# Development of CRB-701 (SYS6002): A Novel Site-Specific Nectin-4 Targeting ADC

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AACR Poster

## Background

- Nectin-4 is a cell adhesion molecule normally involved in adherens junctions and cell-cell interactions. It is overexpressed in several cancers and is linked to cancer progression and poor prognosis.
- Nectin-4 has emerged as a clinically validated tumor-associated antigen by enfortumab (PADCEV) in urothelial carcinoma.
- PADCEV has a short plasma half-life, requiring weekly infusions.
- Patients receiving PADCEV may experience various adverse events (AEs) associated with systemic payload release.
- These AEs (e.g., peripheral neuropathy, skin rash, fatigue) lead to dose interuptions (61%), reductions (34%), and discontinuations (17%)[1].
- CRB-701 (SYS6002) is a next generation Nectin-4-targeting ADC designed to solve these patient challenges by improving PK, safety, and efficacy currently observed with PADCEV.

### CRB-701 Next-gen Nectin-4 Antibody-Drug conjugate

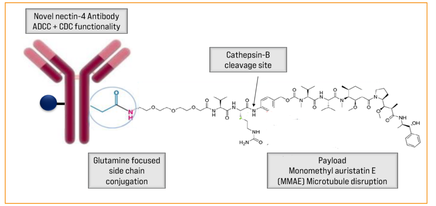
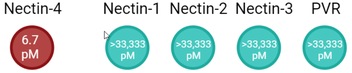



**Figure 1:** CRB-701 contains a novel anti-Nectin-4 monoclonal antibody with a long half-life, affinity, and selectivity. The site-specific cleavable linker employs novel transglutaminase chemistry to yield a homogenous drug product, with a stable drug:antibody ratio DAR of 2.0.

### CRB-701 mAb potency across species


CRB-701 binding is similar across different species used to evaluate PK and toxicity. Binding affinity was measured using ELISA.

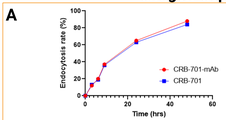
### CRB-701 mAb selectivity




CRB-701 binding is highly specific to the Nectin-4 isoform. PVR - Poliovirus Receptor.

## Results

### CRB-701 undergoes receptor-mediated endocytosis and antigen-dependent MMAE release

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| *In Vitro* MMAE release in PC3 Nectin-4 cells | |
|---|---|
| Time (hrs) | MMAE (ng/ml) |
| 0 | N/A |
| 6 | 0.21 |
| 24 | 0.55 |

**Figure 2: (A)** Endocytosis rates in PC-3 Nectin-4 cells of CRB-701 (blue) and CRB-701-mAb (red) were similar indicating MMAE conjugation did not change antibody uptake. **(B)** MMAE release. No CRB-701 uptake or MMAE release was observed in PC3 parent (non-Nectin-4 expressing) cells, data not shown.

Endocytosis of CRB-701 and CRB-701-mAb (2 ug/mL in culture) was detected using Alexa Fluor488 labeled goat anti-human IgG antibody by flow cytometry (median fluorescence intensity). For MMAE release, cells were incubated with CRB-701 mAb for 0, 6 and 24 hr, then collected and lysed. Intracellular MMAE levels were measured using LC-MS/MS (LLOQ=0.023 ng/mL).

### CRB-701 is internalized at ~ 2-fold the rate of PADCEV

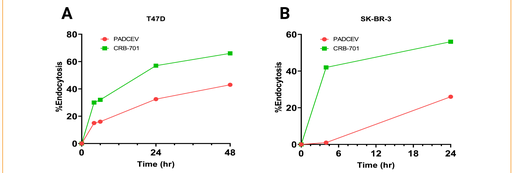



**Figure 3:** Endocytosis rate was measured by confocal microscopy of surface-bound ADC on T47D (A) and SK-BR-3 (B) cells.

### CRB-701 mediates antibody-dependent cellular cytotoxicity (ADCC)

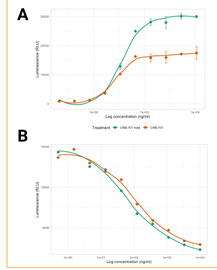



**Figure 4: (A)** Antibody-dependent cellular toxicity (ADCC) EC50=0.13nM (19ng/ml). **(B)** Complement-dependent cytotoxicity (CDC) EC50=0.67nM (105ng/ml). **(C)** CRB-701 has built-in Fc receptor binding activity which can trigger innate immune mediated tumor destruction.

