1078

CX-801, Conditionally Activatable Interferon-alpha 2b Improves Tolerability and Exhibits Preferential Activity in Tumors

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BACKGROUND

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Interferon alpha 2b (IFN) is approved for treatment of multiple tumors, including sarcomas, melanomas, lymphomas and leukemias; however, the toxicity of IFN has limited its clinical use. Using CytomX proprietary Probody® Therapeutics (Pb-Tx) technology, a conditionally activatable IFN-a2b (CX-801) with minimal activity in its prodrug form, was created. CX-801 is conditionally activated in the tumor microenvironment (TME), leading to preferential IFN activity in the TME but not in healthy tissues. CX-801 demonstrated a substantially enhanced tolerability profile compared to standard IFN therapy without compromising its antitumor effects.

METHODS
The Pb-Tx platform technology is designed to attenuate the activity of a biologically active molecule by blocking its active regions with an affinity or steric mask that is recombinantly linked to the molecule. Tumor associated proteases cleave the linker, releasing the mask and restoring biological activity preferentially within the TME.

RESULTS
CX-801 demonstrated considerable reduction (1000-fold or more) of IFN signaling in vitro and significantly reduced immune cell activation. Exposure to viable tumor tissues or tumor-associated proteases in vitro fully restored its bioactivity, including the ability to stimulate tumor-infiltrating immune cells.

Antitumor activity of the CX-801 in xenograft studies is equal to or greater than pegylated-IFN-α2b. In syngeneic mouse tumor models, CX-801 demonstrated significant antitumor activity that was further enhanced by PD-(L)1 blockade. Activation of lymphocytes was observed in tumors but not in secondary lymphoid organs.

Toxicology studies performed in hamsters demonstrated enhanced tolerability of CX-801 compared to the unmasked INF-a2b control. In addition, CX-801 suppressed growth of hamster melanoma tumor model RPMI1846 at dose levels that were above the tolerated dose of the unmasked INF-a2b control.

Biomarkers of IFN signaling were greatly attenuated in non-human primates compared to the unmasked control. In cynomolgus monkey, CX-801 demonstrated linear pharmacokinetics, extended half-life, and was well tolerated at weekly doses up to 60 mg/kg.

CONCLUSIONS
CX-801 shows improved tolerability and antitumor activity in preclinical studies compared to traditional IFN treatment. These data support CX-801 as a promising clinical candidate as both a single agent and in combination with 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

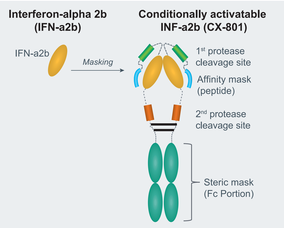


Figure 1. To minimize IFN-a2b toxicity, the molecule was engineered with a dual masking strategy using a cleavable Fc domain at one end of IFN-a2b, and a cleavable affinity peptide mask at the other end.

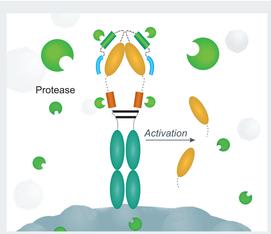


Figure 2. Tumor-associated proteases cleave the masks and Fc domain, releasing the fully active IFN-a2b cytokine. Linkers are cleaved by multiple proteases for utility across tumor types.

Conditional activation creates and enhances therapeutic window

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 Interferon Res.* 1986;405-415.
Howng, B, Winter, MB, LePage, C, et al. Novel ex vivo zymography approach for assessment of protease activity in tissues with activatable antibodies. *Pharmaceutics.* 2021;13:1390.

RESULTS

CX-801, conditionally activatable interferon-alpha 2b

Masking attenuates IFN-a2b activity in vitro

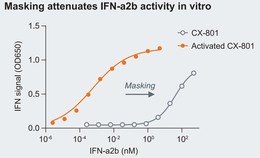


Figure 3. Urokinase-type plasminogen activator (uPA) protease (1:250 molar ratio) activated CX-801. IFN signaling was measured in HEK-blue IFN-a/b reporter cell model. Cells were incubated for 24hr with the indicated test articles.

