ADCT-602 (hLL2-cys-PBD), a new site-specifically conjugated, pyrrolobenzodiazepine (PBD) dimer-based antibody drug conjugate (ADC) targeting CD22-expressing B-cell malignancies

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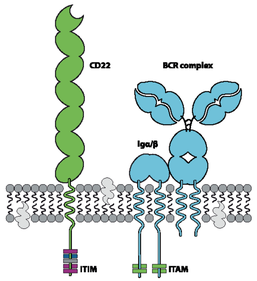
Introduction

1. CD22 is a type I transmembrane sialoglycoprotein, whose expression is restricted to the B-cell lineage [1]. CD22 is also found highly expressed on most malignant mature B cells, including follicular lymphoma (FL), marginal zone lymphoma (MZL), mantle cell lymphoma (MCL), diffuse large B-cell lymphoma (DLBCL), small lymphocytic lymphoma (SLL) and chronic lymphocytic leukemia (CLL) [2, 3]. Moreover, CD22 is expressed in > 90% of cases of B-precursor acute lymphoblastic leukemia (ALL) [4].

Figure 1. CD22 structure



2. The differential and favourable expression profile of CD22 in tumour versus normal tissue, together with its rapid internalization upon binding ligand or antibody [5], make CD22 an attractive target for antibody drug conjugate (ADC)-mediated treatment of B-cell malignancies.
3. ADCT-602 is an ADC composed of an engineered version of the humanized anti-CD22 IgG1 epratuzumab, site-specifically conjugated to the PBD payload tesirine [6] (drug to antibody ratio is 1.7). PBD dimers exert their potent anti-tumor activity by forming highly cytotoxic interstrand cross-links in the DNA minor groove.

Aim of this study

Characterization of the *in vitro* mechanism of action and *in vivo* efficacy, tolerability and pharmacokinetics (PK) of ADCT-602.

Material & Methods

Cytotoxicity of ADCT-602 was determined by the CellTiter® 96 AQueous One Solution Cell Proliferation Assay (MTS) (Promega). Quantitative determination of cell surface CD22 density was done using Bangs Laboratories' Quantum Simply Cellular Anti-Human IgG beads.

The single cell gel electrophoresis (Comet) assay was carried out on Ramos cells treated with ADCT-602 or free warhead. The mean reduction in the product of the tail length and the fraction of total DNA in the tail, i.e. the Olive Tails Moment (OTM) was measured.
Bystander activity was measured via the conditioned media transfer method and cell viability was determined by MTS assay (Promega).

In vivo, ADCT-602 was administered intravenously (i.v.) as single dose to CB.17 SCID mice containing Ramos or WSU-DLCL2 xenografts.

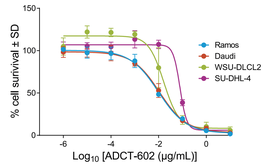
The maximum tolerated dose of ADCT-602 was evaluated in Sprague-Dawley Crl:CD rats and cynomolgus monkeys.

PK analysis of ADCT-602 was performed in cynomolgus monkeys (Mauritian origin). Serum samples were collected for each time point after a single dose administration at 0.6 mg/kg. Total ADCT-602 was measured by ECLIA using biotinylated anti-Fc antibody for capture and a sulfo-TAG labeled anti-Fc antibody for detection. PBD-conjugated ADCT-602 was measured by ECLIA using biotinylated anti-PBD antibody for capture and a sulfo-TAG labeled anti-Fc antibody for detection.

Quantification of platelets and CD20+ cells in cynomolgus monkey was done by flow cytometry.