C

| Fc receptor | Affinity KD (nM) |
|---|---|
| FcgR1 | 4 |
| C1q | 7 |
| FcRn | 29 |

ADCC activity was tested using the Jurkat/hFcγRIIIa-NFAT transgenic cells as the effector cells and the 293T-NECTIN4 cells as the target cells. ADCC activity was detected by a luciferase assay and the relative luminescent unit (RLU) was used as the indicator for cytotoxicity. CDC activity of the test antibodies was measured by the CellTiter-Glo® Luminescent Cell Viability Assay where the RLU was used as the indicator of live cells, using human serum as the complement source and 293T-NECTIN4 cells as the target cells.

### CRB-701 treatment induces a bystander effect

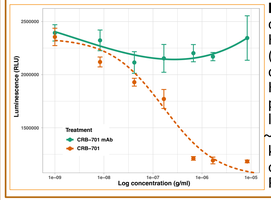



**Figure 5:** Nectin-4 overexpressing PC3 cells were co-cultured for 3 days with HEK293-LUC cells. Bystander effect (viability of non-Nectin-4 expressing cells) was assessed by measuring HEK293-LUC cell proliferation in the presence of CRB-701 or non-MMAE loaded mAb control. Potency was ~5-fold lower for CRB-701 bystander killing [IC50=0.23 ug/mL (1.5 nM)] compared to 0.06 ug/mL (0.40 nM) for PC-3-NECTIN-4 cells.

### CRB-701 demonstrates potent monotherapy in diverse tumors

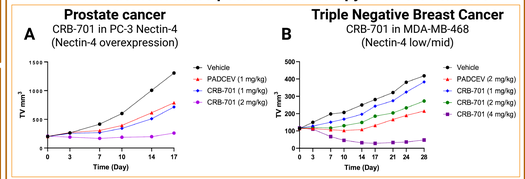
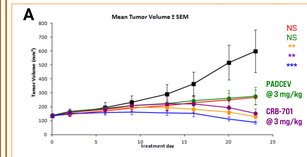



**Figure 6:** CRB-701 has similar efficacy to PADCEV at equivalent doses in two murine cell derived xenograft (CDX) models (A) Human prostate cancer CDX: PC-3-NECTIN4 cells with exogenous NECTIN4 over expression. A single IV administration with PADCEV (1 mg/kg), and CRB-701 (1 and 2 mg/kg) significantly inhibited tumor growth compared to vehicle control (1 mg/kg, p<0.01; 2 mg/kg, p<0.001). (B) Triple negative breast cancer ectopic CDX: MDA-MB-468 cells with endogenous Nectin-4 expression. A single IV administration of CRB-701 dose dependently inhibited tumor growth with minimal effective dose of 2 mg/kg, and similar efficacy to PADCEV (2 mg/kg). Tumor growth inhibition was significant (p<0.001) for PADCEV and CRB-701 (2 and 4 mg/kg).

### CRB-701 is more efficacious than PADCEV in a patient-derived (PDX) tumor model expressing low levels of Nectin-4

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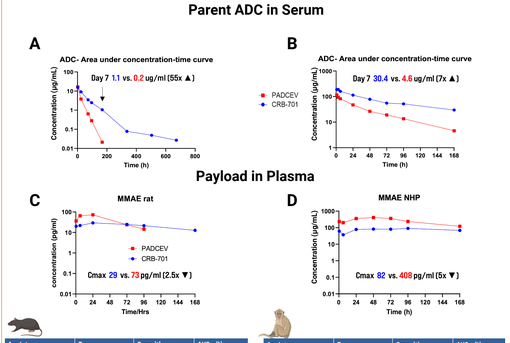
| CRB-701 | PADCEV |
|---|---|
| 74.5% p<0.05 | 53.7% p=0.70 |

**Figure 7:** Anti-tumor activity in a patient-derived xenograft (PDX) bladder cancer model. (A) Tumor growth inhibition in bladder cancer xenograft model BL0597 (HuPrime®). Fragments of tumor from stock mice bearing primary human bladder tumor BL0597 were implanted subcutaneously in the flanks of mice. Once weekly (QWx3) treatments was initiated when the mean tumor size reached 137 $mm^3$. CRB-701 (≥ 2 mg/kg) inhibited tumor growth better than Cisplatin (4 mg/kg) or PADCEV (3 mg/kg). (B) Comparison of TGI values on Day 23 for CRB-701 and PADCEV dosed at 3 mg/kg QWx3.

