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Probody-Interferon-alpha 2b Combines Antitumor Activity with Improved Tolerability

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BACKGROUND

BACKGROUND Type I interferons can exert direct antitumor effects, modulate tumor stroma, and induce de novo antitumor immune responses. They have demonstrated combination activity with PD-(L)1 blockade to potentially expand the benefit to patients with unresponsive tumors. Despite its potential, the toxicity of interferon alpha has limited its clinical use. Here we applied CytomX proprietary Probody® Therapeutics (Pb-Tx) technology to create a conditionally activated IFN-a2b (Pb-IFN-a2b) with minimal activity in its prodrug form. The prodrug is activated in the tumor microenvironment (TME), leading to preferential activity in the TME but not in healthy tissues. Pb-IFN-a2b demonstrated an enhanced tolerability profile compared to standard IFN therapy without compromising its antitumor effects.

METHODS The 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. Pb-IFN molecules were engineered with a dual masking approach combining the effects of steric inhibition by Fc fusion and affinity interference by a peptide mask.

RESULTS Pb-IFN-a2b demonstrated significant reduction (1000-fold or more) of its specific activity in vitro, including antiproliferative effects and immune cell activation. Treatment with tumor-associated proteases or exposure to viable tumor tissues fully restored its activity. Activated but not masked Pb-IFN-a2b induced a gene expression profile consistent with interferon signaling in primary human immune cells. In vitro studies with dissociated human tumors demonstrated the ability of Pb-IFN to activate the tumor immune infiltrate, which could be further enhanced by concomitant PD-L1 blockade.

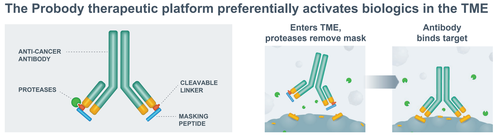
Antitumor activity of the Pb-IFN-a2b in xenograft studies is equal to or greater than Peg-IFN-α2b. Pb-IFN-α2b demonstrated significant antitumor activity in syngeneic mouse tumor models without evidence of toxicity. Consistent with in vitro observations, this antitumor activity was further enhanced by PD-(L)1 blockade.

Toxicology studies performed in hamsters demonstrated enhanced tolerability of the molecule compared to its unmasked control. Pb-IFN-a2b did not cause hematological changes, body weight loss, or mortality associated with unmasked interferon at significantly lower dose level.

In cynomolgus monkey, Pb-IFN-a2b demonstrated linear pharmacokinetics, extended half-life, and was well tolerated at doses up to 15 mg/kg.

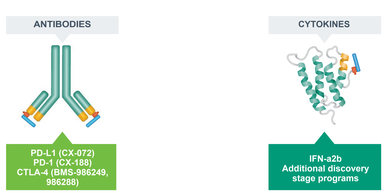
CONCLUSIONS Pb-IFN-a2b shows improved tolerability and antitumor activity in preclinical studies compared to traditional IFN treatment. These data support Probody cytokine therapeutics as a promising addition to current immunotherapy regimens, potentially expanding their benefits to patients with typically unresponsive tumors.

The Probody therapeutic platform preferentially activates biologics in the TME



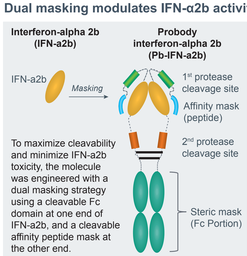
- "Masked" to limit activity in normal tissue
- "Un-masked" by tumor-associated proteases
- Linkers cleaved by multiple proteases for utility across tumor types

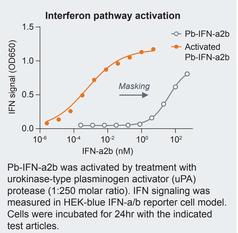
- Improve therapeutic window for validated targets
- Create therapeutic window for undruggable targets
- Applicable to multiple binding modalities



RESULTS

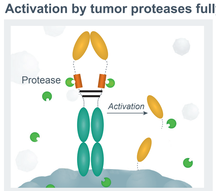
Dual masking modulates IFN-α2b activity in vitro



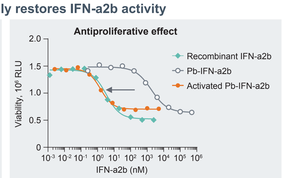


Pb-IFN-a2b was activated by treatment with urokinase-type plasminogen activator (uPA) protease (1:250 molar ratio). IFN signaling was measured in HEK-blue IFN-a/b reporter cell model. Cells were incubated for 24hr with the indicated test articles.

Activation by tumor proteases fully restores IFN-a2b activity

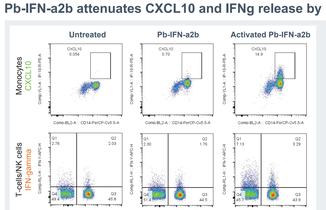


In the TME, tumor-associated proteases activate the molecule by liberating the fully active IFN-a2b cytokine.



