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Preliminary Evidence of Intratumoral Activation and Immunomodulatory Effect of CX-072, a Probody Therapeutic Antibody Prodrug Targeting PD-L1, in a Phase 1/2 Trial

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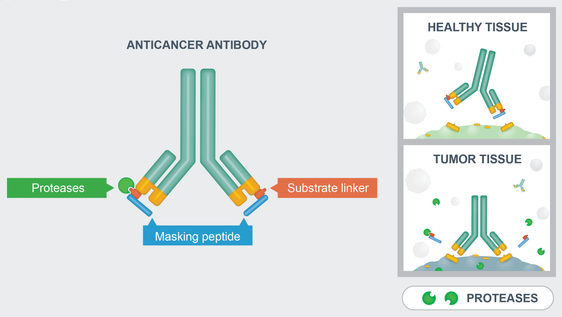
BACKGROUND

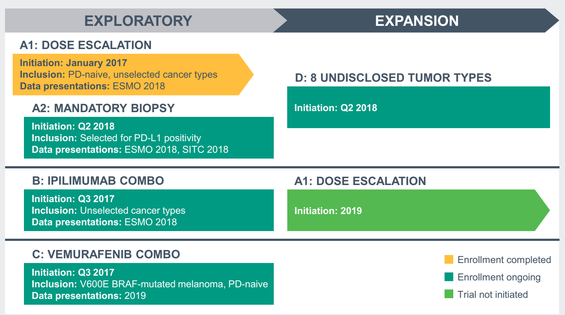
- Programmed cell death ligand 1 (PD-L1) is expressed on many cancer and immune cells. PD-L1 can block cancer immune detection by binding programmed cell death 1 (PD-1), a negative regulator of T-lymphocyte activation[1]
- Monoclonal antibodies targeting the PD-1 pathway have shown anticancer activity in different tumor types,[2] but they also have associated toxicities, especially in combination with other immune-targeted therapeutic agents[2-6]
- Probody™ therapeutics are fully recombinant antibody prodrugs designed to remain relatively inactive systemically and to be activated specifically in the tumor microenvironment by tumor-associated protease activity[7] (**Figure 1**)
- CX-072 is an investigational Probody therapeutic directed against PD-L1 and is designed to have anticancer activity with potentially fewer immune-related adverse events

Figure 1. Probody therapeutics are protease-activatable antibody prodrugs.



- The PROCLAIM-CX-072 study (**PRO**body **CL**inical **A**ssessment **I**n **M**an) is evaluating the tolerability and preliminary antitumor activity of CX-072 as monotherapy or as combination therapy with ipilimumab or vemurafenib in patients with advanced, unresectable solid tumors or lymphoma (ClinicalTrials.gov identifier, NCT03013491) (**Figures 2, 3**)
- Clinical activity of CX-072 as monotherapy and in combination therapy with ipilimumab has been demonstrated previously[8,9]

Figure 2. Clinical trial design for PROCLAIM-CX-072.



ESMO, European Society for Medical Oncology; PD, PD-1/PD-L1 inhibitor; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; SITC, Society for Immunotherapy of Cancer.

Figure 3. Biomarker strategy for PROCLAIM-CX-072.

Potential predictive markers	• PD-L1 levels in tumor (IHC)* • Protease activity in patient biopsies • Tumor mutational burden*
Probody-Tx activation in tumor	• Probody-Tx activation/unmasking: analyzed by capillary electrophoresis immunoassay
Probody-Tx localization in tumor	• ^{89}Zr-PET imaging*
PD-L1/PD-1 pathway inhibition	• Markers of immune system activation: assessed by IHC and mRNA expression

IHC, immunohistochemistry; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; PET, positron emission tomography; Probody-Tx, Probody therapeutic.

*These data are not included in this poster.

