Dose Range Finding Study in Non-human Primates Confirms the Unique Mechanism of Action of CTX-8371, a Novel Bispecific Antibody Blocking PD-1 and PD-L1

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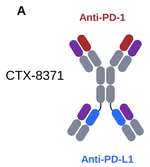
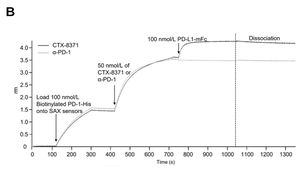
Introduction

Inhibitors of the immune checkpoint pathway, PD-1/PD-L1, have made a critical advance in the treatment of solid tumors. However, not all patients successfully respond to monotherapy making the need for combination strategies apparent. CTX-8371 combines PD-1/PD-L1 targeting in one bispecific, tetravalent molecule. In addition to potently blocking PD-1/PD-L1 interaction, CTX-8371 exhibits a unique mechanism of action (MOA) that involves cleavage of cell surface PD-1. Importantly, *in vivo* cleavage of PD-1 was shown not only in mice, but also in a dose range finding (DRF) study in cynomolgus macaques. In monkeys, a marked decrease in the frequency of PD-1$^+$CD4$^+$ and PD-1$^+$CD8$^+$ T cells in peripheral blood could be observed at the earliest timepoint examined, day 2 after CTX-8371 administration. PK/PD was also examined using tumor bearing mice as well as cynomolgus monkeys. Clearance and half-life of CTX-8371 are within the expected ranges for a human IgG1 antibody in non-human primates (NHP) with a linear PK. The dual blockade of PD-1 and PD-L1, combined with PD-1 cleavage, may offer a superior benefit compared to current checkpoint inhibitor monotherapies. Receptor occupancy data as well as murine and NHP PK/PD analyses will guide a projected human dose for future development.

CTX-8371 is a Common Light Chain Tetravalent Bispecific Antibody Targeting PD-1 and PD-L1

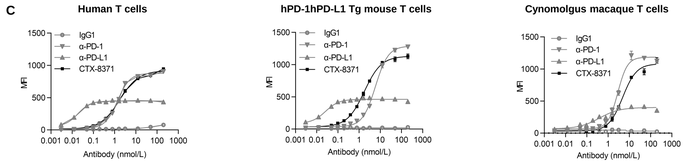


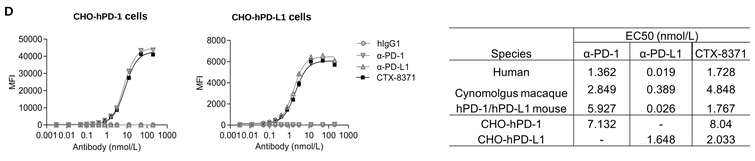


CTX-8371 affinities to PD-1 and PD-L1

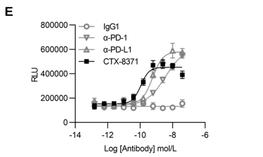
	Binding KD (nmol/L)					
Drug	PD-1			PD-L1		
	Human	Monkey[a]	Mouse[b]	Human	Monkey[a]	Mouse[b]
CTX-8371	3.75	12.4	672	17.3	12.6	134

[a]Cynomolgus monkey; [b]*Mus musculus* reference sequence





Species	EC50 (nmol/L)		
	α-PD-1	α-PD-L1	CTX-8371
Human	1.362	0.019	1.728
Cynomolgus macaque	2.849	0.389	4.848
hPD-1/hPD-L1 mouse	5.927	0.026	1.767
CHO-hPD-1	7.132	-	8.04
CHO-hPD-L1	-	1.648	2.033



Antibody	EC50 (mol/L)
α-PD-1	3.66E-09
α-PD-L1	5.85E-10
CTX-8371	1.11E-10

Figure 1. (A) CTX-8371 structure. **(B)** CTX-8371 simultaneous binding of PD-1 and PD-L1 proteins by Octet bridging assay. **(C)** Binding of CTX-8371 to primary activated CD3$^+$ T cells by flow cytometry. **(D)** CTX-8371 binding to CHO/huPD-1 and CHO/huPD-L1 cells by flow cytometry. The average MFI of triplicates at each concentration is shown. **(E)** CTX-8371 blockade of PD-1/PD-L1 interaction was measured using an NFAT reporter assay. TCR activation caused by PD-1/PD-L1 blockade is detected in a PD-1$^+$ Jurkat-NFAT reporter cell line after incubation with PD-L1$^+$CHO-K1 cells in the presence of increasing concentrations of CTX-8371, Anti-PD-1, Anti-PD-L1, or IgG1 isotype control. The luminescence signal resulting from NFAT activation is plotted as relative light units versus antibody concentration (mean ± SD; *n* = 3).

