A Multi-Analyte HPLC-MS/MS Approach to Assessing Exposure of a Probody™ Drug Conjugate in Preclinical Studies

Laura P. Serwer[1], Shweta Singh[1], Claus Krebber[1], Shouchun Liu[1], Niharika Chauhan[2], Robert Leanna[2], Hong Lu[1], Ilaria Badagnani[4], Tracy Henriques[2], Susan Morgan-Lappe[2], William Mylott Jr.[3], Sridhar Viswanathan[1], Jennifer H. Richardson[1], W. Michael Kavanaugh[1]

[1]CytomX Therapeutics, Inc., South San Francisco, CA; [2]AbbVie, North Chicago, IL; [3]PPD Laboratories, Richmond, VA, [4]Former AbbVie Employee

B103

ABSTRACT

CX-2029 is a protease-activatable antibody prodrug (Probody™ Therapeutic) targeted against CD71 (transferrin receptor) and conjugated to a vcMMAE cytotoxic payload with a purified Drug to Probody Ratio (DPR) of 2. In the intact, prodrug form, each light chain of CX-2029 contains an N-terminal prodomain which masks the target binding region of the parental antibody and decreases antigen binding. In vivo proteolytic cleavage of the prodomain in the tumor microenvironment exposes the target binding region, yielding the active antibody. In this way, Probody therapeutics are designed to avoid on-target toxicity in normal tissues while preserving anti-tumor activity.

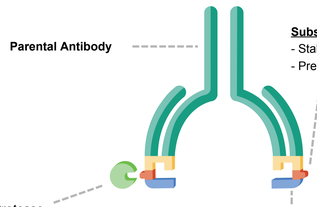
In vivo, CX-2029 may be present in several forms as a result of activation of the antibody prodrug, as well as deconjugation of the cytotoxic payload. We have developed a multi-analyte HPLC-MS/MS approach to monitor levels of four analytes (Intact Probody Therapeutic, Total Probody Therapeutic, Probody-Conjugated MMAE, and Unconjugated MMAE) in cynomolgus monkey plasma to evaluate the exposure of both the intact CX-2029 and activated antibody prodrug, as well as to monitor changes in the Drug to Probody Ratio (DPR) over time. To understand the impact of Anti-Drug Antibodies (ADA) on exposure, a bridging assay was developed to monitor the formation of ADA.

These assays were used to assess exposure of CX-2029 in an ascending dose toxicity study (6, 12, and 18 mg/kg/dose) in cynomolgus monkeys, in support of dose selection for an IND-enabling study. CX-2029 was administered as an intravenous bolus dose to groups of 3 monkeys (2 male, 1 female) once every three weeks for a total of two doses. Samples were collected for pharmacokinetic analysis at time points spanning 21 days after the first dose and 7 days after the second dose. Samples were collected for ADA analysis pre-study and 7 days after the second dose. Dose-dependent increases in Cmax were observed between 6 and 18 mg/kg for the analytes measured. Half-life estimates were similar for Intact Probody Therapeutic, Total Probody Therapeutic, and Probody-Conjugated MMAE and ranged from 2.5 to 6.3 days. The ratio of Intact Probody Therapeutic to Total Probody Therapeutic was used to assess stability of the cleavable prodomain in the cynomolgus monkey over time. By 7 days post-dose, approximately 80% of CX-2029 in plasma was in the intact, prodrug form. The average Drug to Probody Ratio (DPR) was evaluated over three weeks in vivo. Average DPR decreased from 2 shortly after dosing to approximately 0.5 by 21 days post-dose. ADA were detected in 3 of 9 animals dosed with CX-2029.

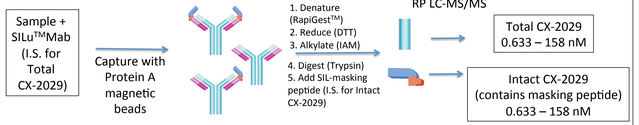
In cynomolgus monkeys, CX-2029 exposure is maintained throughout the 21-day dosing interval, and the majority of CX-2029 in circulation is intact. CX-2029 is currently under development, with an IND filing expected in 2018.

