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Conditional Cytokine Therapeutics for Tumor-Targeted Biological Activity: Preclinical Characterization of a Dual-Masked IFN-a2b

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BACKGROUND

Background Cytokines have been shown to elicit broad anti-tumor activity in preclinical models. These results have translated into the approval for clinical use of IFN-a and IL-2. Therapeutic effects of IFN-a have been confirmed for many human cancers; however, the clinical success of cytokines has been limited by systemic toxicity and poor exposure.

CytomX Therapeutics has developed a new class of antibodies called Probody® therapeutics (Pb-Tx), designed to widen the therapeutic window by minimizing binding to targets in healthy tissue while being preferentially activated in the tumor microenvironment (TME) by tumor-associated proteases. CytomX has applied the Pb-Tx platform across multiple modalities including traditional antibodies, antibody-drug conjugates, and T-cell engaging bispecifics, and has advanced multiple programs into clinical studies. Here we have expanded the Pb-Tx platform to a conditionally activated cytokine, IFN-a2b, that has the potential to improve the therapeutic index of IFN-a therapy and allow systemic delivery.

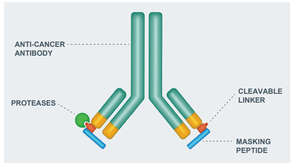
Methods We engineered an IFN-a2b with a dual masking strategy using a cleavable Fc domain at one end of IFN-a2b, and a cleavable affinity peptide mask at the other end. The construct was optimized to both maximize cleavability and minimize IFN-a2b toxicity.

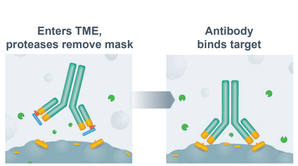
Results The optimized IFN-a2b conditionally activated (Pb-IFN-a2b) cytokine strongly reduced IFN-a2b activity in vitro (5,000X) in its dual-masked form. Its activity was fully restored upon protease activation. Transcriptional profiling of in vitro treated PBMC confirmed reduction of interferon-mediated activities of the masked molecule. In vitro studies with dissociated tumors indicated its ability to activate tumor immune infiltrate, that could be further enhanced by concomitant PD-L1 blockade. In mouse xenograft studies, conditionally activated IFN-a2b cytokines induced complete regression at doses as low as 0.1 mg/kg (activity comparable to peginterferon). Surrogate conditionally activated IFN-a2b molecules were also highly potent in syngeneic mice in vivo efficacy studies. Finally, we established an in vivo safety model in hamster, which has been shown to be sensitive to IFN-a mediated toxicity in the liver and bone marrow. In hamster, we showed that conditionally activated IFN-a2b cytokines are well tolerated up to 15 mg/kg and have reduced systemic IFN-a2b mediated toxicity as compared to the unmasked cytokine.

Conclusions Taken together, these preclinical data further support the continued development of conditionally activated IFN-a2b with the potential to improve the therapeutic index of IFN-a therapy and to enable single agent and combination treatment in multiple clinical settings.

Ethics Approval All animal experiments were reviewed and approved by CytomX IACUC.

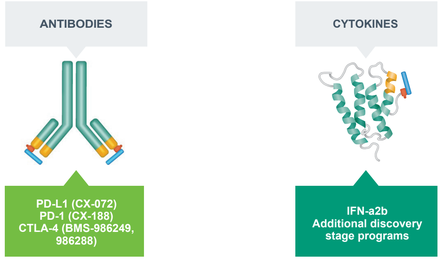
The Probody therapeutic platform preferentially activates biologics in the TME





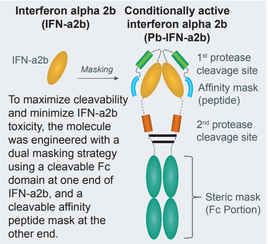
- "Masked" to limit activity in normal tissue
- "Un-masked" by tumor-associated proteases
- Linkers cleaved by multiple proteases for utility across tumor types