CTX-8371 Mechanisms of Action

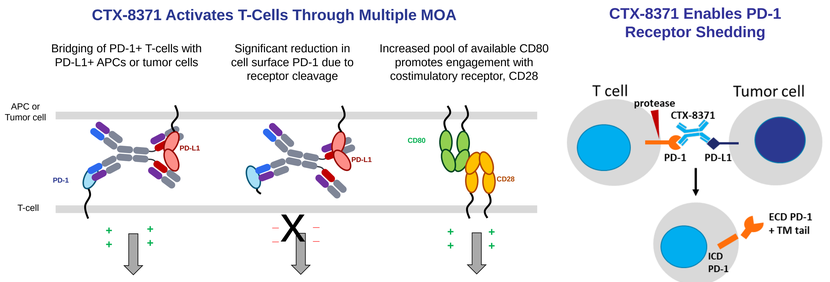


Figure 2. Studies have shown that CTX-8371 uniquely drives the loss of cell surface PD-1 protein on activated T cells in addition to blocking PD-1 and PD-L1 interaction and CD28 liberation.

CTX-8371 Treatment Leads to PD-1 Loss in mice

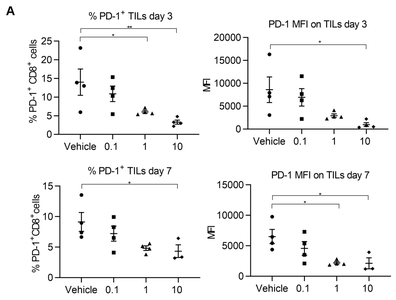
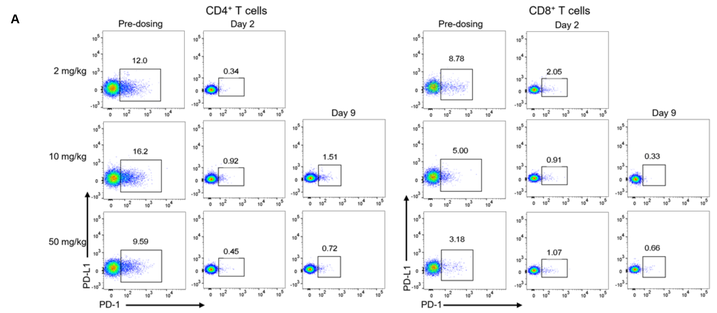


Figure 3. Transgenic hPD-1hPD-L1 mice were inoculated with 1x10^6 MC38-hPD-L1 cells s.c. and randomized when the average tumor size reached aprox.350 mm^3. Mice received a single injection of CTX-8371 at three dose levels and tumors were collected on days 3 and 7 after dosing. Single cell suspensions were prepared from tumors and PD-1 and PD-L1-expression on CD8$^+$TILs were measured by FACS.
(A) Analysis of PD-1$^+$ CD8$^+$ T cell frequencies and PD-1 intensity in all mice. Data are shown as mean ± SEM, n=4. **, P<0.001, *, P<0.05, One-way ANOVA and Dunnett's multiple comparisons test. **(B)** Western blot of PD-1 expression in tumor lysates on day 3.

CTX-8371 Drives Loss of PD-1 in Cynomolgus Macaques



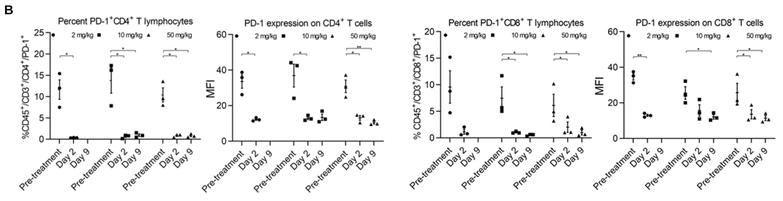
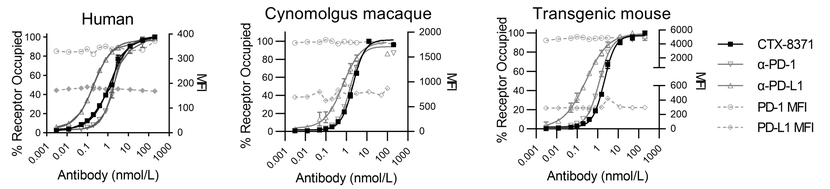


Figure 4. (A) Representative dot plots of cynomolgus macaque PD-1$^+$ CD4$^+$ and CD8$^+$ T cells at baseline and on days 2 and 9 after dosing with 2, 10, or 50 mg/kg of CTX-8371. Cynomolgus macaques in the 10 and 50 mg/kg groups received a second dose on day 8 after the first dose.
(B) CTX-8371 treatment of cynomolgus monkey drives PD-1 loss on peripheral blood T lymphocytes. Analysis of systemic PD-1$^+$ CD4$^+$ and CD8$^+$ T cell frequencies and PD-1 intensity in all monkeys. Data are presented as mean ± SEM, n=3. **, P<0.001, *, P<0.05, One-way ANOVA and Dunnett's multiple comparisons test.