### CRB-701 has a longer half-life, higher ADC exposure, and lower systemic MMAE release than PADCEV



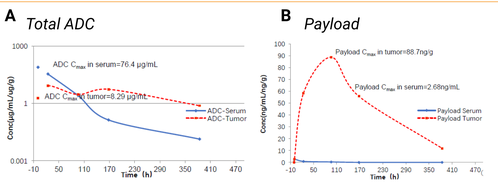

| Analyte | $T_{1/2}$ (days) | $C_{max}$[a] | $AUC_{0-t}$[b] [h*ug/mL] |
|---|---|---|---|
| CRB-701 | 2.42 | 24.9 | 931 |
| MMAE | | 32.5 | 2750 |
| Padcev | 0.58 | 24.5 | 274 |
| MMAE | | 71.4 | 3888 |

| Analyte | $T_{1/2}$ (days) | $C_{max}$[a] | $AUC_{0-t}$[b] [h*pg/mL] |
|---|---|---|---|
| CRB-701 | 3.94 | 189 | 11604 |
| MMAE | | 90.2 | 12941 |
| PADCEV | 1.95 | 116 | 4113 |
| MMAE | | 408 | 44651 |

[a] Units for $C_{max}$: CRB-701 & PADCEV: [mg/mL], MMAE: pg/mL; [b] Units for AUC: CRB-701 & PADCEV: h*mg/mL, MMAE: h*pg/mL

**Figure 8:** PK profiles of parent ADC in serum and free MMAE in plasma, following a single IV administration of PADCEV or CRB-701 in Sprague Dawley rats (1 mg/kg) and cynomolgus monkeys (6 mg/kg).

### CRB-701 preferentially targets payload to a breast cancer tumor xenograft, localizing cell killing while limiting systemic exposure

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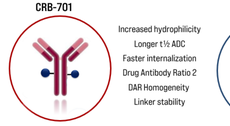
| Measurement | Tumor Cmax | Serum Cmax | Tumor/ Serum AUC ratio |
|---|---|---|---|
| CRB-701 ug/ml | 8.3 | 76.4 | 0.5X |
| MMAE payload ng/ml | 88.7 | 2.7 | 164X |

**Figure 9:** Serum and tumor levels of the parent drug **(A)** and the MMAE payload **(B). (C)** CRB-701 distribution to tumor vs. serum.

## Conclusions

- CRB-701 leverages a novel site-specific conjugation technology, yielding an ADC with a drug-antibody ratio (DAR) of 2.0.
- The glutamine peptide bond ensures greater linker stability than maleimide conjugation, leading to reduced free MMAE concentrations.
- This linker enables CRB-701 to selectively delivers MMAE to tumors.
- Reduced free MMAE concentrations should improve patient tolerability.
- Faster drug uptake of CRB-701 may offer increased potency versus PADCEV, especially considering drug load differences.
- Effector functionality (ADCC and CDC) and a robust bystander effect potentially enhance *in vivo* efficacy.
- In Nectin-4 expressing tumor models, CRB-701 showed potency and efficacy comparable to PADCEV, irrespective of expression levels.
- CRB-701 exhibited enhanced potency and efficacy compared to PADCEV in a low Nectin-4 expressing bladder patient derived tumor mode.
- CRB-701 boasts a longer half-life than PADCEV, suggesting possible extended dosing intervals (q3w instead of qw).
- Plasma MMAE levels for CRB-701 were notably lower than PADCEV, hinting at a safer profile by potentially decreasing associated toxicities.
- CRB-701 (SYS6002) is under clinical investiagion in a Phase 1 dose escalation study in China, with completion expected by the end of 2023.
- Phase 1 dosing in the U.S./EU to be initiated in Q1 2024.

**CRB-701 is designed to establish a wider therapeutic index**

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[1] PADCEV safety information

## Disclosures and Acknowledgements

CRB-701 was designed and produced by CSPC and is being evaluated in Phase 1 clinical studies in China. All nonclinical studies reported here were conducted by CSPC.

We also thanks Huili Sun (CSPC) for her help in reviewing this poster