Conditionally activated IFNa induces an inflammatory tumor microenvironment in preclinical models and increases efficacy in combination with checkpoint blockade

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Abstract

Background:

Interferon alpha 2b (IFNa) is an approved immunotherapy for the treatment of multiple tumors; however, the toxicity of IFNa has limited its clinical use. The CytomX proprietary Probody® Therapeutics (Pb-Tx) platform technology attenuates activity of a molecule by blocking its active regions through affinity or steric interference. Such blockade, termed masking, is reversed upon proteolytic cleavage of a substrate-containing linker between the molecule and the mask by tumor-associated proteases. Using CytomX Pb-Tx technology, a conditionally active, mouse cross reactive IFNa (Pb-IFNa-A/D) with minimal activity in its prodrug form, was generated to enable the study of cancer immunobiology in the mouse. Pb-IFNa-A/D is activated by elevated protease activity associated with the tumor microenvironment (TME), leading to preferential IFNa activity in the TME but not in healthy tissues. Pb-IFNa-A/D has displayed robust activity in a range of tumors, including checkpoint non-responsive models.

Methods:

To investigate the pharmacodynamic activity and evaluate biomarkers related to response to Pb-IFNa-A/D, we screened 25 syngeneic murine tumor models with both monotherapy and PD-1 checkpoint blockade (PD-1) combination. In addition to monitoring efficacy outcomes, tumor tissue and peripheral blood were collected 48 hours post administration for pharmacodynamic response measurement.

Results:

Pb-IFNa-A/D monotherapy demonstrated anti-tumor activity in a range of syngeneic tumor models, including some refractory to PD-1 blockade. We found significantly higher tolerability of masked Pb-IFNa-A/D in comparison to unmasked IFNa. Unmasked IFNa significantly increased T-cell activation in normal tissues including lymph node and spleen, while masked Pb-IFNa-A/D did not. To evaluate the on-tumor changes in response to Pb-IFNa-A/D, we performed RNA-seq on tumor tissue. We observed an increase in interferon stimulated genes with Pb-IFNa-A/D and combination treatment. In agreement with peripheral observations, Pb-IFNa-A/D and combination treatment similarly increased Granzyme-B and CXCL10 expression demonstrating that Pb-IFNa-A/D inflames the tumor microenvironment. To analyze changes in lymphocyte activation we performed peripheral blood immunophenotyping. Pb-IFNa-A/D and combination treatment demonstrated a significant increase in peripheral blood lymphocyte activation as measured by CD69 and Granzyme B staining and were correlated with response.

Background

Overview of the Probody Therapeutic Platform

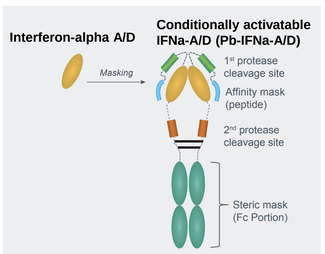

Figure 1. To minimize IFNa-A/D toxicity, the molecule was engineered with a dual masking strategy using a cleavable Fc domain at one end of IFNa-A/D, and a cleavable affinity peptide mask at the other end.

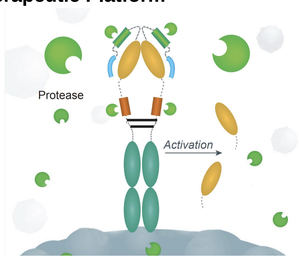

Figure 2. Tumor-associated proteases cleave the masks and Fc domain, releasing the fully active IFNa-A/D cytokine. Linkers are cleaved by multiple proteases for utility across tumor types.

Results

Efficacy of PD-1 Inhibition is Enhanced by Addition of Pb-IFNa-A/D

Model	Tumor type
A20; EL4	Lymphoma
CT26.WT; Colon26	Colorectal cancer
EMT6; 4T1; JC	Breast cancer
B16-BL6; B16-F10; Clone M-3	Melanoma
H22; Hepa1-6	Liver cancer
KLN205; LL/2(LLC1)	Lung cancer
Renca	Renal cancer
MPC-11; J558	Myeloma
MBT-2	Bladder cancer
Pan02	Pancreatic cancer
RM-1	Prostate cancer
WEHI-164	Fibrosarcoma
P815	Mastocytoma
Neuro-2a	Neuroblastoma
GL261	Glioma

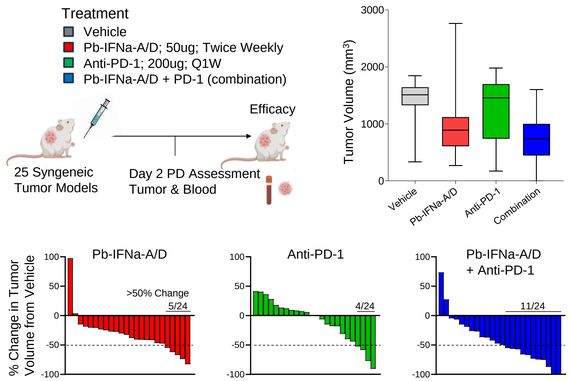

Figure 3. Left, list of models tested with tumor type. Center, study design. Mice treated with vehicle, Pb-IFNa-A/D, PD-1, or combination with PD assessment of blood and tumor on Day 2. Upper right, Tumor volume at the end of study. Bottom, the % change in tumor volume from vehicle treated controls for indicated treatments, calculated from end of study for vehicle animals.

