Preclinical development of ADCT-212, a PSMA-targeted antibody-drug conjugate employing the pyrrolobenzodiazepine dimer SG2000 for PSMA-expressing cancers

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INTRODUCTION

- Prostate-specific membrane antigen (PSMA) is a membrane-bound glutamate carboxypeptidase (**Figure 1**) that is highly expressed in nearly all prostate cancers with the highest expression in metastatic castration-resistant prostate cancer[1, 2].
- Moreover, PSMA is expressed in the neovasculature that supplies most non-prostatic solid tumors, including carcinomas of the lung, colon, breast, kidney, liver, and pancreas[3].
- ADCT-212 is an antibody-drug conjugate composed of the human IgG1 antibody 2A10 directed against human PSMA, site-specifically conjugated using GlycoConnect™ technology[4] to PL1801, which contains Hydraspace™, a valine-alanine cleavable linker and the pyrrolobenzodiazepine (PBD) dimer warhead SG2000 (drug-antibody ratio of ~1.8) (**Figure 2**).

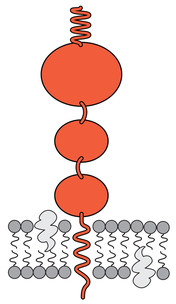


Figure 1. PSMA.

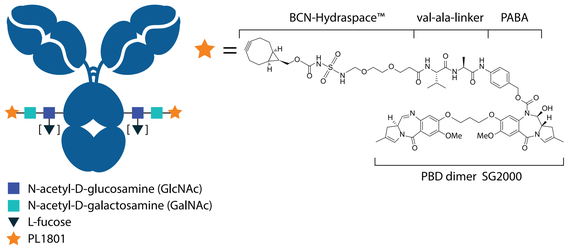


Figure 2. ADCT-212.

AIM OF THE STUDY

The purpose of this study was to characterize the *in vitro* mechanism of action and *in vivo* efficacy of ADCT-212 and to determine its safety, tolerability and pharmacokinetics (PK) in the rat.

MATERIALS AND METHODS

- Cytotoxicity of ADCT-212, the free PBD dimer SG2000 and isotype control ADC (B12-PL1801) was determined by the CellTiterGlo® assays (Promega). Quantitative determination of cell-surface PSMA density was done using Bangs Laboratories Quantum™ MESF kit.
- The single cell gel electrophoresis (Comet) assay was carried out on LNCaP and PC-3 cells treated with ADCT-212, B12-PL1801 or free warhead SG2000. The mean reduction in the product of the tail length and the fraction of total DNA in the tail, i.e. The Olive Tail Moment (OTM) in irradiated and unirradiated control cells were both measured.
- Binding, internalization and trafficking to lysosomes were visualized by standard immunofluorescence techniques.
- *In vivo*, ADCT-212 was administered intravenously (i.v.) as single dose to athymic nude or SCID mice containing LNCaP, CWR22Rv1 or PC-3 s.c. xenografts.
- PK analysis of ADCT-212 was performed in male Crl:CD(SD) rats. Quantitation of total antibody or PBD-conjugated antibody was performed by ECLIA.

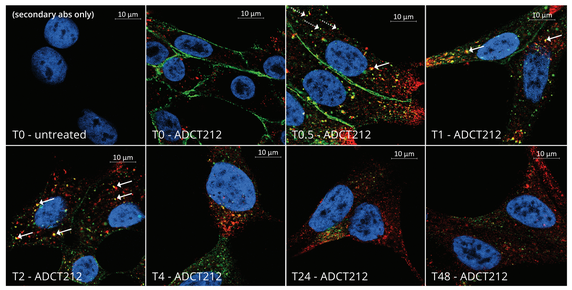
RESULTS

Table 1. *In vitro* cytotoxicity

	LNCaP	C4-2	22Rv1	PC-3	P4E6	DU145
Mean PSMA antigen density (SEM)	1,116,628 (66,748)	764,487 (17,222)	13,583 (610)	BLLQ	BLLQ	BLLQ
Mean SG2000 IC50 pM (SEM)	749 (160)	173 (8.01)	83.6 (9.9)	2,690 (777)	974 (31.4)	1,680 (333)
Mean ADCT-212 IC50 pM (SEM)	882 (425)	540 (46.7)	4,000 (1110)	N/D	42,500 (3,550)	1.29E+07 (1.27E+07)
Mean B12-PL1801 IC50 pM (SEM)	98,900 (30,800)	27,200 (1,760)	13,100 (2,980)	N/D	40,300 (6,470)	7.96E+06 (7.92E+06)

