Pre-clinical activity of ADCT-601, a novel pyrrolobenzodiazepine (PBD) dimer-based antibody-drug conjugate (ADC) targeting AXL-expressing tumors

no. 2792A

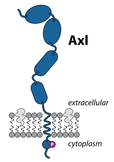
Francesca Zammarchi[1], Karin Havenith[1], Simon Chivers[1], Paul W. Hogg[1], Charlie Britten[1], Sandamali Dissanayake[1], Peter Tyrer[2], Francois Bertelli[2], Ian Hutchinson[2], Luke Masterson[2], Phil Howard[2], John A. Hartley[3], Patrick H. van Berkel[1]

[1]ADC Therapeutics SA, Epalinges, Switzerland, [2]Spirogen, Medimmune, London, United Kingdom; [3]University College London, London, United Kingdom.

Introduction

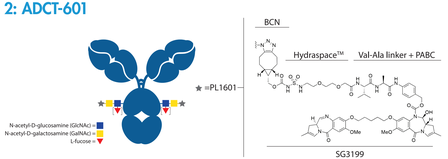
- AXL, a member of the tyrosine kinase receptor family TAM, is a transmembrane receptor containing an extracellular (N-terminal) domain comprising two Ig-like and two fibronectin type III motifs and an intracellular (C-terminal) tyrosine kinase domain (Figure 1). AXL is found overexpressed in many solid (e.g. lung, breast, pancreas, glioma and esophageal)[1] and hematological malignancies (acute myeloid and chronic lymphocytic leukemia)[2] and its overexpression is maintained in both primary tumors and metastasis. Expression and activation of AXL is associated with poor clinical prognosis and several studies suggest that overexpression of AXL results in resistance to conventional chemotherapy and targeted therapies[3]. All these features make AXL an attractive target for the development of an ADC to treat AXL-expressing cancers.

Figure 1: Axl structure



- ADCT-601 is an ADC composed of a humanized IgG1 antibody against human AXL, site-specifically conjugated using GlycoConnect™ technology[4] to PL1601, which contains Hydraspace™, a valine-alanine cleavable linker and the PBD dimer cytotoxin SG3199. The drug to antibody ratio (DARR) is ~2 (Figure 2).

Figure 2: ADCT-601



Aim of this study

The purpose of this study was to characterize the *in vitro* and *in vivo* anti-tumor activity of ADCT-601 in human cancer cell lines and xenograft models and to determine its safety and tolerability in the rat.

Material & Methods

- Cytotoxicity of ADCT-601, the free PBD dimer SG3199 and isotype-control ADC (DAR 1.9; obtained using the same GlycoConnect™ technology and PL1601 payload) was determined by the CellTiterGlo® assays (Promega). Quantitative determination of cell surface AXL density was done using Bangs Laboratories' Quantum Simply Cellular Anti-Human IgG beads.
- *In vivo*, ADCT-601 was administered intravenously (i.v.) as single dose to athymic nude mice containing MDA-MB-231 or PAXF1657 patient-derived xenografts (PDX) and to CB.17 SCID mice containing SN12C or Karpas299 xenografts. The activity of ADCT-601 was compared to that of an isotype control PBD-ADC.
- PK analysis of ADCT-601 was performed in male Sprague-Dawley CD/IS (SD) rats. Serum samples were collected for each time point after a single dose administration (3 or 6 mg/kg). Quantitation of total antibody, AXL antigen binding antibody and PBD-conjugated antibody was performed by ECLIA using a biotinylated anti-human IgG-Fc antibody, an AXL antigen and a biotinylated anti-PBD mouse antibody as a capture, respectively. For both the total and AXL-binding antibody assays, anti-human IgG-Fc-sulfotag conjugated antibody was used for detection, whereas for the PBD-conjugated antibody assay an anti-idiotypic sulfotag-conjugated antibody was used.
- Analysis of AXL expression on FFPE tumor section from PAXF1657 PDX was performed by immunohistochemistry (IHC) using the parental mouse monoclonal anti-human AXL antibody (employed in ADCT-601 in humanized format).

