Pharmacodynamic and Pharmacokinetic Activity of CX-801, a Masked IFNα2b PROBODY® Cytokine, in Patients with Advanced Melanoma

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Abstract

Background: Interferon-alpha2b (IFNα2b) has shown meaningful clinical activity in combination with checkpoint inhibitors in melanoma (60.5% ORR), but toxicity has limited its use (48.8% grade 3/4 treatment-related adverse events)[1,2]. CX-801 is a masked PROBODY® cytokine therapeutic designed to deliver interferon-alpha2b (IFNα2b) preferentially to the tumor microenvironment, thereby minimizing systemic toxicity while enhancing local immune activation. We report preliminary results demonstrating pharmacodynamic (PD) activity and pharmacokinetics (PK) of CX-801 from an ongoing, first-in-human, dose escalation study in patients with advanced melanoma.

Methods: PD and PK assessments in an ongoing trial (NCT06462794) supporting CX-801 mechanism of action described herein were performed on tumor biopsies and peripheral blood samples collected from patients treated with monotherapy CX-801.

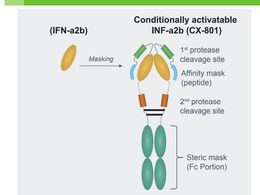
Results: PK analysis demonstrated dose-proportional exposure of CX-801, which remained predominantly in its intact (masked) form in circulation. CX-801 plasma half-life from the first three cohorts ranged from 8.9 - 11.5 days in comparison to 51 hours for unmasked IFNα2b[3].

Gene expression analysis of pre- and post-treatment tumor biopsies demonstrated consistently increased expression of interferon-stimulated genes (ISGs, n=5/5 patients). Evidence of T-cell and NK cell activation as well as immune checkpoint upregulation was observed. There was a sustained elevation of CXCL10 in the tumor but not the blood, suggesting preferential CX-801 activity in the tumor versus the periphery.

Conclusions:

- CX-801 induces interferon signaling in the tumor microenvironment, activating both innate and adaptive immune responses.
- Masked CX-801 demonstrates tumor localized ISG and chemokine induction, consistent with its design to minimize systemic toxicity.
- CX-801 has been well tolerated at doses exceeding the approved dose of unmasked IFNα2b[3].
- The half-life and exposure of CX-801 exceed those of unmasked IFNα2b[3].
- These findings support the continued investigation of CX-801 as a novel immunotherapeutic agent for advanced solid tumors, including melanoma.
- Dose escalation of CX-801 as monotherapy and in combination with pembrolizumab is ongoing.

Background



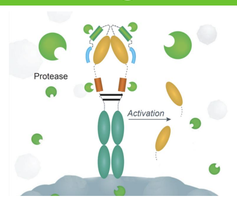
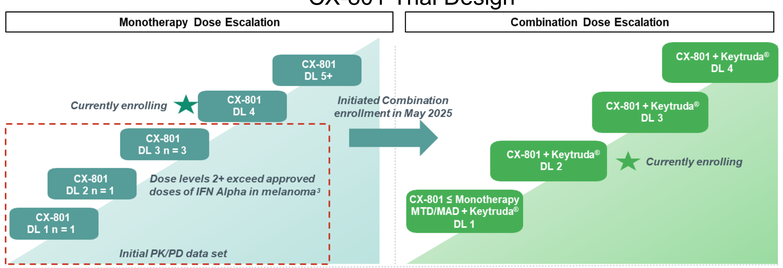


Figure 1 CX-801 is engineered with a dual masking strategy using a cleavable Fc domain for masking and half-life extension, and a cleavable affinity peptide mask. Tumor-associated proteases cleave the masks and Fc domain, releasing fully active IFNa2b.

CX-801 Trial Design



KEYTRUDA® to be administered on approved dose and schedule; KEYTRUDA® is a registered trademark of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA

Results

CX-801 Pharmacokinetics

Dose Level	n	Half-life (days)	Cycle 1 AUC (h*nM)
1	1	11.5	77.6
2	1	9.5	108
3	3	8.9	473

Data are presented as geometric mean where n>1

Figure 2. Following cycle 1, CX-801 has pharmacokinetic properties of:
- A half-life between 8.9 and 11.5 days.
- CX-801 exposure is approximately linear with dose within the dose range tested.
- The majority of CX-801 remains in its fully masked form in the periphery (data not shown).

CX-801 Selectively Induces ISG Expression in Tumor Biopsies

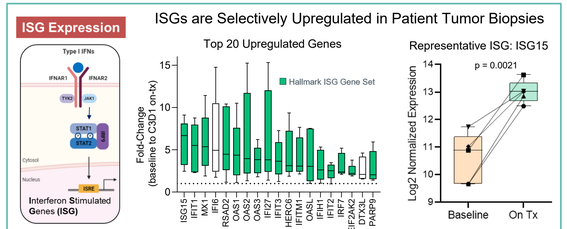


Figure 3. CX-801 tumor localized activation of interferon stimulated genes (ISGs). Left, IFN signaling pathway[4]. Middle, NanoString gene expression analysis of baseline and on-treatment tumor biopsies (n=5). 18/20 (90%) of the most highly increased genes are in the hallmark ISG gene set (green)[5]. Right, the top upregulated gene ISG15 is significantly increased in individual paired on-treatment biopsies.