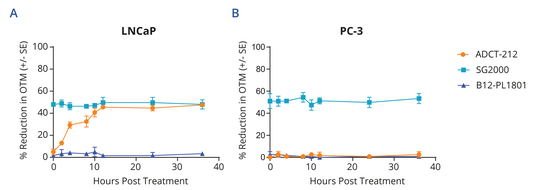
Mean PSMA molecules/cell in a panel of prostate cancer cell lines and mean IC50 values (pM) of PBD warhead SG2000, ADCT-212, and B12-PL1801 (isotype-control ADC). Data are presented as mean and standard error of the mean (SEM), calculated from 3 independent experiments. BLLQ, below lower limit of quantitation. N/D, not possible to determine.

Figure 3: Internalization and trafficking to lysosomes



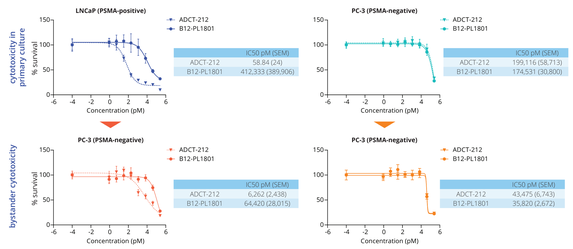
Immunofluorescence images of LNCaP cells stained for nuclei (blue), LAMP-1 (red) and human IgG antibody (green). Solid white arrows show co-localised signal (yellow) of ADCT-212 with LAMP1 protein. Dashed arrows show intracellular ADC (no-co-localisation). Length of ADC incubation with cells at 37°C (T) is expressed in hours.

Figure 4: DNA interstrand cross-linking



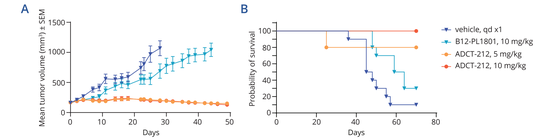
Time course of DNA interstrand cross-link formation following a 2-hour treatment with ADCT-212, B12-PL1801 or SG2000 in LNCaP cells (**A**) or PC-3 cells (**B**). Results are presented as mean % decrease in OTM ± SEM (n=3).

Figure 5: Bystander killing



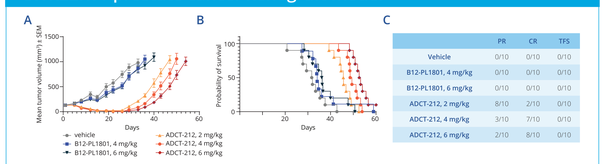
Primary and bystander ADCT-212 cytotoxicity IC50 in LNCaP and PC-3 cultures. Data shown are from one representative experiment. IC50 values are mean of three independent experiments ± SEM.

Figure 6: *In vivo* anti-tumor activity in the PSMA-expressing LNCaP prostate cancer xenograft model



A. ADCT-212 and B12-PL1801 (isotype-control ADC) were administered i.v. (day 0) as single dose to treatment groups of 10 mice. A vehicle-treated group served as control. **B**. Kaplan-Meier analysis of survival.

