A Novel Splicing- Targeted ADC Drives Immune Activation, Synergy with Checkpoint Inhibitors, and Enhanced Therapeutic Potential beyond Cytotoxicity

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Abstract:

Background: Thailanstatins are naturally occurring anti-proliferative compounds that target spliceosomes and pre-mRNA splicing. PH1 is a proprietary Thailanstatin analog that has anti-tumor activity in preclinical xenograft and syngeneic models as Her2 and Trop2 ADCs [1,2]. Treatment of NCI-N87 gastric cancer cells with PH1 stimulated intron retention and neopeptides with high affinity to MHC Class I, as predicted by MHC flurry analysis. Trastuzumab PH1 ADCs induced complete regression (CR) of MC38-hHer2 colon carcinoma tumors and CR mice rejected a rechallenge with a fresh bolus of tumor cells suggesting immune memory [1]. The current work investigates immunomodulatory mechanisms of PH1 contributing to tumor regression and memory.

References:

1. https://doi.org/10.1158/1538-7445.AM2021-1832
2. https://doi.org/10.1158/1538-7445.AM2023-6297

A Payload Classes of Different FDA-Approved ADCs

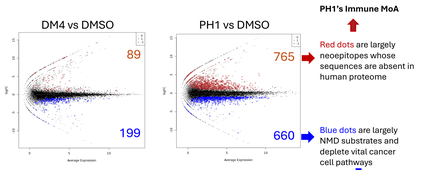
	Product	Payload	Mechanism of Action
Solid Tumors	TRODELVY	SN38	Topoisomerase I inhibitor
	ENHERTU	DXd	
	tivdak	MMAE	Microtubule Inhibitor
	Kadcyla	DM1	
	PADCEV	MMAE	
	ELAHERE	DM4	
Blood cancers	POLIVY	MMAE	
	ADCETRIS	MMAE	
	Zynlonta	Tesirine (PBD)	DNA Damaging Agent
	BESPONSA	Ozogamicin	
	MYLOTARG	Ozogamicin	

PH1 is an investigational payload targeting spliceosomes

B PH1 Payload Generates Retained Intron (RI) and Frameshift Peptide (FSP) Neoepitopes

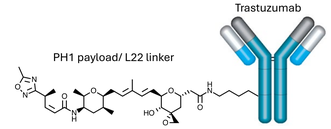
NCI-N87 gastric carcinoma cells were treated with DMSO, DM4, or PH1 methyl ester derivative as naked payloads at IC90 concentrations for 2 hours at 37° C. RNASeq was performed and transcriptome generated using HiSAT, StringTie and StringTie Merge as described in Pertea M et al (2016) *Nature Protocols* 11, 1650-1667. AS transcripts were annotated using knownAlt track of UCSC table browser that contains AS in nine categories.
PH1 Treatment induced aberrant transcripts rich in retained introns (RI), Overlapping (bleeding) exons, Exon Skipping (ES), Mutually Exclusive Exons (MXE) and alternate promoter usage. Transcripts in these categories have higher frequency of frameshifts and larger non-self regions due to retention of large introns relative to those discovered on cancer cells.

PH1-payload induced neoepitopes are different from cancer neoantigens



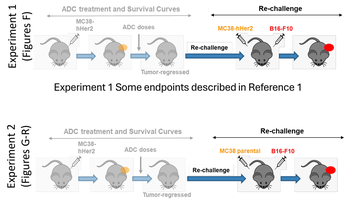
Where AS= Alternatively spliced transcripts, PH1= is the linker toxin L22 in Ref 2 and in conjugated form is represented by the schematic below., and NMD= Nonsense Mediated Decay

C Schematic Representation of ADC



Tras PH1 ADC
Drug-to-Antibody Ratio (DAR= 3.5 -4.0) ± 10%

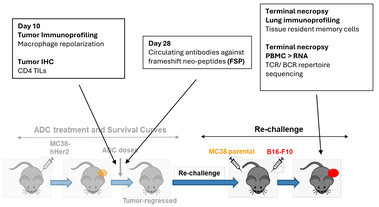
D Set of Two Experiments to Understand Immune Mechanism and Epitope Spreading