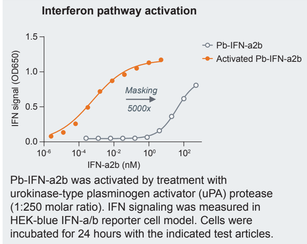
- Improve therapeutic window for validated targets
- Create therapeutic window for undruggable targets
- Applicable to multiple binding modalities



RESULTS

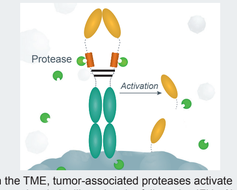
Dual masking modulates IFN-α2b activity in vitro



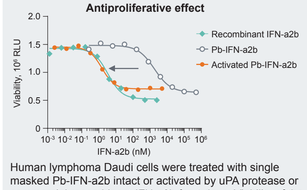


Pb-IFN-a2b was activated by treatment with urokinase-type plasminogen activator (uPA) protease (1:250 molar ratio). IFN signaling was measured in HEK-blue IFN-a/b reporter cell model. Cells were incubated for 24 hours with the indicated test articles.

Activation by tumor proteases fully restores IFN-a2b activity

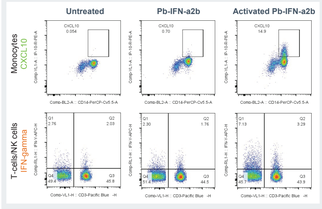


In the TME, tumor-associated proteases activate the molecule by liberating the fully active IFN-a2b cytokine.



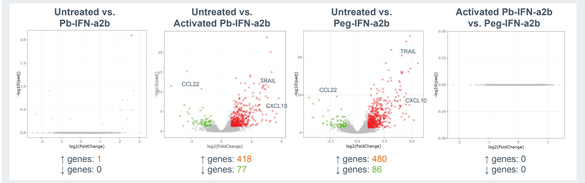
Human lymphoma Daudi cells were treated with single masked Pb-IFN-a2b intact or activated by uPA protease or commercial recombinant IFN-a2b for 4 days. Viability of the cells was measured using Cell Titer-Glo.

Pb-IFN-a2b induces CXCL10 and IFN-gamma release by PBMC in an activation-dependent manner



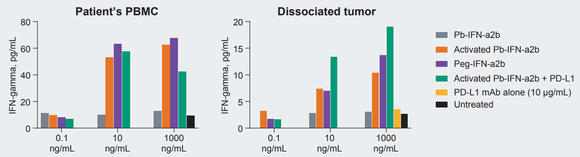
Healthy donor PBMC were treated with Pb-IFN-a2b or Pb-IFN-a2b activated by in vitro protease treatment for 5 hours in the presence of Brefeldin A. Cells were stained for CD3/CD19/CD14, fixed/permeabilized, and stained for intracellular expression of CXCL10 and IFN-gamma. Gated on viable monocytes treated 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



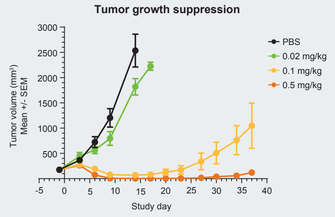
PBMC from healthy donors (N=4) were treated in vitro with 10 ng/mL of Pb-IFN-a2b, uPA-activated Pb-IFN-a2b, or pegylated IFN-a2b (Merk, USA) for 24 hours. mRNA from treated cells was used for HT RNAseq. Genes with an adjusted $P<0.05$ and absolute log2 fold change >1 were called as differentially expressed.

Pb-IFN-a2b activates immune infiltrate of dissociated tumors



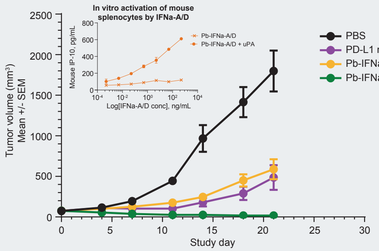
PBMC or dissociated tumor cells from a patient with renal carcinoma were treated with Pb-IFN-a2b, activated Pb-IFN-a2b, Peg-IFN-a2b, or combination of activated Pb-IFN-a2b with PD-L1 mAb for 24 hours. IFN-gamma release was measured by MSD multiplex assay.