OBJECTIVE

- To investigate the molecular mechanism of the Probody therapeutic CX-072 in cancer patients

METHODS

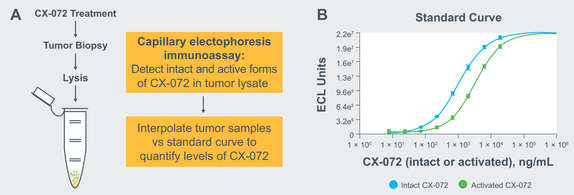
- Tumor biopsies from a subset of PROCLAIM-CX-072 patients were collected during screening and at 3-5 days after the first dose or after 4-6 weeks of CX-072 therapy
- Tumor-associated protease activity was measured by incubating frozen **predose** biopsy sections with a fluorescence-labeled Probody molecule (distinct from CX-072) (**Figure 4**)
  - Cleavage and activation/unmasking of the indicator molecule was assessed by capillary electrophoresis
  - Tumor biopsies with protease activity above the lower limit of quantification were categorized as positive

Figure 4. Measurement of protease activity in pretreatment biopsies.

Probody-Tx, Probody therapeutic.

- Levels of intact and activated/unmasked CX-072 in **postdose** tumor biopsy lysates were measured by capillary electrophoresis immunoassay (**Figure 5**)
  - Biopsies were lysed and separated by electrophoresis (**Figure 5A**)
  - Intact and activated/unmasked forms of CX-072 were detected with an anti-idiotypic antibody, and concentrations were determined (**Figure 5B**)

Figure 5. Measurement of Probody therapeutic activation/unmasking in tumor samples from CX-072–treated patients.



ECL, electrochemiluminescence.

- Fractional target occupancy was calculated using the following equation

$$\frac{[\text{activated intratumoral CX-072}]}{[\text{activated intratumoral CX-072}] + K_d \text{ of activated CX-072 for PD-L1}}$$

- To estimate the molar ratio of activated antibody to total tumoral PD-L1, PD-L1 expression was analyzed by ELISA in a reference set of 50 commercially obtained tumors
- The MC-38 mouse model was used to assess the efficacy of a CX-072 surrogate
  - Efficacy groups were dosed twice weekly; the percentage of tumor growth inhibition (TGI) was calculated 17-18 days after the initiation of dosing for 3 independent MC-38 studies
  - Tumors were obtained from satellite animals 4 days after the initial dose, and levels of intact and activated CX-072 surrogate in murine tumors were assessed as described for patient biopsies in **Figure 5**
- CD8 expression and cell density were evaluated using the Dako C8/144B assay
- Gene expression analysis was performed using the PanCancer Immune Profiling Panel on the NanoString™ platform

RESULTS

Measurement of Intratumoral Protease Activity

- Because CX-072 is designed to be a protease-activated prodrug, we investigated whether protease activity could be detected in patient tumor biopsies
- 18 predose biopsies from PROCLAIM-CX-072 patients were analyzed using a novel assay (**Figure 4**)
- Protease activity was detected in 15 of 18 samples (83%)
- 3 predose biopsy samples had no detectable signal; this may represent tumors with very low protease activity or false negatives because of sample quality or other factors

CX-072 Activation in Patient Tumors and Estimated Target Occupancy

- Tumor biopsies from patients treated with CX-072 were analyzed to determine levels of intratumoral intact and activated/unmasked CX-072 (**Table 1**) (see **Figure 5** for the method)

Table 1. Activated/Unmasked CX-072 Is Detected in Human Tumors at Doses ≥1 mg/kg

CX-072 Dose, mg/kg	Total CX-072, nM	Activated CX-072, nM
30	734.0	221.0
10	206.7	104.7
10	165.3	65.4
10	120.8	31.0
10	57.7	10.2
10	72.5	6.6
3	55.6	8.5
3	13.0	3.8
3	28.2	3.8
3	13.3	Not detectable
3	2.6	Not detectable
3	1.1	Not detectable
3	Below LLOQ	Not detectable
3	Below LLOQ	Not detectable
1	18.8	6.4
1	6.5	Not detectable
1	Not detectable	Not detectable
1	Not detectable	Not detectable
0.3	8.7	Not detectable
0.3	0.7	Not detectable
0.3	0.5	Not detectable
0.3	Below LLOQ	Below LLOQ
0.3	Not detectable	Not detectable

LLOQ, lower limit of quantification.