INTRODUCTION

Probody Therapeutics are Protease-Activatable Antibody Prodrugs



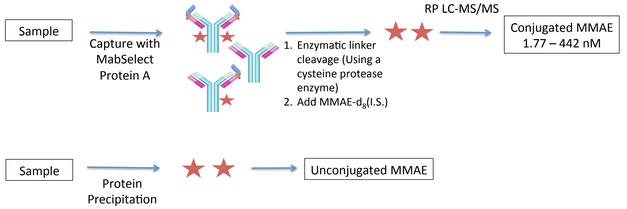
BIOANALYTICAL ASSAY DESIGN



Surrogate Peptides

Intact CX-2029 Peptide: Peptide at the N-terminus of the light chain (masking peptide).

Total CX-2029 Peptide: Peptide from the heavy chain, present in both activated (binding) and intact (non-binding) molecules.



Samples from CX-2029 treated animals are divided into three aliquots and processed as shown above to yield concentration information for Total CX-2029, Intact CX-2029 (contains masking peptide), Conjugated MMAE, and Unconjugated MMAE. I.S. = Internal Standard, RP = Reverse Phase, DTT = Dithiothreitol, IAM = Iodoacetamide.

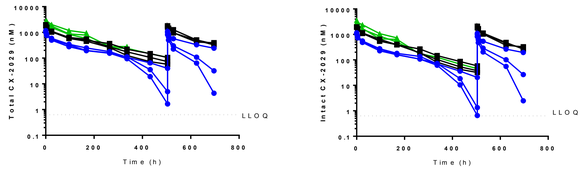
TOXICITY STUDY DESIGN

Group	Test Article	Dose (mg/kg)	# Doses (Schedule)	# Animals	Sampling Time Points Relative to Dose (days)
1	Vehicle	NA	2 (Q3W)	3 (2M/1F)	Day 1: + 0.003, 0.17, 1, 2, 4, 7, 11, 14, 18, 21 Day 22: + 0.003, 0.17, 1, 2, 4, 7
2	CX-2029	6			
3	CX-2029	12			
4	CX-2029	18	1		Day 1: + 0.003, 0.17, 1, 2, 4, 7, 11, 14, 18, 21

Cynomolgus monkeys were dosed with either vehicle or CX-2029 by slow IV bolus at the dose levels and schedules shown. Blood samples for toxicokinetic analysis were processed to plasma and stored at -80°C prior to analysis. Serum samples were obtained for anti-drug analysis pre-dose and 7 days after the second dose for Groups 1-3 and 22 days after the first dose for Group 4. All groups contained three animals (2 males and 1 female).

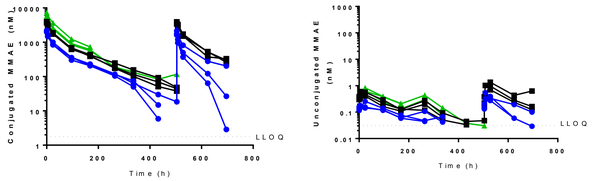
RESULTS

Figure 1: Exposure of Total CX-2029 and Intact CX-2029 is generally maintained throughout 21 day dosing interval



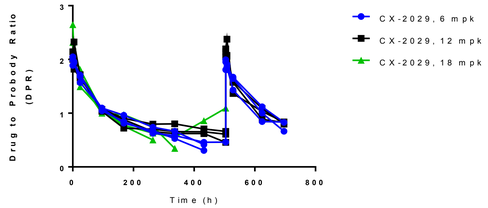
Individual plasma concentration curves for Group 2 (6 mg/kg, ●), Group 3 (12 mg/kg, ■), and Group 4 (18 mg/kg, ▲) for Total CX-2029 (binding and non-binding species, left panel) and Intact CX-2029 (non-binding species only, right panel). Lower limit of quantitation (LLOQ, dashed line) for both analytes was 0.633 nM. Intact CX-2029 contains the masking peptide and has reduced binding to CD-71.

Figure 2: Conjugated MMAE exposure is substantially higher than unconjugated MMAE levels at all dose levels tested



Individual plasma concentration curves for Group 2 (6 mg/kg, ●), Group 3 (12 mg/kg, ■), and Group 4 (18 mg/kg, ▲) for conjugated MMAE (left panel) and unconjugated MMAE (right panel). Lower limit of quantitation (LLOQ, dashed line) was 1.77 nM for conjugated MMAE and 0.31 nM for unconjugated MMAE.