Results

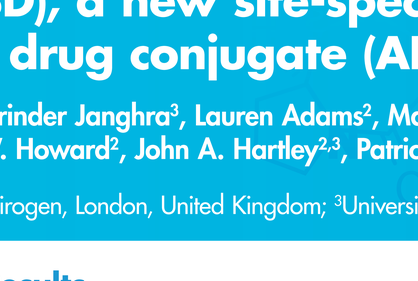
Figure 2: *In vitro* cytotoxicity



	Ramos	Daudi	WSU-DLCL2	SU-DHL-4
Tumor type	BL	BL	DLBCL	GCB-DLBCL
Mean EC_{50} µg/mL	0.010	0.011	0.016	0.078
Mean CD22 molecules/cells (± SEM)	52,604 (± 3,142)	107,264 (± 5,203)	138,300 (± 5,183)	34,957 (± 25,670)

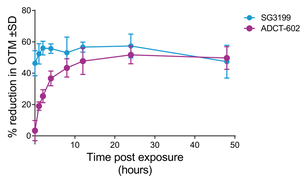
In vitro cytotoxicity of ADCT-602 after 96-hour exposure on four lymphoma cell lines. BL, Burkitt's lymphoma; GCB-DLBCL, Germinal center B-cell-DLBCL; SEM, standard error of the mean.

Figure 3: Internalization



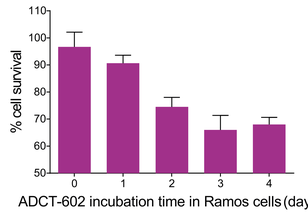
Confocal microscopy images of Ramos cells treated with 2 µg/ml ADCT-602 and stained for nuclei (blue), LAMP-1 (red) and human IgG antibody (green). **A.** Untreated cells, **B.** Immediately following exposure to ADCT-602, **C.** 1 hour post-incubation, **D.** 2 hours post-incubation, **E.** 4 hours post-incubation, **F.** 24 hours post-incubation. Co-staining between LAMP-1 and IgG is observed as yellow and it is indicated by asterisks.

Figure 4: DNA cross-linking



Time course of DNA cross-link formation in Ramos cells exposed to 10 nM ADCT-602 or 10 pM SG3199. Cells were incubated for 2 hours, washed and incubated in fresh medium for a further 24 hours. Data are shown as percentage reduction in OTM relative to untreated Ramos cells. For ADCT-602, the peak of DNA cross-linking occurred at around 12 hours and persisted for 48 hours.

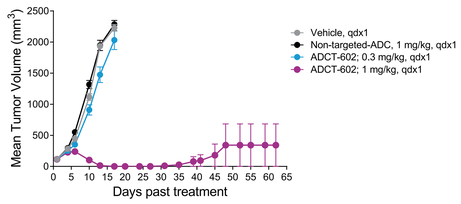
Figure 5: Bystander killing



Histograms depicting percentage of viable CD22-negative Karpas 299 cells after exposure for 0 to 4 days to conditioned medium from Ramos cells treated with ADCT-602.

Figure 6: *In vivo* antitumor efficacy in Ramos xenograft model

A



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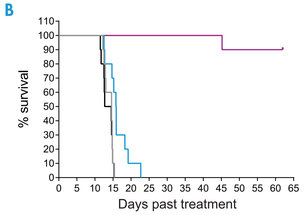


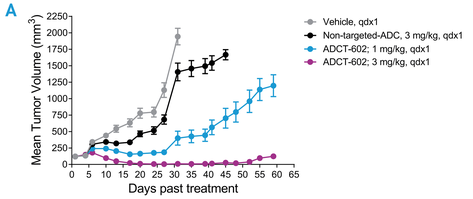
C

	PR	CR	TFS
Vehicle	0	0	0
Non-targeted ADC, 1 mg/kg	0	0	0
ADCT-602, 0.3 mg/kg	0	0	0
ADCT-602, 1 mg/kg	0	10	9

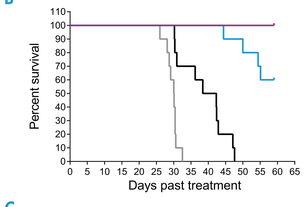
A. ADCT-602 was administered i.v. (day 1) as a single dose at 0.3 or 1 mg/kg to treatment groups of 10 mice. An isotype-control, non-targeted ADC (administered as single dose at 1 mg/kg) and a vehicle (PBS) treated group served as controls.
B. Kaplan-Meier analysis of survival.
C. Response summary. CR, complete response; PR, partial response; TFS, tumor-free survivors.