Experiment 1 Some endpoints described in Reference 1

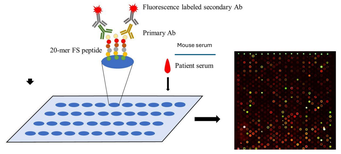
Experiment 2 Endpoints described here

E Endpoints in MC38-hHer2 Syngeneic Model Study

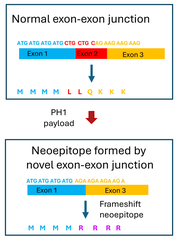


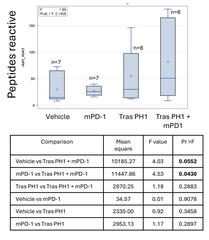
F Evidence For Circulating Antibodies Against Frameshift Neoepitopes by ADC Treatment in MC38-hHer2 Model

Day 28 serum samples from MC38-hHer2 study
Probed on proprietary peptide array



Peptides contain novel exon-exon junction sequence

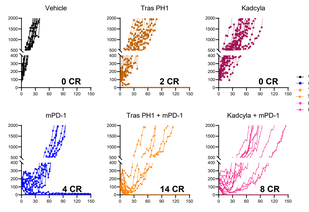




Comparison	Mean square	F value	Pr >F
Vehicle vs Tras PH1 + mPD-1	10185.27	4.03	**0.0552**
mPD-1 vs Tras PH1 + mPD-1	11447.86	4.53	**0.0430**
Tras PH1 vs Tras PH1 + mPD-1	2970.25	1.18	0.2883
Vehicle vs mPD-1	34.57	0.01	0.9078
Vehicle vs Tras PH1	2330.00	0.92	0.3458
mPD-1 vs Tras PH1	2953.13	1.17	0.2897

Statistical analyses were performed in SAS 9.4.

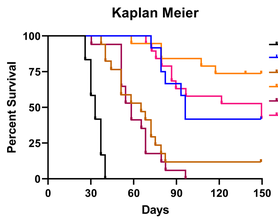
G Increased Rate of Complete Regressions (CRs) of Colon Tumors Treated with Combination of Tras PH1 ADC with Checkpoint Inhibitor



Comparison of CR rates across treatments using Chi Squared test

	Vehicle	mPD-1	Tras PH1	Tras PH1 +mPD-1
mPD-1	0.0285	-	n.s	0.0266
Tras PH1	n.s.	n.s	-	0.0002
Kadcyla	n.s.	0.0104	n.s.	<0.0001
Tras PH1 + mPD-1	<0.0001	0.0266	0.0002	-
Kadcyla + mPD-1	0.0001	n.s.	0.0425	0.0497

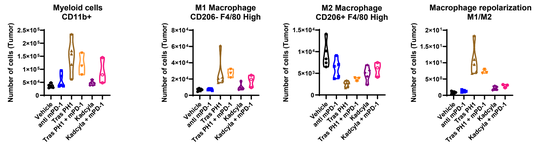
H Tras PH1 Combines with Anti-mPD-1 to Improve OS in MC38-hHer2 Model



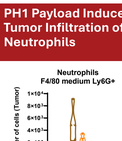
Arms	Median Survival	Fraction surviving (D149)
Vehicle	33 days	0.0%
mPD-1	96 days	41.7%
Tras PH1	65 days	11.8%
Tras PH1 + mPD-1	>149 days	73.7%
Kadcyla	58 days	0.0%
Kadcyla + mPD-1	149 days	42.1%

Log Rank (Mantel Cox) comparison of Survival Curves
- All treatment arms significantly improved OS relative to Vehicle at p<0.0001
- OS of Tras PH1 + Kadcyla as single agent (p0.4284)
- OS of mPD-1> Tras PH1 (p=0.0685)
- No OS benefit of Kadcyla+mPD1 vs mPD-1 (p=0.8958)
- OS of Tras PH1 +mPD-1 > Kadcyla +mpD-1 (p=0.0672)

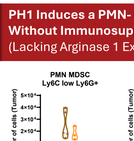
I PH1 Payload Induces Macrophage Polarization to Anti-Tumor M1 Type



J PH1 Payload Induces Tumor Infiltration of Neutrophils



K PH1 Induces a PMN- MDSC Population Without Immunosuppressive Properties (Lacking Arginase 1 Expression)