Figure 7: *In vivo* anti-tumor activity in the PSMA-expressing CWR22Rv1 prostate cancer xenograft model

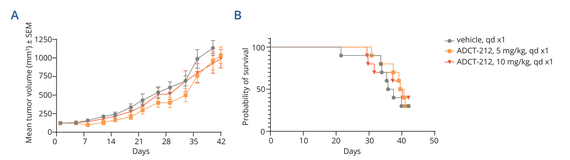


C

	PR	CR	TFS
Vehicle	0/10	0/10	0/10
B12-PL1801, 4 mg/kg	0/10	0/10	0/10
B12-PL1801, 6 mg/kg	0/10	0/10	0/10
ADCT-212, 2 mg/kg	8/10	2/10	0/10
ADCT-212, 4 mg/kg	3/10	7/10	0/10
ADCT-212, 6 mg/kg	2/10	8/10	0/10

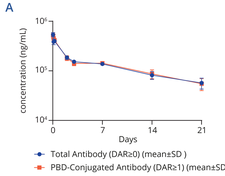
A. ADCT-212 and B12-PL1801 (isotype-control ADC) were administered i.v. (day 1) as single dose to treatment groups of 10 mice. A vehicle-treated group served as control. **B**. Kaplan-Meier analysis of survival. **C**. Response summary. PR, partial response; CR, complete response; TFS, tumor-free survivors.

Figure 8: Lack of anti-tumor activity in the PSMA-negative PC-3 prostate cancer xenograft model



A. ADCT-212 was administered i.v. (day 1) as single dose to treatment groups of 10 mice. A vehicle-treated group served as control. **B**. Kaplan-Meier analysis of survival.

Figure 9: PK analysis in rat



B

	Cmax ng/mL	Tmax hours	Clast hours	Tlast hours	half life hours	half life days
NCA parameters @ 20 mg/kg						
mean total antibody	536946	1	57046	504	292	12.2
mean PBD-conjugated Ab	520570	1	55004	504	289	12.0

PK following a single IV dose (20 mg/kg) of ADCT-212 to rats (n=3/group). **A**. Serum exposure to total (conjugated and unconjugated) Ab and PBD-conjugated Ab determined by ECLIA using anti-human IgG-Fc and/or anti-PBD antibodies (mean ± SD is shown). **B**. PK parameters determined by non-compartmental analysis (NCA).

CONCLUSIONS

- *In vitro*, ADCT-212 demonstrated potent and target-mediated cytotoxicity in a panel of PSMA-positive prostate cancer cell lines.
- ADCT-212 was efficiently internalized by PSMA-expressing LNCaP cells, trafficked to the lysosomes and it produced DNA interstrand cross-links that peaked by 12 hours and persisted for up to 36 hours post-treatment.
- ADCT-212 showed indirect bystander killing activity of PSMA-negative PC-3 cells incubated with conditioned medium from ADCT-212-treated LNCaP cells.
- *In vivo*, ADCT-212 showed strong antitumor activity against CWR22Rv1 and LNCaP prostate cancer xenograft models, while it did not have activity in the PSMA-negative PC-3 xenograft model, highlighting its target-mediated antitumor activity.
- ADCT-212 was tolerated as a single 20 mg/kg dose in male rats, with exposure data being indicative of a linear PK profile with a half-life of approximately 12 days.
- In conclusion, ADCT-212 demonstrated potent and specific *in vitro* and *in vivo* anti-tumor activity while it was stable and well tolerated in the rat, warranting further development of ADCT-212 into the clinic.

ACKNOWLEDGEMENTS

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REFERENCES

1. Wright, G.L., Jr., et al., Upregulation of prostate-specific membrane antigen after androgen-deprivation therapy. Urology, 1996. 48(2): p. 326-34.
2. Tsourlakis, M.C., et al., PSMA expression is highly homogenous in primary prostate cancer. Appl Immunohistochem Mol Morphol, 2015. 23(6): p. 449-55.
3. Rizzo, A., et al., PSMA Radioligand Uptake as a Biomarker of Neoangiogenesis in Solid Tumours: Diagnostic or Theragnostic Factor? Cancers (Basel), 2022. 14(16).
4. Verkade, J.M.M., et al., A Polar Sulfamide Spacer Significantly Enhances the Manufacturability, Stability, and Therapeutic Index of Antibody–Drug Conjugates. Antibodies, 2018. 7(1).