Activation by tumor proteases fully restores IFN-a2b activity

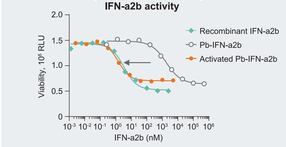


Figure 4. Human lymphoma Daudi cells were treated with single masked CX-801 that was either intact or activated by uPA protease, or commercial recombinant IFN-a2b for 4 days. Viability of the cells was measured using Cell Titer-Glo.

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

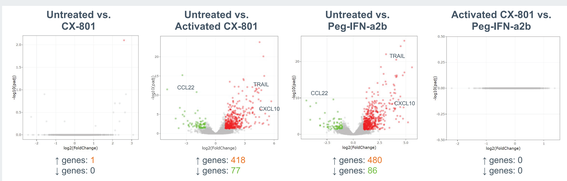


Figure 5. PBMC from healthy donors (n=4) were treated in vitro with 10 ng/mL of CX-801, uPA-activated CX-801, or pegylated-IFN-a2b for 24hr. mRNA from treated cells was used for HT RNAseq. Genes with an adjusted *P*<0.05 and absolute log2 fold change >1 were called as differentially expressed.

CX-801 is activated by tumor tissues

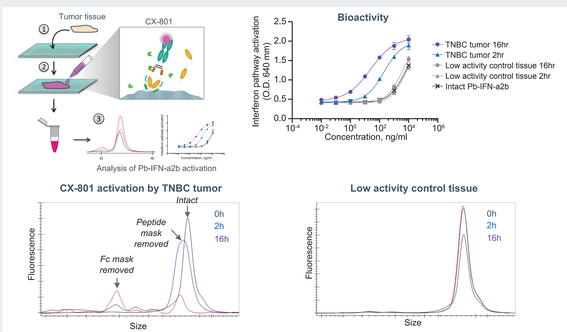


Figure 6. Fluorescently labeled CX-801 was incubated on tumor tissue sections at 37°C (Howng et al. 2021). Recovered solution was analyzed with capillary electrophoresis, enabling quantification of CX-801 in situ cleavage (activation) or using HEK-blue IFNA reporter assay. A protease activity-low tissue was used as a negative control.

Antitumor efficacy of CX-801 surrogate could be enhanced by PD-L1 blockade

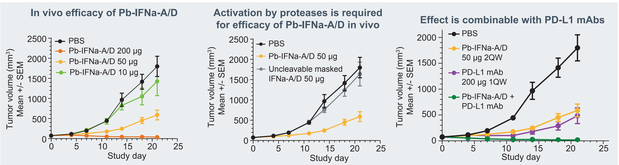


Figure 7. IFNa-A/D is a hybrid IFNa cytokine with human/mouse cross-reactivity (Rehberg et al. 1982). Pb-IFNa-A/D shows similar masking efficiency to human IFN-a2b. In vivo studies with Pb-IFNA-A/D were performed in the MC38 model as described to the right.

Figure 8. Mice (n=5 per group) were implanted subcutaneously with 1.5×10⁶ MC38 cells and treated when the average tumor volume reached ~80 mm³. Indicated doses of Pb-IFNa-A/D were administered twice weekly for 3 weeks. Control uncleavable molecule was constructed by replacing protease cleavage sites with uncleavable linker sequence.

CX-801 suppresses tumor growth

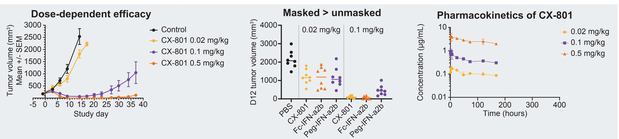


Figure 9. Beige/SCID mice (n=8 per group) were implanted subcutaneously with 10×10⁶ human lymphoma (Daudi) cells and treated when the average tumor volume reached ~200 mm³. Indicated doses were administered i.p. once weekly.