- The amount of total CX-072 (intact + activated CX-072) and of activated CX-072 detected in patient biopsies increased with dose
- The intratumoral concentration of activated/unmasked CX-072 at doses ≥10 mg/kg (the selected dose for the monotherapy expansion cohort) was estimated to be at a ≥10× molar excess versus a median PD-L1 concentration derived from a reference set (**Table 2**)
- Doses of CX-072 ≥3 mg/kg were estimated to achieve >98% target occupancy in tumors (**Table 2**)

Table 2. Estimated Intratumoral Target Occupancy of PD-L1 by Activated/Unmasked CX-072 Exceeds 98% at Doses ≥3 mg/kg

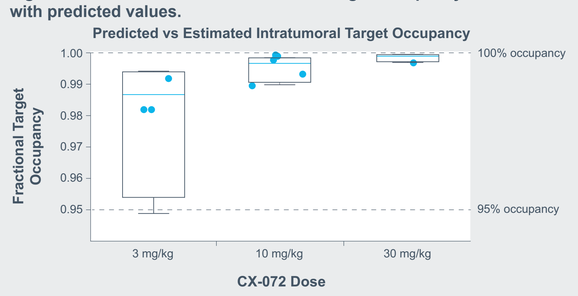
CX-072 Dose, mg/kg	Molar Ratio Activated CX-072: Median Reference PD-L1	Estimated Target Occupancy, %
30 (n = 1)	271	99.97
10 (n = 5)	116	99.65
3 (n = 3*)	9	98.87

PD-L1, programmed cell death ligand 1.

*For 3-mg/kg biopsy samples, **Table 2** shows data only from 3 of 8 biopsies for which activated/unmasked CX-072 was detectable.

- **Figure 6** shows that the estimated intratumoral CX-072 target occupancy (**Table 2,** shown as colored circles) correlated well with predicted values (box plots) derived from a quantitative systems pharmacology[10] model

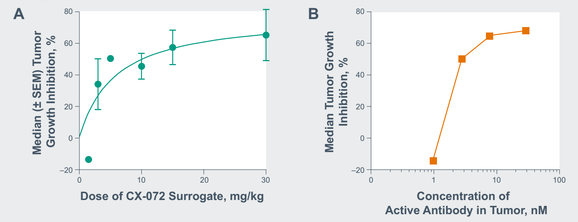
Figure 6. Estimated intratumoral CX-072 target occupancy correlates with predicted values.



Preclinical Data Suggest That Efficacious Levels of CX-072 Are Achieved in Patients

- TGI in the MC-38 mouse model of colorectal cancer correlated with dose and with the intratumoral concentration of activated CX-072 surrogate (**Figure 7**)
  - Panel A: Median %TGI (for a given dose) of 3 independent study iterations is plotted versus dose
  - Panel B: Median %TGI for a single study iteration is plotted versus the median intratumoral concentration of activated CX-072 surrogate

Figure 7. Concentration of active antibody correlates with efficacy in a syngeneic mouse model of colorectal cancer.

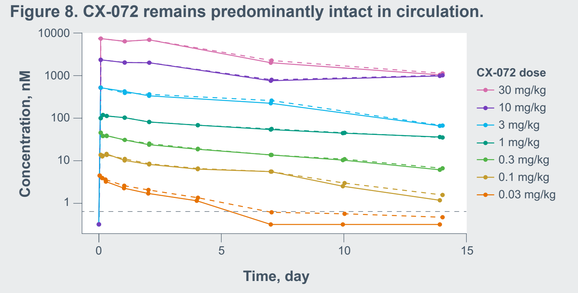


- The concentration range of activated/unmasked intratumoral CX-072 that was associated with statistically significant efficacy in MC-38 tumors was 2.8-126.5 nM (range median, 21 nM)
- This range was similar to that observed in patients who were dosed with CX-072 at 10-30 mg/kg (6.6-221 nM) (**Table 1**)

Stability of CX-072 in Circulation

- The general concordance of the intact and total CX-072 profiles over time demonstrates that CX-072 is largely stable in systemic circulation (**Figure 8**)

Figure 8. CX-072 remains predominantly intact in circulation.