Figure 3: Change in Drug to Probody Ratio (DPR) is consistent at all dose levels tested



Test Article	Dose (mpk)	DPR, 5 min	DPR, 4 hr	DPR, 24 hr	DPR 4 days	DPR, 7 days
CX-2029	6	1.98 ± 0.081	1.95 ± 0.008	1.65 ± 0.075	1.08 ± 0.029	0.874 ± 0.077
	12	2.05 ± 0.080	2.00 ± 0.283	1.69 ± 0.030	1.06 ± 0.023	0.835 ± 0.100
	18	2.34 ± 0.295	2.01 ± 0.072	1.63 ± 0.164	1.03 ± 0.030	0.835 ± 0.119

Individual Drug to Probody Ratio (DPR) curves for Group 2 (6 mg/kg, ●), Group 3 (12 mg/kg, ■), and Group 4 (18 mg/kg, ▲) were generated by dividing the concentration of conjugated MMAE by the concentration of Total Probody Therapeutic. Average group values ± standard deviation are shown at several time points post-dose in the table above.

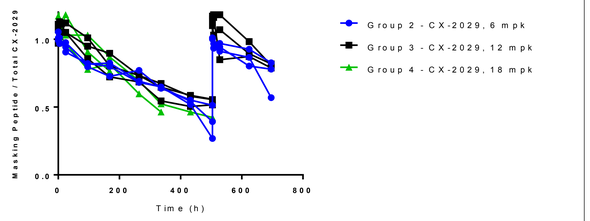
Figure 4: Pharmacokinetic Parameters for Total CX-2029, Intact CX-2029, Conjugated MMAE, and Unconjugated MMAE

Article	Analyte	Mean C_{max} (nM)			Mean AUC_{0-7} (day·nM)		
		18 mg/kg	12 mg/kg	6 mg/kg	18 mg/kg	12 mg/kg	6 mg/kg
CX-2029	Total CX-2029	2610	1810	1060	5560	5490	2850
	Intact CX-2029	2530	1190	1080	4804	4970	2520
	Conjugated MMAE	6090	3820	2090	12500	7850	4000
	Unconjugated MMAE	0.696	0.487	0.195	1680	1170	531

AUC_{0-7} = area under the plasma concentration-time curve to Day 7; C_{max} = maximum observed plasma concentration

Half-Life estimates for Total CX-2029, Intact CX-2029, and pc-MMAE ranged from 2.5 – 6.3 days. Intact CX-2029 contains the masking peptide and has reduced binding to CD-71. Half-life estimates were not generated for unconjugated MMAE.

Figure 5: Majority of the masking peptide is present in CX-2029 for 11 days post-dose



Test Article	Dose (mpk)	Intact Ratio 5 min	Intact Ratio 4 hr	Intact Ratio 24 hr	Intact Ratio 7 days	Intact Ratio 11 days
CX-2029	6	1.02 ± 0.032	0.997 ± 0.024	0.942 ± 0.035	0.783 ± 0.051	0.714 ± 0.049
	12	1.05 ± 0.072	1.06 ± 0.059	1.07 ± 0.040	0.807 ± 0.087	0.716 ± 0.027
	18	1.21 ± 0.102	1.06 ± 0.035	1.06 ± 0.107	0.817 ± 0.053	0.646 ± 0.069

The concentration of the masking peptide divided by the total concentration of CX-2029 is shown for Group 2 (6 mg/kg, ●), Group 3 (12 mg/kg, ■), and Group 4 (18 mg/kg, ▲). Average group values ± standard deviation are shown at several time points post-dose in the table above.

SUMMARY/CONCLUSIONS

- The exposure of CX-2029, a novel Probody Drug Conjugate, was assessed by multi-analyte LC-MS/MS following dosing in cynomolgus monkeys at multiple dose levels.
- Two surrogate peptide analytes were used to measure both the total levels of CX-2029 as well as the levels of masked CX-2029 in cynomolgus monkey plasma samples.
- CX-2029 exposure was generally maintained throughout the 21 day dosing interval and was proportional to dose at the 6 and 12 mg/kg levels.
- Unconjugated MMAE levels were low (<1% of conjugated MMAE levels) at all time points measured.
- The change in average Drug to Probody Ratio (DPR) was consistent at all dose levels tested. Average DPR decreased to 1 approximately 96 hours post-dose.
- The majority of circulating CX-2029 contained the masking peptide for 11 days post-dose.
- For additional information on the efficacy and safety of CX-2029, please see Poster B116.