Pb-IFNa-A/D preferentially activates immune cells in tumor, not draining lymph nodes

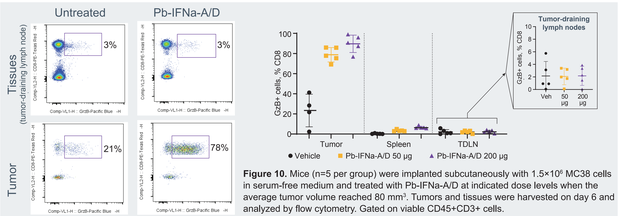
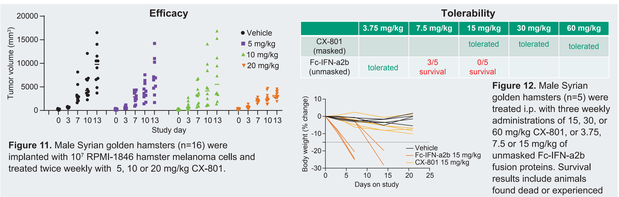


Figure 10. Mice (n=5 per group) were implanted subcutaneously with 1.5×10⁶ MC38 cells in serum-free medium and treated with Pb-IFNa-A/D at indicated dose levels when the average tumor volume reached 80 mm³. Tumors and tissues were harvested on day 6 and analyzed by flow cytometry. Gated on viable CD45+CD3+ cells.

CX-801 demonstrates a favorable predicted therapeutic index in a preclinical model



	3.75 mg/kg	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg
CX-801 (masked)			tolerated	tolerated	tolerated
Fc-IFN-a2b (unmasked)	tolerated	3/5 survival	0/5 survival		

Figure 11. Male Syrian golden hamsters (n=16) were implanted with 10⁷ RPMI-1846 hamster melanoma cells and treated twice weekly with 5, 10 or 20 mg/kg CX-801.

Figure 12. Male Syrian golden hamsters (n=5) were treated i.p. with three weekly administrations of 15, 30, or 60 mg/kg CX-801, or 3.75, 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%.

CX-801 is well tolerated in cynomolgus monkey

Objective
- Characterize toxicity, toxicokinetics, and biomarker changes of Pb-IFN-a2b after multiple weekly administrations to cynomolgus monkeys

Study design
- Three weekly i.v. doses on Day 1, 8, 15 followed by observation period and bioactivity assessment (Cytokines, RNAseq, TK)
- Dose groups: 7.5, 15, 30, and 60 mg/kg
- n=4 per dose group (2 males + 2 females)

CX-801 is well tolerated in cynomolgus monkey

	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg
Clinical observations	-	-	-	-
CRP increase	+	+	++	+++
Albumin/globulin decrease	+	+	+	+
Lipid parameters decrease	+	+	+	+
WBC decrease	+/-	+	++	++
IP-10 induction	++	++	++	++

NOAEL for Peg-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.

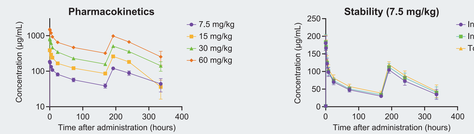


Figure 13. Plasma concentrations of CX-801 measured by LC-MS assay.

Observations
- CX-801 is well tolerated at doses up to 60 mg/kg
- CX-801 demonstrated linear pharmacokinetics and extended half-life

CX-801 demonstrates attenuated IP-10 release in cynomolgus monkeys

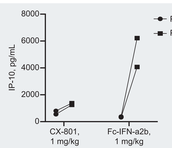


Figure 14. Cynomolgus monkey (n=2 per group) were treated with 1 mg/kg subcutaneous administration of CX-801 or unmasked control Fc-IFN-a2b. Concentrations of IP-10 in serum were measured by MSD V-plex assay.

CONCLUSIONS

1. CX-801 has significantly reduced specific activity that is fully restored through activation by tumor-associated proteases.
2. CX-801 surrogate demonstrated single agent activity as well as enhanced activity when combined with PD-(L)1 blockade. The effect is associated with immune activation in the TME, but not tumor-draining lymph nodes or spleen, showing effective localization via conditional activation.
3. CX-801 demonstrated linear pharmacokinetics, high stability of masking, and an extended half-life, and was well tolerated at multiple weekly doses of at least up to 60 mg/kg in cynomolgus monkeys and hamsters. No MTD was reached.
4. CX-801 demonstrates a favorable predicted therapeutic index with potential for an enhanced clinical profile as monotherapy and in combinations.

CX-801 is in IND-enabling studies