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Evidence of Intratumoral Localization, Activation, and Immunomodulatory Effect of CX-072, a PROBODY Therapeutic Targeting PD-L1, in a Phase 1/2 Trial

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BACKGROUND

- Programmed death ligand 1 (PD-L1) is expressed on many cancer and immune cells, and can block cancer immune detection by binding the receptor programmed death protein 1 (PD-1), a negative regulator of T-lymphocyte activation
- Monoclonal antibodies targeting the PD-1/PD-L1 pathway have demonstrated efficacy in a broad array of tumor types, but can also generate organ-specific immune reactions associated with systemic immune activation, especially in combination with other immune-targeted therapeutic agents
- Probody therapeutics (Pb-Tx) are fully recombinant antibody prodrugs that are designed to remain mostly inactive systemically and in healthy tissue, and to be selectively activated in the tumor microenvironment by tumor-associated proteases (**Figure 1**)
- CX-072 is an investigational Probody therapeutic directed against PD-L1, and is designed to maintain anticancer activity while potentially reducing systemic immune-related adverse events
- The PROCLAIM-CX-072 study is designed to evaluate the tolerability and preliminary antitumor activity of CX-072 as monotherapy or combination therapy with ipilimumab in patients with advanced, unresectable solid tumors or lymphoma (ClinicalTrials.gov identifier, NCT03013491) (**Figure 2**)
- The clinical activity of CX-072 as monotherapy[1,2] and in combination with ipilimumab has been demonstrated previously[3,4] and current data are presented in ASCO 2020 abstract 3005 (Thistlethwaite F, et al)
- We present updated results of translational studies (**Figure 3**) designed to illustrate the mechanism of action of CX-072 in PROCLAIM-CX-072 patients

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

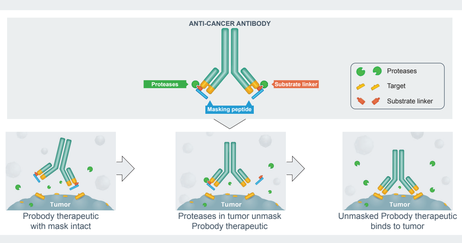
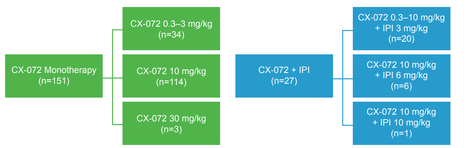


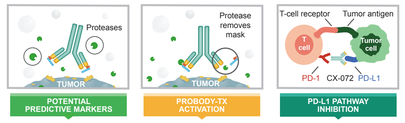
Figure 2. Clinical trial design for PROCLAIM-CX-072.



- Phase 1: monotherapy every 2 weeks (Q2W)[1,2]
  - CX-072: 0.03–30 mg/kg
  - Maximum tolerated dose (MTD) was not reached at a dose of 30 mg/kg
- Phase 1: combination with ipilimumab[3,4]
  - CX-072: 0.3–10 mg/kg every 3 weeks (Q3W)
  - IPI: 3–10 mg/kg Q3W
  - MTD was CX-072 10 mg/kg + IPI 3 mg/kg
- Phase 2 (monotherapy)[5]
  - 10 mg/kg Q2W in patients with
    - Anal squamous cell carcinoma
    - Cutaneous squamous cell carcinoma
    - Triple-negative breast cancer
    - Small bowel adenocarcinoma
    - Undifferentiated pleomorphic sarcoma
    - High tumor mutational burden (as assessed locally)
    - Thymoma or thymic cancers

IPI, ipilimumab.

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



IHC, immunohistochemistry.

METHODS

- Tumor biopsies were collected during the screening phase (1–30 days prior to treatment), and also 3–5 days after the first dose (combination therapy, n=5) or third dose (monotherapy, n=24) of 0.3–30 mg/kg CX-072. One biopsy (combination therapy) was collected 7 days after the second dose of CX-072

Figure 4. Measurement of protease activity in pre-treatment biopsies by tissue zymography.

Pb-Tx, Probody therapeutic.