Masking of IFNa Significantly Increases Murine Tolerability and Lowers Peripheral Activity

Tolerability of Masked and Unmasked IFNa-A/D

	5 ug	20 ug	600 ug	1200 ug	2400 ug
Pb-IFNa-A/D (Masked)			Tolerated	Tolerated	Not tolerated
IFNa-A/D (Unmasked)	Tolerated	Not tolerated			

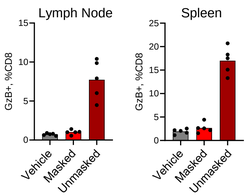

Figure 4. Tolerability and peripheral activity of masked and unmasked IFNa. Left, masked Pb-IFNa-A/D is significantly more tolerable than unmasked IFNa-A/D (1200 ug vs 5 ug). Right, spleen and lymph node were collected prior to toxicity in mice treated with 50ug of masked or unmasked IFNa-A/D. Unmasked IFNa-A/D has significant peripheral T-cell activation in lymph node and spleen in comparison to masked IFNa-A/D.

Pb-IFNa-A/D Inflames the Tumor Microenvironment

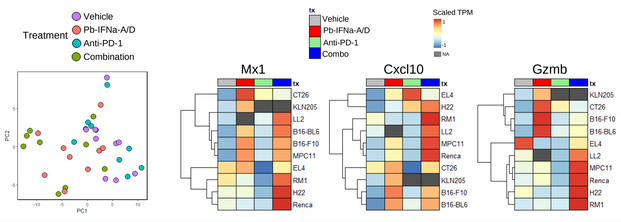

Figure 5. RNA-seq analysis of tumors collected from animals 48 hours post treatment. Left, principal component analysis of hallmark interferon-alpha gene set shows differential expression following Pb-IFNa-A/D treatment. Right, Scaled gene expression of interferon response genes (ISGs) Mx1 and Cxcl10, and T-cell activation gene Gzmb following indicated treatment for each model. Pb-IFNa-A/D monotherapy and combination increases tumor ISG and Gzmb expression, with distinct expression signatures.

Pb-IFNa-A/D treatment increases activated circulating CD8+ T-cells

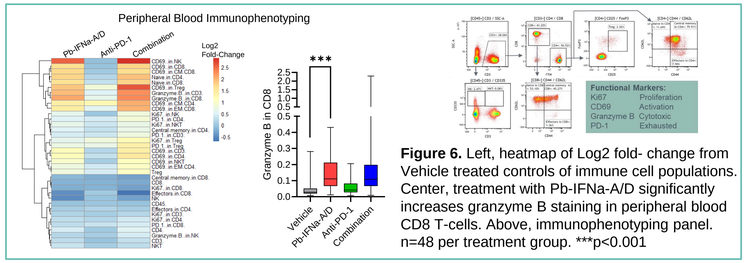

Figure 6. Left, heatmap of Log2 fold- change from Vehicle treated controls of immune cell populations. Center, treatment with Pb-IFNa-A/D significantly increases granzyme B staining in peripheral blood CD8 T-cells. Above, immunophenotyping panel. n=48 per treatment group. ***p<0.001

Peripheral Blood CD8 T-cell Activation Correlates with Response

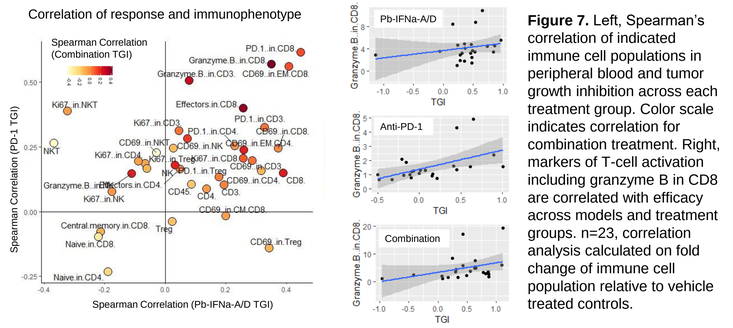

Figure 7. Left, Spearman's correlation of indicated immune cell populations in peripheral blood and tumor growth inhibition across each treatment group. Color scale indicates correlation for combination treatment. Right, markers of T-cell activation including granzyme B in CD8 are correlated with efficacy across models and treatment groups. n=23, correlation analysis calculated on fold change of immune cell population relative to vehicle treated controls.

Conclusions

- Pb-IFNa-A/D demonstrated robust anti-tumor activity in a range of syngeneic tumor models.
- Masked IFNa (Pb-IFNa-A/D) has significantly higher tolerability and lower peripheral activity than unmasked IFNa.
- Pharmacodynamic activity in the periphery and tumor demonstrates an IFN-stimulated immune response.
- Although IFN stimulation appeared to be the main driver of peripheral PD activity, combination with checkpoint blockade showed superior efficacy to checkpoint blockade alone in most models.
- These data support development of a conditionally active IFNa as a promising clinical candidate, including in combination with immune modulating agents including checkpoint blockade, for the treatment of malignancies.

References

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