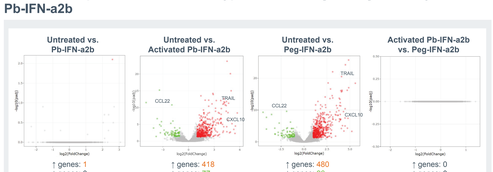
Human lymphoma Daudi cells were treated with single masked Pb-IFN-a2b that was either intact or activated by uPA protease, or commercial recombinant IFN-a2b for 4 days. Viability of the cells was measured using Cell Titer-Glo.

Pb-IFN-a2b attenuates CXCL10 and IFNg release by PBMC



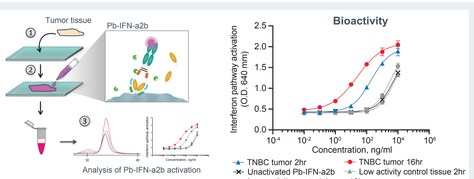
Healthy donor PBMC were treated with Pb-IFN-a2b or Pb-IFN-a2b activated by in vitro protease treatment for 5hr in the presence of Brefeldin A. Cells were stained for CD3/CD19/CD14, fixed/permeabilized, and stained for intracellular expression of CXCL10 and IFN-gamma. Cells were gated on viable monocytes treated with 1 ng/mL of IFN-a2b molecules (top row) or viable CD19-negative lymphocytes treated with 10 ng/mL.

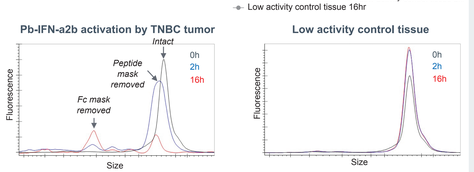
Activation-dependent induction of type I interferon gene signature by Pb-IFN-a2b



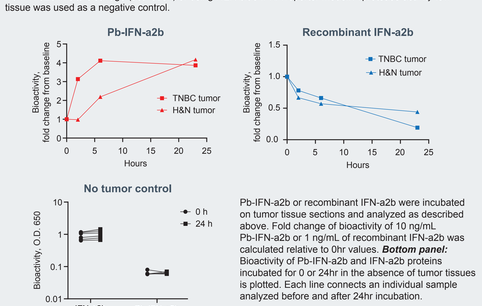
PBMC from healthy donors (N=4) were treated in vitro with 10 ng/mL of Pb-IFN-a2b, uPA-activated Pb-IFN-a2b, or pegylated IFN-a2b (Merck, USA) for 24hr. mRNA from treated cells was used for HT RNAseq. Genes with an adjusted *P*<0.05 and absolute log2 fold change >1 were called as differentially expressed.

Pb-IFN-a2b is activated by tumor tissues



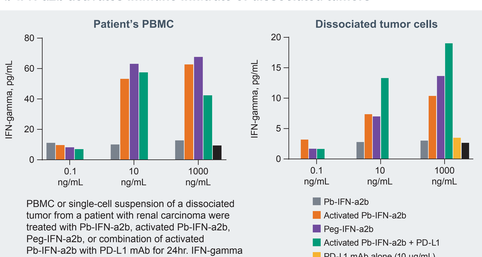


Fluorescently labeled Pb-IFN-a2b was incubated on tumor tissue sections at 37°C (Howng et al. 2021). Recovered solution was then analyzed through capillary electrophoresis, enabling quantification of Pb-IFN-a2b in situ cleavage (activation) or using HEK-blue IFNA reporter model. A protease activity-low tissue was used as a negative control.



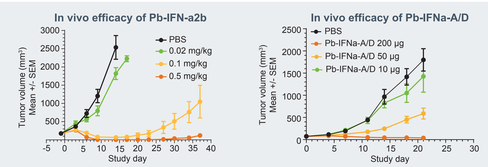
Pb-IFN-a2b or recombinant IFN-a2b were incubated on tumor tissue sections and analyzed as described above. Fold change of bioactivity of 10 ng/mL Pb-IFN-a2b or 1 ng/mL of recombinant IFN-a2b was calculated relative to 0hr values. ***Bottom panel:*** Bioactivity of Pb-IFN-a2b and IFN-a2b proteins incubated for 0 or 24hr in the absence of tumor tissues is plotted. Each line connects an individual sample analyzed before and after 24hr incubation.

Pb-IFN-a2b activates immune infiltrate of dissociated tumors



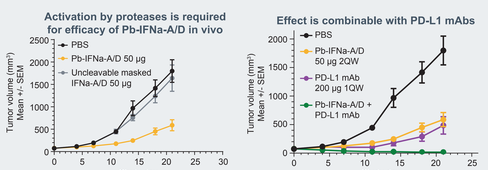
PBMC or single-cell suspension of a dissociated tumor from a patient with renal carcinoma were treated with Pb-IFN-a2b, activated Pb-IFN-a2b, Peg-IFN-a2b, or combination of activated Pb-IFN-a2b with PD-L1 mAb for 24hr. IFN-gamma release was measured by MSD multiplex assay.