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

- Fractional target occupancy was calculated as follows:

$$\text{Fractional occupancy} = \frac{[\text{activated CX-072 in biopsy}]}{[\text{activated CX-072 in biopsy}] + K_d \text{ of activated CX-072 for PD-L1}}$$

- To calculate the ratio of intratumoral activated CX-072 to intratumoral PD-L1, PD-L1 was measured by an ultrasensitive enzyme-linked immunosorbent assay and resultant PD-L1 concentrations were compared to those of activated CX-072, measured in the same biopsy lysate
- Concentrations of intact and total CX-072 in plasma were measured by peptide quantification with liquid chromatography–mass spectrometry following affinity capture (see ASCO 2020 pharmacokinetics poster, abstract 3602 [Stroh M, et al])
- Patient serum was serially collected at designated intervals following the first 3 doses, and levels of circulating markers at these time points were measured using the Myriad RBM ExplorerMAP Luminex panel
- For IHC analysis of CD8, fixed pre-dose and on-treatment biopsy sections were stained using the Dako C8/144B antibody

RESULTS

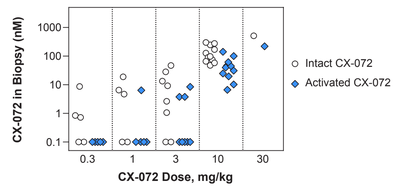
The Majority of Patient Tumors Have Detectable Protease Activity

- Because CX-072 is designed to be a protease-activated prodrug, we investigated whether protease activity could be detected in patients' tumor biopsies
- 26 of 30 (87%) pre-dose biopsies from CX-072 patients were positive for protease activity, as measured in situ by tissue zymography (see assay description in **Figure 4**)
- 1 biopsy that was negative for protease activity had insufficient tumor cells; the remaining 3 negative samples may represent tumors with protease activity below the sensitivity limit of the assay, or may be false negatives due to poor sample quality
- These data are consistent with prior results (data not shown) demonstrating that ~90% of 300 commercially sourced human tumor samples scored positive in a similar tissue zymography assay

CX-072 Is Activated in Tumors But Largely Intact in Circulation

- 30 tumor biopsies from patients treated with CX-072 were analyzed to determine levels of intratumoral intact/masked and activated/unmasked CX-072 (**Figure 6**)

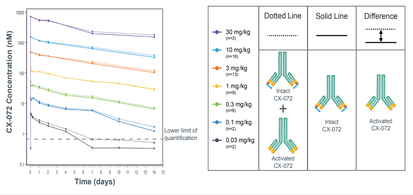
Figure 6. Activated CX-072 is detected in patient biopsies at doses ≥1 mg/kg.



Samples in which CX-072 was below the lower limit of quantification (LLOQ) are plotted as 0.1 nM.

- Activated/unmasked CX-072 was detected in patient biopsies at doses ≥1 mg/kg
  - Intact and activated CX-072 were measured by capillary electrophoresis immunoassay (CEI), as described in **Figure 5**
  - CEI cannot distinguish between Probody molecules with mask cleavage on only 1 light chain (1-arm activation) vs cleavage on both light chains (2-arm activation)
  - The standard curve for activated CX-072 utilizes 2-arm-activated Pb-Tx—so the CEI may under-represent both the amount of 1-arm-activated Pb-Tx and the total amount of activated Pb-Tx
- Total (intact + activated) and intact levels of CX-072 in patient plasma were analyzed by a mass spectrometry assay; representative results of pharmacokinetic profiles after a single dose of up to 30 mg/kg CX-072 are shown in **Figure 7** (see also ASCO 2020 pharmacokinetics poster, abstract 3602 [Stroh M, et al])
  - The concentration profiles for intact CX-072 and total CX-072 appear similar at a given dose, suggesting that CX-072 circulates predominantly in the intact (masked) form

Figure 7. CX-072 remains predominantly intact in circulation.



The horizontal gray dashed line represents the lower limit of quantification (LLOQ) for the CX-072 PK assay; samples below the LLOQ were assigned a value of LLOQ/2. Curves show median concentrations.