Pb-IFN-a2b is active in vivo

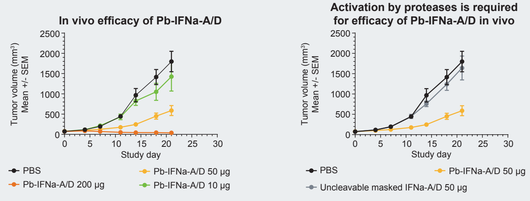


Beige/SCID mice were implanted subcutaneously with 10×10^6 Daudi cells in serum-free medium (1:1 Matrigel) and tumor measurements were recorded twice weekly for the duration of the study. When the average tumor volume reached ~200 mm³, mice (n=8 per group) were treated with indicated doses of Pb-IFN-a2b once weekly for 5 weeks.

Pb-IFNa-A/D suppresses syngeneic tumor growth

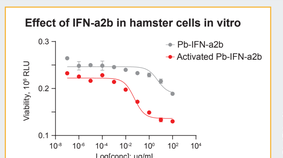


IFNa-A/D is a hybrid IFNa cytokine with human/mouse cross-reactivity (Rehberg et al. 1982). Pb-IFNa-A/D shows similar masking efficiency to human IFN-a2b. Control uncleavable molecule was constructed by replacing protease cleavage sites with uncleavable linker sequence: mouse splenocytes were treated for 24 hours. IP-10 level in supernatant was measured by ELISA.

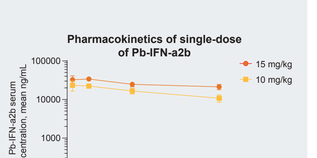


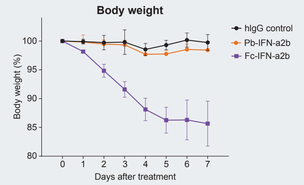
Mice (n=5 per group) were implanted subcutaneously with 1.5×10^6 MC38 cells in serum-free medium. Body weights and tumor measurements were recorded twice weekly for the duration of the study and treated when the average tumor volume reached 80 mm³.

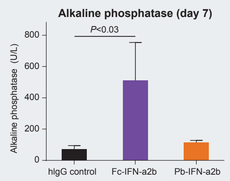
Conditionally active IFN-a2b is well tolerated in hamsters



Hamster melanoma RPMI1846 cells were treated with Pb-IFN-a2b or activated Pb-IFN-a2b for 96 hours.







Male Syrian golden hamsters were treated with i.p. Pb-IFN-a2b or unmasked Fc-IFN-a2b fusion protein. Clinical observations, body weights, and temperature were measured prior to dosing, and at 6, 24, 48, 72, 96, 120, and 144 hours post-dose (prior to blood sampling at each time point) for each animal. Whole blood was collected at 6, 24, 72, and 144 hours post-dose for each animal.

CONCLUSIONS

- Dual masked, conditionally active IFN-a2b demonstrated ~5000-fold reduced IFN signaling measured by reporter assay. Activation by tumor-associated proteases restores full function of the molecule.
- Pb-IFN-a2b directly activates primary immune cells, including immune infiltrate in dissociated human tumors.
- Pb-IFNa-A/D suppresses growth of syngeneic murine tumors in vivo. Co-administration of Pb-IFNa-A/D with PD-L1 mAb enhances the effect.
- Conditionally active IFN-a2b cytokine is well tolerated up to 15 mg/kg in hamsters compared with the unmasked cytokine.
- Pb-IFN-a2b cytokine is generally well tolerated in cynomolgus monkeys. Further preclinical evaluation of the in vivo pharmacology and toxicology for the conditional IFN-a program is underway.
- The CytomX platform is being extended to additional cytokine families.

REFERENCES

Rehberg, et al. Specific molecular activities of recombinant and hybrid leukocyte interferons. *J Biol Chem.* 1982;257:11497-502.

Altrock, et al. Antiviral and Antitumor Effects of a Human Interferon Analog, IFN-αCon1, Assessed in Hamsters. *J. of Interferon Research.* 1986;405-415.