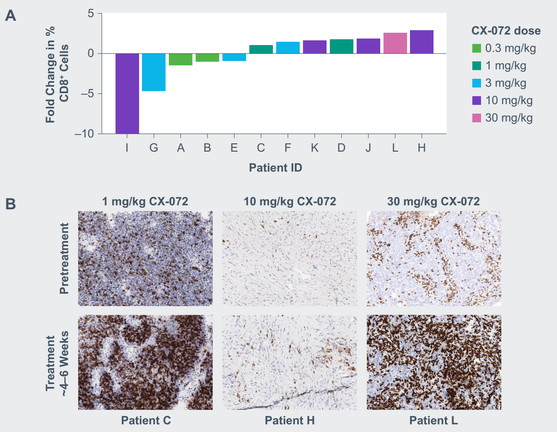
LLOQ, lower limit of quantitation.

Dose 1 median concentrations of intact (solid lines) and total (dashed lines) CX-072 are plotted versus time after administration of up to 30 mg/kg CX-072. The gray dashed line represents the LLOQ; samples below the LLOQ are assigned a value of LLOQ/2.

PD-L1 Pathway Inhibition

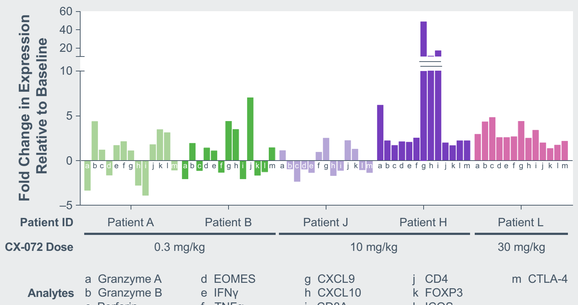
- The percentage of CD8$^+$ cells was assessed by immunohistochemistry (IHC) in 12 evaluable CX-072 monotherapy patient biopsies
- 7 biopsies showed an increase in tumor infiltration of CD8$^+$ T cells relative to the predose baseline (**Figure 9A**; see IHC examples in **Figure 9B**), consistent with inhibition of the PD-1/PD-L1 signaling pathway

Figure 9. CX-072 treatment increases levels of CD8$^+$ T cells in some patient tumors.



- mRNA expression of immune markers in 5 evaluable predose and postdose biopsies was profiled by NanoString analysis (**Figure 10**)
  - An increase in markers of T-cell activation was seen in multiple patients, consistent with inhibition of the PD-1/PD-L1 signaling pathway

Figure 10. CX-072 treatment increases mRNA expression of markers of T-cell activation.



CTLA-4, cytotoxic T-lymphocyte–associated protein 4; EOMES, eomesodermin; IFNγ, interferon gamma; TNFα, tumor necrosis factor α.

CONCLUSIONS

- Preliminary translational studies demonstrate that the PD-L1 Probody therapeutic CX-072 appears to function in cancer patients as designed
- Results are consistent with previous clinical observations showing safety and activity of CX-072 as monotherapy[8]
  - Proteolytic activation of CX-072
    - There is protease activity in the majority of patient tumors
    - CX-072 is activated/unmasked in human tumors
    - CX-072 is predominantly intact in circulation
  - Biological activity of CX-072
    - In patients treated with ≥3 mg/kg CX-072, intratumoral concentrations of activated/unmasked Probody therapeutic are estimated to be sufficient for high-level target occupancy
    - Similar concentrations of activated CX-072 are sufficient for antitumor efficacy in a syngeneic mouse model
    - CX-072 has biological activity in human subjects, as demonstrated by expansion of intratumoral CD8$^+$ T cells and by an increase in markers of T-cell activation
- These results demonstrate additional proof-of-mechanism for the Probody platform and support continued development of CX-072 and of a broad pipeline of Probody therapeutics

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