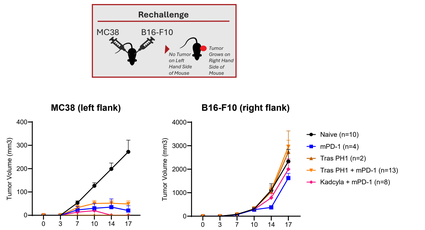


In above violin plots, the absolute number of a cell type per tumor replicate tested are represented. Since the total number of CD45+ hematopoietic cells in the tumors were higher in Tras PH1 and Tras PH1 +mPD-1 tumors, it was possible that the infiltration would be underrepresented if one normalized for total hematopoietic content. However, results are unchanged if presented as percentage of CD45+ ve cell type

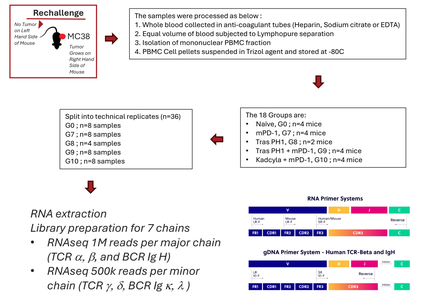
Only cell types showing statistically significant changes in Tras PH1 and Tras PH1 + mPD1 immune profiles are shown above.

Suppression of Arginase 1 in PMN-MDSC was associated with therapeutic effects in NSCLC lung cancer model. Miret et al (2019) Journal for Immunotherapy of Cancer 7:32.

L MC38 Parental Cell Rechallenge is Rejected Evidence of Epitope Spreading

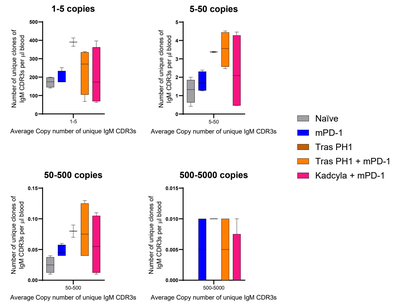


M Workflow for TCR/ BCR Sequencing Analysis



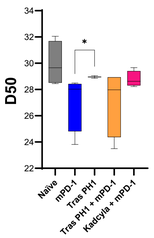
RNA extraction
Library preparation for 7 chains
- *RNAseq 1M reads per major chain (TCR α, β, and BCR Ig H)*
- *RNAseq 500k reads per minor chain (TCR γ, δ, BCR Ig κ, λ)*

N Tras PH1 Treatment Arms Have a Higher Diversity of Unique CDR3 IgM . This is Observed Even in the Presence of Selection and Expansions of IgM Clones

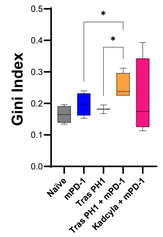


Similarly, increased IgG1 and IgG2 diversity associated with Tras PH1 (greater Shannon Entropy)

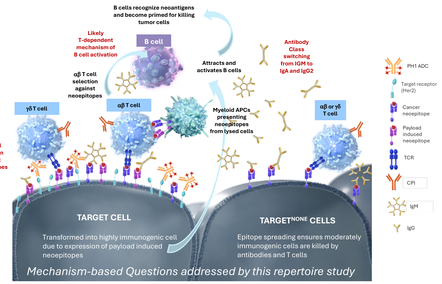
O mPD-1 Induces Clonal Expansions of TCR Beta Chains



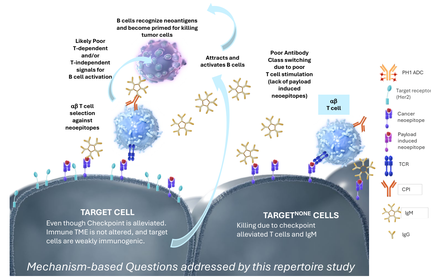
P Tras PH1+ mPD-1 Combination Stimulates TCR Delta Expansions



Q Mechanistic Model for Enhanced Efficacy of Tras PH1 + mPD1 Combination Resulting in Activation of B Cells, TCR αβ and TCR γδ Cells



R Mechanistic Model of mPD1 Alone Arm Activating TCR αβ and IgM Clonal Expansions in the Absence of Payload Induced Neoepitopes



Conclusions:

When combined, the MOAs of the two agents complemented each other where Tras PH1 increased neoantigens, innate myeloid MOAs, and stimulated IgM antibodies whereas mPD-1 activated αβ T cells and promoted antibody class switching. The synergy of Tras PH1+mPD-1 may be due to each agent potentially improving the other's MOA or may be due to expansion of MHC non-restricted γδ T cells which neither single agent was able to achieve on its own.