Development of a splicing modulator-based ADC payload class with immune stimulatory properties for cancer therapy

*Satyajit K. Mitra[1], Vasu Jammalamadaka[1], Jeffrey Kang[1], Teodora Losic[1], Greg Tuffy[1], Tony W. Liang[1,*3], Kim Tipton[1,*4], Adriana Lopez[2], Scott Savage[1,*5], William Monteith[1], William E. Haskins[1], Melissa S. Jurica[2], Sanjeev Satyal[1], Mary Do[1].*

[1]pH Pharma Inc., South San Francisco, CA and [2]University of California, Santa Cruz, CA; [*]Currently at [3]PTM Therapeutics, San Carlos, CA; [4]CRISPR Therapeutics, San Francisco, CA; [5]Neuron23 Inc., South San Francisco, CA

pH PHARMA

Abstract 1832

BACKGROUND

Thailanstatins are naturally occurring anti-proliferative compounds that target spliceosomes and modulate pre-mRNA splicing. Alterations in splicing machinery and mRNA splicing is common in cancer and represents a potential susceptibility that can be exploited by targeted delivery of a splicing modulator to tumors with antibody drug conjugates (ADCs).

- Lee SC, Abdel-Wahab O. *Nat Med*. 2016;22(9):976-986.
- Effenberger KA, Urabe VK, Jurica MS. *Wiley Interdiscip Rev RNA*. 2017;8(2):10.1002/wrna.1381.
- Nicolaou KC, Rhoades D, Kumar SM. J Am Chem Soc. 2018;140(26):8303-8320.

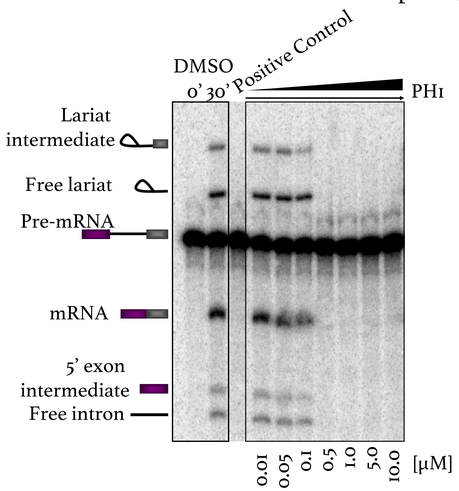
A. PH1 payload is a novel derivative of Thailanstatin optimized for metabolic stability and anti-tumor activity.

Cell line (source)	BT474 (Breast)	MCF7 (Breast)	HCT116 (Colon)	HT-29 (Colon)	NCI-N87 (Gastric)	SW480 (Colon)	A549 (Lung)	NCI-H23 (Lung)	SK-MES-1 (Lung)	SKOV3 (Ovary)	DU145 (Prostate)	A431 (Skin)
Thailanstatin Analog	IC50 (nM)											
PH1	0.5	4.6	1.1	3.4	1.0	1.3	8.8	0.5	12.4	35.5	5.3	1.1

0-1 nM | 1-50 nM | 50-100 nM

RESULTS (Payload PH1)

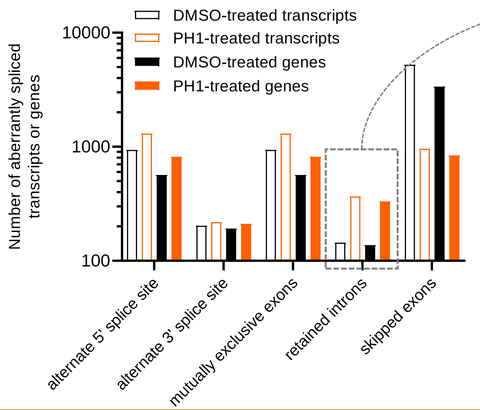
B. PH1 is an efficient modulator of splicing



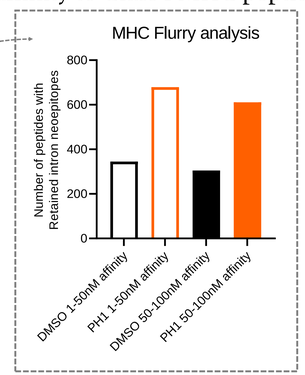
Positive Control is Spliceostatin A

In vitro splicing assay described at Effenberger et. Al. RNA 22: 350–359

C. Genome-wide transcriptome analysis of PH1-treated NCI-N87 cells revealed multiple classes of mis-splicing events including elevated expression of transcripts with skipped exons (3364 genes/ 4x change) and retained introns (332 genes/ 2.5x change).

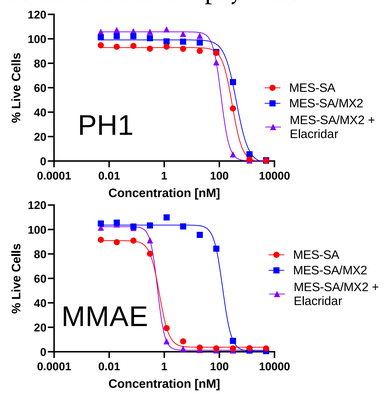


D. Translation of intron-retained transcripts predict increase in PH1-induced neoepitope peptides with high affinity to Class I MHC peptides.