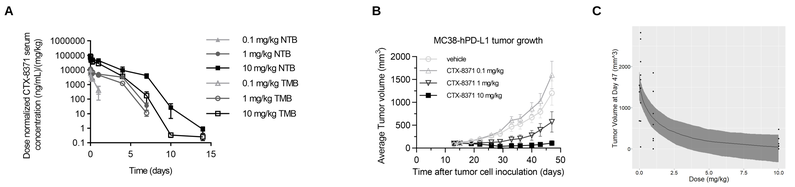
In vitro Receptor Occupancy for CTX-8371



Antibody	Target	RO EC50 [nmol/L] human	RO EC50 [nmol/L] cynomolgus macaque	RO EC50 [nmol/L] mouse
CTX-8371	PD-1/PD-L1	1.313	2.0	2.088
α-PD-1	PD-1	1.975	1.45	1.209
α-PD-L1	PD-L1	0.205	0.54	0.2695

Figure 5. To evaluate RO, PBMCs from human, cynomolgus macaque, or splenocytes from transgenic hPD-1/hPD-L1 mice were stimulated for 3 days with anti-CD3 and anti-CD28 antibodies then treated with the listed antibodies for 1h at 4ºC. PD-1 and PD-L1 receptor occupancy *in vitro* was determined by FACS. EC50 values are shown for each antibody. Data shown as mean ± SD, n=3.

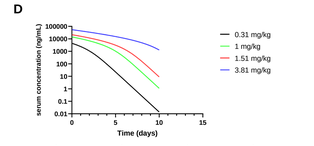
PK/PD Relationship in Tg hPD-1hPD-L1 mice



PK parameters of CTX-8371 in Tg mice

Dose mg/kg	Mouse	HL_Lambda_z Day	Cmax ng/mL	Cmax_D (ng/mL)/(mg/kg)	AUClast Day*ng/mL	CL_obs mL/Day/kg	Vss_obs mL/kg
0.1	NTB	0.31	1025.07	10250.68	211.07	429.4	147.39
1	NTB	1	8881.67	8881.67	23392.92	42.65	91.45
10	NTB	0.9	651931.5	65193.15	1581694	6.32	11.77
0.1	TMB	0.26	839.35	8393.55	231.67	405.12	113.99
1	TMB	0.77	11459.92	11459.92	17044.21	58.63	83.22
10	TMB	0.71	825677	82567.7	938615.6	10.65	12

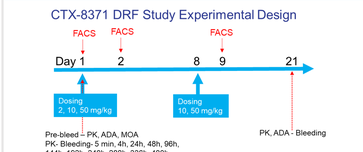
AUClast (day*ng/mL), area under the curve from time 0 to last measurable concentrations; CL_obs (mL/day/kg), clearance observed; Cmax (ng/mL), maximum observed serum concentration; Cmax_D ((ng/mL)/(mg/kg)), dose normalized Cmax; HL_Lambda_Z (Day), terminal half-life; Vss_obs (mL/kg), volume of distribution at steady state



Dose (mg/kg)	0.31	1	1.51	3.81
AUC0-last (ng/mL*day)	5955.99	31958.30	56983.61	192813.10

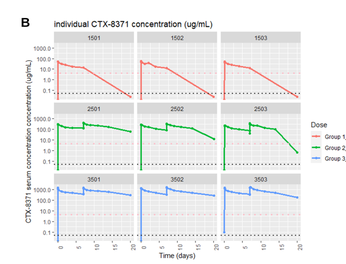
Figure 6. (A) Dose-normalized CTX-8371 serum concentrations in non-tumor bearing (NTB) vs tumor bearing (TMB) mice. Female Tg hPD-1hPD-L1 mice were inoculated with 1x10^6 MC38-hPD-L1 cells subcutaneously in the right flank. Treatment started when tumors reached an average volume of ~100 mm^3. Mice were serially bled at the indicated time points. CTX-8371 levels in sera were determined using MSD ELISA. Data are shown as mean ± SEM, n=3. **(B-C)** Tumor growth inhibition was used as a biomarker of activity to create a dose-response curve for CTX-8371. The dots on (C) represent individual tumor volumes on Day 47, solid line is the fitted curve using Emax model, and the shaded area is the 95% confidence interval for the Emax curve. **(D)** Projected CTX-8371 PK in TMB mice at various dose.