Figure 7: *In vivo* antitumor efficacy in WSU-DLCL2 xenograft model

A



B



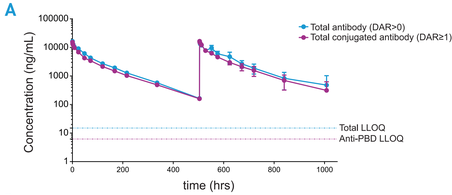
C

	PR	CR	TFS
Vehicle	0	0	0
Non-targeted ADC, 3 mg/kg	0	0	0
ADCT-602, 1 mg/kg	0	0	0
ADCT-602, 3 mg/kg	3	7	2

A. ADCT-602 was administered i.v. (day 1) as a single dose at 1 or 3 mg/kg to treatment groups of 10 mice. An isotype-control, non-targeted ADC (administered as single dose at 3 mg/kg) and a vehicle (PBS) treated group served as controls.
B. Kaplan-Meier analysis of survival.
C. Response summary. CR, complete response; PR, partial response; TFS, tumor-free survivors.

Figure 8: PK analysis in cynomolgus monkey

A

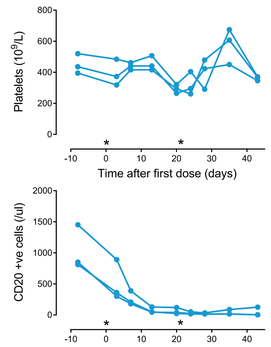


B

	Tmax (hr)	Cmax (ng/ml)	Tlast (hr)	Clast (ng/ml)	Half life (days)
Total ab	0.5	16348.8	504.0	163.3	4
Total conjugated Ab	0.5	15289.0	504.0	159.5	4.5

A. Quantitation of total and PBD-conjugated antibody in cynomolgus monkey serum. The graphs show the mean ± SD (n=3).
B. Tables with PK parameters according to a non-compartimental PK analysis (NCA). Calculations based on the first dose, from t=0 to t=504 hours.

Figure 9: *In vivo* pharmacodynamics



Quantification of **A.** platelets and **B.** CD20+ (B cells) cells in cynomolgus monkey over the course of the tolerability study. Asterisks indicate dosing.

Conclusions

1. ADCT-602 showed potent *in vitro* cytotoxicity in CD22-expressing Ramos, Daudi, SU-DHL-4 and WSU-DLCL2 lymphoma cell lines.
2. ADCT-602 was efficiently internalized into Ramos cells and trafficked to the lysosomes. It induced DNA cross-links, which persisted for at least 48 hours *in vitro*, and it mediated bystander kill of CD22-negative Karpas 299 cells by transfer of conditioned media from Ramos cells treated with ADCT-602.
3. *In vivo*, single doses of ADCT-602 demonstrated remarkable and dose-dependent anti-tumor efficacy in Ramos and WSU-DLCL2 xenografts.
4. A single dose of ADCT-602 at 2 mg/kg or 0.6 mg/kg was well tolerated in rat and cynomolgus monkey, respectively. Importantly, in monkey, ADCT-602 was pharmacologically active (expected rapid B-cell depletion) while it did not induce myelosuppression.
5. PK analysis of ADCT-602 in cynomolgus monkeys showed a half-life of 4 and 4.5 days for total and PBD conjugated ADCT-602, respectively, indicating that ADCT-602 has excellent stability *in vivo*.
6. Together, these data demonstrate the potent *in vitro* and *in vivo* anti-tumor activity of ADCT-602 against CD22-positive hematological tumours and warrants the rapid development of this ADC into the clinic.

Acknowledgements

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