Antigen presenting cells use MHC Class I molecules as co-receptors to prime cytotoxic T cells against immunogenic peptides

E. PH1 is a poor substrate for MDR multi-drug transporters responsible for resistance to current payloads

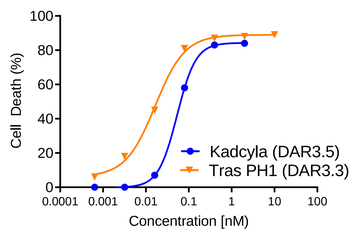


MES-SA - parental MDR^{low} cell line
MES-SA/MX2 - MDR^{high} cell line
Elacridar - MDR inhibitor
MMAE- Monomethyl Auristatin E, anti-mitotic microtubule inhibitor
MMAE is a good substrate of MDR1 transporter

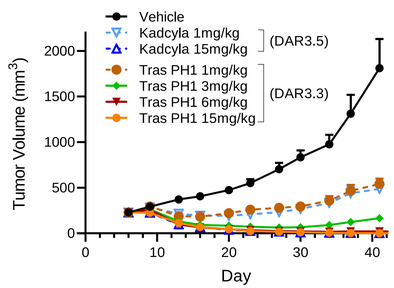
RESULTS (Antibody Drug Conjugate- Tras PH1)

F-I. Trastuzumab conjugated ADC termed Tras PH1 exhibited nanomolar potency specific to HER2-expressing NCI-N87 cells *in vitro*. Dose-proportional and durable anti-tumor efficacy was observed against NCI-N87 xenograft tumors and ADC pharmacokinetic exposure was favorable.

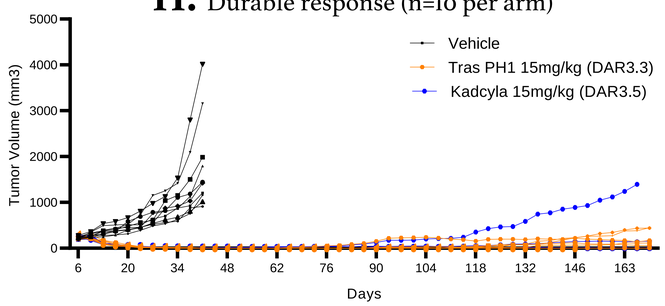
F. Cytotoxicity in vitro



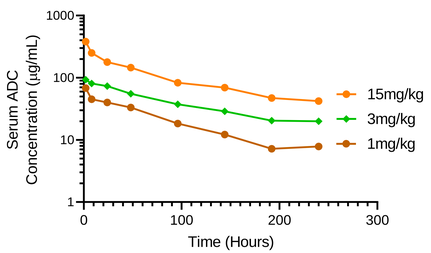
G. Dose response (n=10 per arm)



H. Durable response (n=10 per arm)

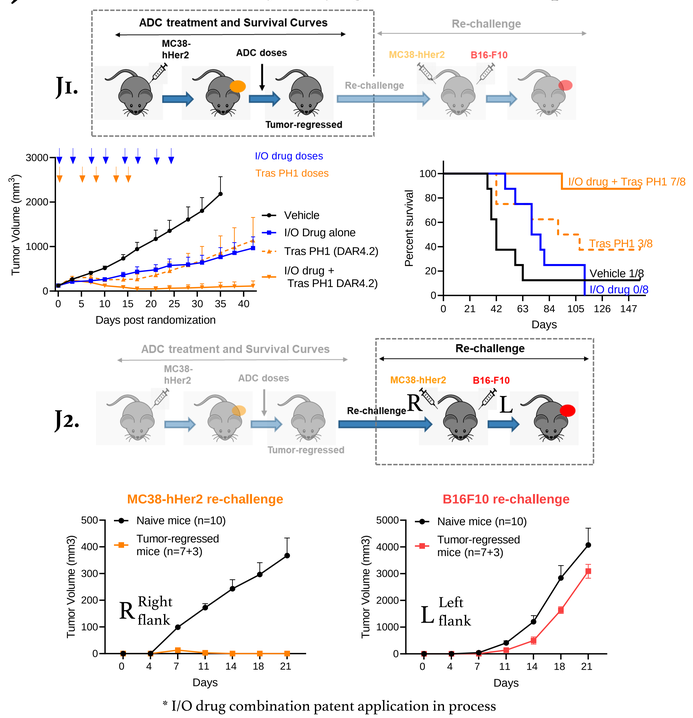


I. Pharmacokinetics (n=3 per dose)



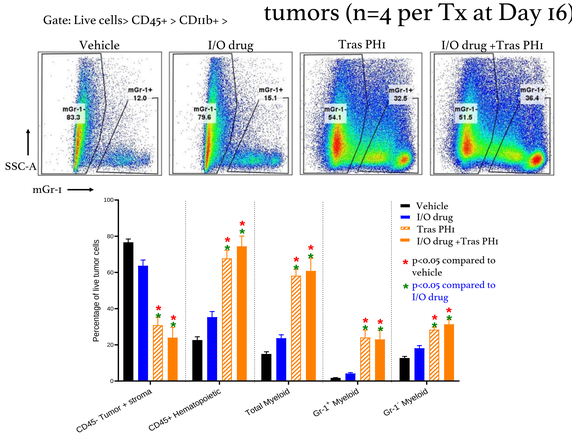
As measured by anti-PH1 antibody capture and anti-human Fc assay

J. Combination Efficacy in Syngeneic model (n=8 per arm)



* I/O drug combination patent application in process

K. Increased myeloid cell recruitment to Tras PH1 tumors (n=4 per Tx at Day 16)



CONCLUSIONS

We present the development of a splicing modulator-based payload class with the ability to target tumor mRNA splicing, induce tumor neoepitopes, recruit myeloid cells and generate anti-tumor immunity. These findings support the development of ADCs using this novel class of immunostimulatory payload.

Presented at Therapeutic antibodies, including Engineered antibodies session, AACR 2021