CX-801 Induces Innate and Adaptive Immune Response

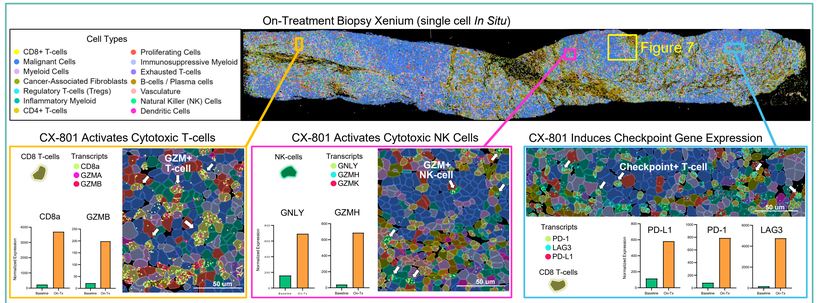
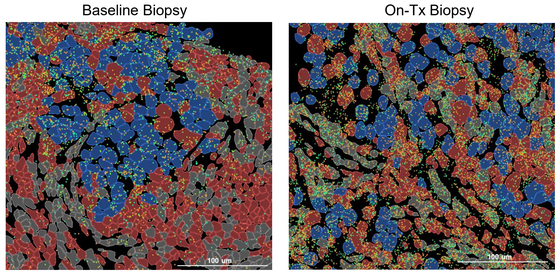


Figure 6. Spatial analysis of immune response in CX-801 patient biopsy. Top, single on-treatment biopsy core with single cells identified from clustering in Fig. 5. Bottom left, representative CD8+ T-cells infiltrate the tumor and express high levels of Granzyme-A/B. NanoString expression from the paired baseline and the same on-treatment biopsy shows significant increase in CD8+ T-cells, and cytotoxic activity (GZMB). Middle, representative cytotoxic NK-cells infiltrating the tumor, with corresponding NanoString showing increased NK cell (GNLY) and cytotoxic expression (GZMH) . Right, significant checkpoint gene expression represented by PD-L1, PD-1, and LAG3 induced by CX-801, with corresponding NanoString expression.

CX-801 Induces Strong ISG Expression In Tumor Biopsy in Multiple Cell Types



Cell Type
Melanoma Cells
Immune Cells
Stromal Cells

ISG Transcript	Transcript Count Baseline	On-Tx
ISG15	1,061	2,886
MX1	1,185	5,189
IFIT3	882	2,602

Figure 4. Multiplex In Situ Hybridization Imaging of interferon pathway activation. Left, baseline and on-treatment biopsies show interferon activity following treatment with CX-801. ISG transcripts ISG15 (cyan), MX1 (orange), and IFIT3 (green) are significantly higher in on-treatment biopsy. As expected due to widespread IFNAR1/2 expression, ISG activation occurs throughout melanoma (blue) and surrounding immune (red) and stromal (grey) cells.

Tumor Specific Chemokine Induction by CX-801 Drives Lymphocyte Infiltration

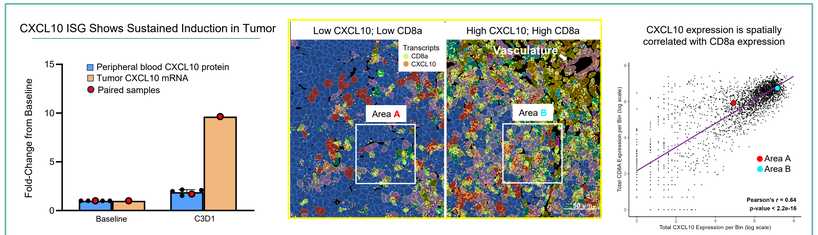


Figure 7. CX-801 induces tumor specific chemokine expression. Left, paired timepoint biopsy expression analysis shows significant increase in tumor expression of CXCL10 with minimal change in peripheral blood CXCL10. Middle, Xenium analysis from the same on-treatment tumor biopsy shows areas of high CXCL10 expression surrounding tumor vasculature with high CD8 infiltration and CXCL10 expression (Area B), and regions with low CXCL10/CD8 (Area A). Right, spatial quantification of CXCL10 and CD8a show strong spatial correlation with representative high and low ROIs from the middle panel.

CX-801 Induces an Interferon Response in All Cell Types and Drives T-Cell Activation

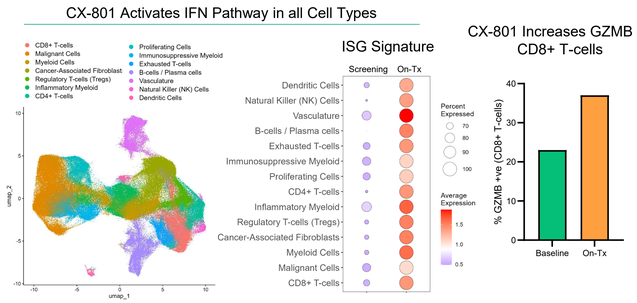


Figure 5. Single cell analysis reveals universal interferon activity and T-cell activation. Left, UMAP of single cells from three patient biopsies. Clustering analysis revealed 14 main cell types present in melanoma tumors. Middle, higher interferon activity can be seen in all cell types identified by single cell clustering in on-treatment biopsy. Right, CX-801 increased Granzyme-B expression in CD8+ T-cells compared to baseline.

References

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