Median Estimated Target Occupancy for CX-072 in Patient Biopsies Exceeds 98%

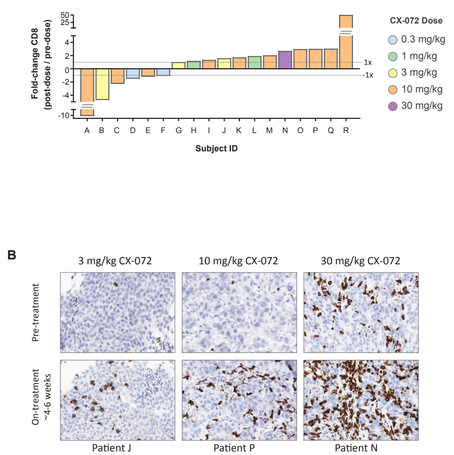
Table 1. Estimated CX-072 target occupancy

CX-072 Dose (mg/kg)	Median Molar Ratio Activated CX-072:PD-L1	Median Calculated Target Occupancy
30 (n=1 biopsy sample)	Not available	**99.97%**
10 (11/11 biopsies had detectable activated CX-072)	**259x** Range: 14–12430x	**99.70%**
3 (3/8 biopsies had detectable activated CX-072)	**7x** Range: 7–984x	**98.18%**

PD-L1 Pathway Inhibition: CX-072-Induced Changes in Tumor CD8

- Pre-dose and on-treatment biopsies from CX-072 monotherapy patients were analyzed by IHC to assess changes in the T-cell activation marker CD8
- **Figure 8A** shows the changes in tumor-associated CD8 in on-treatment vs pre-dose biopsies; **Figure 8B** shows examples of increased CD8 positivity upon PD-L1 inhibition with CX-072, similar to what has been reported for the PD-L1 inhibitor atezolizumab[6]
- 11 of 18 evaluable monotherapy biopsy pairs showed an increase in CD8 relative to the pre-dose baseline, suggesting tumor infiltration of CD8+ T cells, consistent with inhibition of the PD-1/PD-L1 signaling pathway

Figure 8. CX-072 treatment is associated with increased levels of CD8 in some patient tumors.



CONCLUSIONS

- Protease activity is detectable in the majority of PROCLAIM-CX-072 patients' tumors
- CX-072 is unmasked/activated in tumors, and PD-L1 target occupancy exceeds 98% at the 10 mg/kg dose chosen for expansion cohorts
- On-treatment pharmacodynamic changes are consistent with PD-1/PD-L1 pathway activation in PROCLAIM-CX-072 patients
- Updated clinical results from PROCLAIM-CX-072 are presented separately (ASCO 2020 pharmacokinetics poster, abstract 3602 [Stroh M, et al]; ASCO 2020 clinical oral presentation, abstract 3005 [Thistlethwaite F, et al])
- Taken together, these data establish that a PD-L1–directed Probody therapeutic performs as designed, and support continued development of CX-072

PD-L1 Pathway Inhibition: CX-072-Induced Changes in Circulating Analytes

Figure 9. CX-072 treatment increases expression of circulating markers of T-cell activation in patient serum.

C1D1, cycle 1, day 1; IL, interleukin.
Data are graphed as the mean $\log_2$-fold change of each analyte (+/− the standard error of the mean), with respect to the level measured on the first day of dosing (C1D1).
Data points represent the average of 46–70 patient samples.

- The RBM Myriad Luminex ExplorerMAP panel was used to analyze pre-dose and on-treatment serum samples from CX-072 monotherapy patients dosed at 10 mg/kg (**Figure 9**)
  - Results showed an increase in circulating markers of T-cell activation such as interleukin (IL)-18, IL-2 receptor alpha, and CXCL9 as well as in the cytokine CCL18; Il-18 levels have previously been reported to be elevated in patients treated with atezolizumab[6]
  - Increases in circulating immune markers are consistent with widespread intratumoral PD-1/PD-L1 pathway inhibition in metastatic lesions and/or with activated, tumor antigen–specific T cells migrating out of tumors after therapy

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