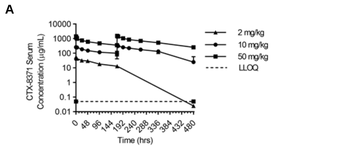
PK of CTX-8371 in Cynomolgus Macaques



Group No.	Test Material	Dose Level (mg/kg/dose)	Dose Volume (mL/kg)	Infusion Rate (mL/kg/h)	Dose Concentration (mg/mL)	Number of Animals Main Study Females
1[a]	CTX-8371	2	2	10	1	3
2[a]	CTX-8371	10	5	10	2	3
3[b]	CTX-8371	50	5	10	10	3

[a] Single dose, 12 minutes infusion
[b] Two doses, Days 1 and 8, 30 minutes infusion





PK parameters of CTX-8371 in cynomolgus macaques

Dose (mg/kg)	t_{max}[a] (hr)	C_{max} (µg/mL)	C_{max}/Dose (µg/mL/(mg/kg))	$AUC_{(0-tlast)}$[b,f] or $AUC_{(0-168.5\ hr)}$ [c]/(hr*µg/mL)	$AUC_{(0-tlast)}$/Dose[e,f] or $AUC_{(0-168.5\ hr)}$ / Dose [c]/(hr*µg/mL/(mg/kg))	$T_{1/2}$[b] (hr)
Day 1						
2[d]	0.2 (0.2 - 0.7)	55.9 ± 3.16	28.0 ± 1.58	4000 ± 232	2000 ± 116	NC
10[d]	1.5 (0.5 - 4.5)	274 ± 45.7	27.4 ± 4.57	24100 ± 3510	2410 ± 351	NC
50[d]	0.5 (0.5 - 0.5)	1440 ± 109	28.9 ± 2.17	95500 ± 4280	1910 ± 85.5	NC
Day 8						
10[d]	0.5 (0.5 - 1)	377 ± 48.0	37.7 ± 4.80	34000 ± 5940	3400 ± 594	75.9 ± 50.6[g]
50[d]	0.5 (0.5 - 1.5)	1650 ± 86.3	33.0 ± 1.73	137000 ± 1590	2730 ± 31.8	118[h]

[a] Median (Min – Max), time Post SOI; [b] $T_{1/2}$ could not be determined on Day 1; [c] length of infusion = 12 minutes [d] length of infusion = 30 minutes; [e] $AUC_{(0-168.5)}$ for Group 1 and $AUC_{(0-tlast)}$ for Groups 2 and 3, which are equivalent to AUC_{last} for Day 1; [f] tlast = 168.2 hr for 2mg/kg dose; 168.5 hr for 10 and 50 mg/kg dose for Day 1 [g] = for Day 8; [h] Summary of $T_{1/2}$ for profiles with $R^2 > 0.800$, and $\%AUC_{extrap} < 20\%$ of the total area; NC, Not calculated

Figure 7. (A) Dose-normalized CTX-8371 serum concentrations in cynomolgus macaques following a single IV dose of 2 mg/kg and repeated IV doses of 10 mg/kg and 50 mg/kg on Day 1 and Day 8. Data are shown as mean ± SD, n=3. **(B)** Individual CTX-8371 concentration (µg/mL), Black dashed line: LLOQ 50 ng/mL, BLQ values were replotted as half of LLOQ, Pink dashed line: *in vitro* RO EC90

Conclusions

- Treatment with CTX-8371 leads to PD-1 loss *in vivo* on intra-tumoral T cells in tumor-bearing Tg mice and on peripheral blood T cells in cynomolgus macaques.
- Unique MOA differentiates CTX-8371 from other PD-1/PD-L1 blockers.
- CTX-8371 had non-dose proportional PK in both NTB and TMB mice as evidenced by decreasing CL with increased dosing.
- CTX-8371 cleared faster in TMB compared to NTB mice at doses ≥ 1 mg/kg resulting in a shorter half-life and faster CL.
- A dose-response curve in MC38-hPD-L1 TMB mice showed an ED50 of 1-2 mg/kg.
- PK data of CTX-8371 in cynomolgus monkey correlated with *in vitro* RO.
- On day 8, the individual t1/2 ranged between 26.5 and 128 hours.
- Systemic exposure to CTX-8371, as described by mean Cmax and AUC(0-168.5) or AUC(0-168.5), increased with increasing dose in a dose-proportional manner on both days, 1 and 8.
- Following repeated IV infusion, systemic exposure (AUC0-168.5 and Cmax) to CTX-8371 was similar on Day 8 relative to Day 1 showing no apparent accumulation after a second dose, at 10 and 50 mg/kg.
- Efficacious dose in mice, mouse and monkey PK data will be used to predict the human efficacious dose range for CTX-8371.

Acknowledgements

- We acknowledge the contribution of Charles River Laboratories, Montreal for conducting the DRF study in cynomolgus monkeys.
- We thank Biocytogen for the engineered Tg hPD-1hPD-L1 mice, the help with PK/PD study, and the MC38-hPD-L1 cell line.
- We recognize the work of Dr. Rong Deng for the DRF studies and human dose prediction.
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