Pb-IFNa suppresses tumor growth



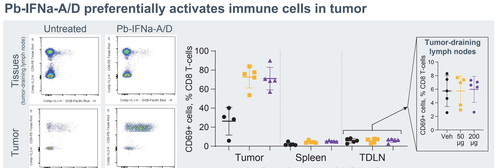
Beige/SCID mice (n=8 per group) were implanted subcutaneously with 10×10^6 human lymphoma (Daudi) cells and treated when the average tumor volume reached ~200 mm³. Indicated doses of Pb-IFN-a2b were administered i.p. once weekly for 5 weeks.

IFNa-A/D is a hybrid IFNa cytokine with human/mouse cross-reactivity (Rehberg et al. 1982). Pb-IFNa-A/D shows similar masking efficiency to human IFN-a2b. In vivo studies with Pb-IFNa-A/D were performed in the MC38 model as described below.



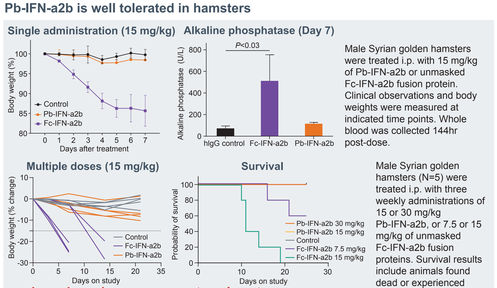
Mice (n=5 per group) were implanted subcutaneously with 1.5×10^6 MC38 cells and treated when the average tumor volume reached ~80 mm³. Indicated doses of Pb-IFN-a2b were administered s.c. twice weekly for 3 weeks. Control uncleavable molecule was constructed by replacing protease cleavage sites with uncleavable linker sequence.

Pb-IFNa-A/D preferentially activates immune cells in tumor



Mice (N=5 per group) were implanted subcutaneously with 1.5×10^6 MC38 cells in serum-free medium and treated with Pb-IFNa-A/D at indicated dose levels when the average tumor volume reached 80 mm³. 6 days after the treatment tumors and tissues were harvested and analyzed by flow cytometry. Gated on viable CD45+CD3+ cells.

Pb-IFN-a2b is well tolerated in hamsters



Male Syrian golden hamsters were treated i.p. with 15 mg/kg of Pb-IFN-a2b or unmasked Fc-IFN-a2b fusion protein. Clinical observations and body weights were measured at indicated time points. Whole blood was collected 144hr post-dose.

Male Syrian golden hamsters (N=5) were treated i.p. with three weekly administrations of 15 or 30 mg/kg Pb-IFN-a2b, or 7.5 or 15 mg/kg of unmasked Fc-IFN-a2b fusion proteins. Survival results include animals found dead or experienced body weight loss >15%.

Pb-IFN-a2b is well tolerated in cynomolgus monkey

Objective

- Characterize toxicity, toxicokinetics, and biomarker changes of Pb-IFN-a2b after single subcutaneous administration to cynomolgus monkeys

Study design

- Single subcutaneous dose on Day 1 with a 30-day observation period (Hematology, Clinical Chemistry, Flow, Cytokines, RNAseq, TK)
- Doses; 0.03, 0.3, 3.0 and 15 mg/kg
- N=2 per dose group

Results

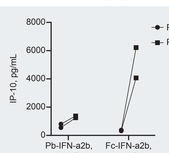
	0.03 mg/kg	0.3 mg/kg	3 mg/kg	15 mg/kg
Adverse effects	–	–	–	–
AST elevation	–	–	–	+/–
WBC reduction	–	–	–	+/–
Ki67 increase in NK, T-cells	–	–	+	+
IP-10 elevation	–	–	+	++

NOAEL for Pep-IFN-a2b in a historic benchmark study was ~2.5 mg/kg (in females).
A single s.c. dose of Peg-IFN-a2b at ~9.8 mg/kg (117721 mg/m²) resulted in mortality.

Observations

- Pb-IFN-a2b is well tolerated at doses up to 15 mg/kg
- Pb-IFN-a2b demonstrated linear pharmacokinetics and extended half-life

Pb-IFN-a2b attenuates IP-10 release in cynomolgus monkeys



Cynomolgus monkey (N=2 per group) were treated with 1 mg/kg subcutaneous administration of Pb-IFN-a2b or its unmasked control Fc-IFN-a2b. Concentrations of IP-10 in serum were measured by MSD V-plex assay.

CONCLUSIONS

1. Pb-IFN-a2b has significantly reduced (1000-fold or more) specific activity that is fully restored through activation by tumor-associated proteases
2. Pb-IFNa-A/D suppresses growth of syngeneic murine tumors in vivo. The effect is associated with immune activation in TME, but not TDLN
3. Pb-IFN-a2b is well tolerated in hamsters compared with unmasked cytokine, including tolerability of multiple administrations of 30 mg/kg
4. In cynomolgus monkey, Pb-IFN-a2b demonstrated linear pharmacokinetics and an extended half-life, and was well tolerated at doses up to 15 mg/kg with an attenuated IFN response compared to activated IFNa

The CytomX platform is being extended to additional cytokine families.

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