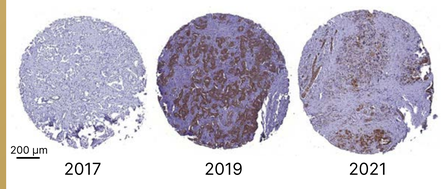


Pin1 in Human CCA Tumor Microarray

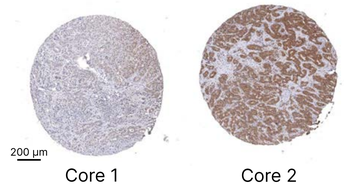
Examples of Staining Patterns



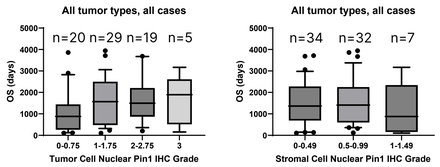
Temporal variation in Pin1 expr.



Spatial variation in Pin1 expr.



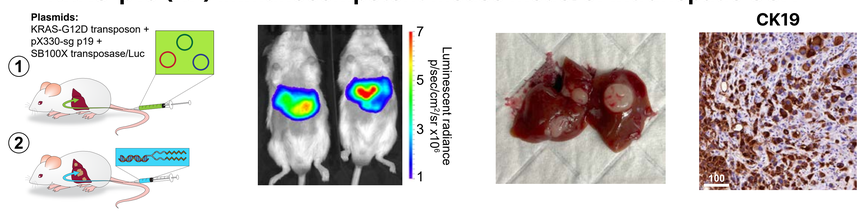
Pin1 intensity vs. overall survival



Analyses by sex, tumor stage/resectability, and tumor location (intrahepatic vs. extrahepatic) are in process, as well as different outcome measures including cancer recurrence and treatment response. Grading by cytoplasmic rather than nuclear expression is also underway.

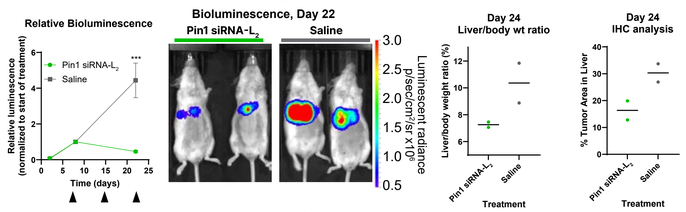
Pin1 siRNA-L_2 in Transgenic Models of CCA

KRAS-p19 (KP) immunocompetent mouse model of intrahepatic CCA[7]

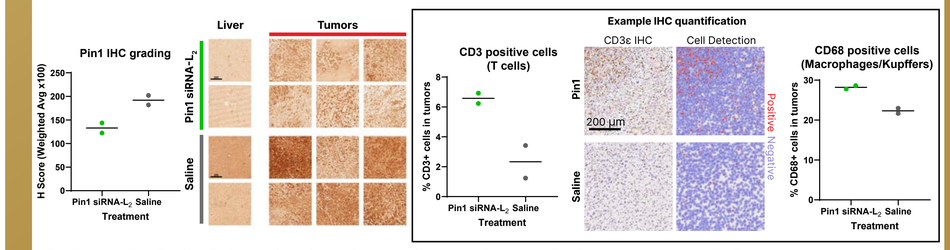


Pilot study of *Pin1* siRNA-L_2 monotherapy *in vivo*

Mice with KP tumors were matched based on baseline bioluminescence and treated with Pin1 siRNA-L_2 (10 mg/kg i.v. weekly) or saline (n=2 per group)



Tumor burden is reduced, as measured by *in vivo* imaging (IVIS), liver/body wt ratio, and quantification of tumor area on cross-sectional H&E



Preliminarily, Pin1 siRNA-L_2 treatment leads to reductions in Pin1 expression by IHC and increased percentage of T cells and, to a lesser degree, macrophages/Kupffer cells within the tumor

Conclusions & Future Directions

Conclusions:

1. We have identified potent siRNA sequences for knockdown of human and murine Pin1
2. siRNA-L_2 distributes into CCA tumors, including tumor and stromal cells
3. Pin1 siRNA-L_2 is well-tolerated at therapeutic dosing in healthy mice
4. Preliminarily, Pin1 siRNA-L_2 reduces tumor burden in a transgenic model of CCA, with trends towards reduced Pin1 expression and increased infiltration of T cells
5. Pin1 staining of annotated human CCA tumor microarray shows that a subset of CCA tumors overexpress Pin1, but further analysis is needed to better understand the prognostic implications of Pin1 overexpression

Ongoing and Future Work:

1. Study underway comparing control, gemcitabine/cisplatin/anti-PD-L1 chemoimmunotherapy,[8] Pin1 siRNA-L_2, and Pin1 siRNA-L_2+chemoimmunotherapy in male and female KP mice.
2. Single cell RNASeq to determine levels of mRNA knockdown in specific cell types
3. CD8 depletion studies to determine role of cytotoxic T cells in mediating effects of Pin1 silencing

References and Acknowledgments

References:

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