Results

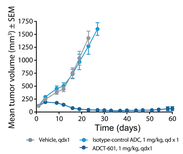
Figure 3: *In vitro* cytotoxicity

	A-431	MDA-MB-157	Panc-1	A-172	SK-LU-1	MDA-MB-231	SK-OV-3	NCI-H1299	SN12C	MDA-MB-468	Karpas 299
Copy number (SEM)	6,600 (± 1876)	11,000 (± 4654)	20,000 (± 8340)	23,000 (± 3910)	24,000 (± 3996)	36,000 (± 6257)	46,000 (± 8488)	79,000 (± 9276)	88,000 (± 6882)	BLLQ	BLLQ
ADCT-601 IC_{50} nM (±SEM)	18.64 (±0.6)	64.99 (±5.62)	0.47 (±0.05)	0.59 (±0.08)	0.02 (±0)	0.35 (±0.06)	0.11 (±0.02)	2.2 (±0.05)	0.83 (±0.12)	9.29 (±0.12)	14.62 (±0.22)
Isotype-control ADC IC_{50} nM (±SEM)	15.02 (±0.96)	N/A	54.05 (±6.23)	37.26 (±3.13)	3.67 (±0.19)	14.19 (±0.56)	79.89 (±1.82)	N/A	12.29 (±0.47)	4.17 (±0.04)	11.59 (±0.27)
SG3199 IC_{50} pM (±SEM)	16.73 (±2.41)	239.8 (±12.15)	60.6 (±3.83)	22.79 (±1.61)	19.06 (±1.37)	110.5 (±10.24)	62.59 (±6.36)	88.68 (±7.58)	57.63 (±5.27)	174.1 (±29.09)	9.18 (±0.45)

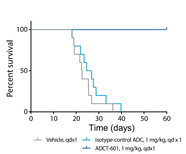
Mean AXL molecules/cell in a panel of solid tumor cell lines and mean IC_{50} values of ADCT-601, isotype-control ADC, and PBD warhead SG3199. Data are presented as mean and standard error of the mean, calculated from 3 independent experiments. BLLQ, below lower limit of quantitation; SEM, standard error of the mean; N/A, not applicable.

Figure 4: *In vivo* anti-tumor activity in the MDA-MB-231 TNBC xenograft

A



B



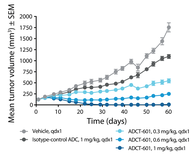
C

	PR	CR	TFS
Vehicle	0	0	0
Isotype-control ADC, 1 mg/kg, qdx1	0	0	0
ADCT-601, 1 mg/kg, qdx1	5	4	4

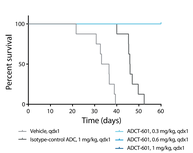
A. ADCT-601 and isotype-control ADC were administered i.v. (day 1) to treatment groups of 10 mice. A vehicle-treated group served as control. **B.** Kaplan-Meier analysis of survival. **C.** Response summary. *PR*, partial response; *CR*, complete response; *TFS*, tumor-free survivors. *TNBC*, triple-negative breast cancer.

Figure 5: *In vivo* anti-tumor activity in the SN12C renal cancer xenograft

A



B



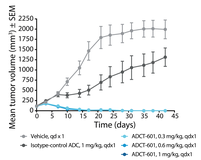
C

	PR	CR	TFS
Vehicle	0	0	0
Isotype-control ADC, 1 mg/kg, qdx1	0	0	0
ADCT-601, 0.3 mg/kg, qdx1	0	0	0
ADCT-601, 0.6 mg/kg, qdx1	2	0	0
ADCT-601, 1 mg/kg, qdx1	1	7	6

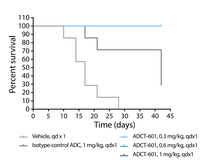
A. ADCT-601 and isotype-control ADC were administered i.v. (day 1) to treatment groups of 8 mice. A vehicle-treated group served as control. **B.** Kaplan-Meier analysis of survival. **C.** Response summary. *PR*, partial response; *CR*, complete response; *TFS*, tumor-free survivors.

Figure 6: *In vivo* anti-tumor activity in PAXF1657 pancreatic cancer PDX

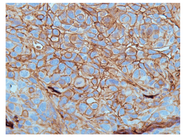
A



B



C



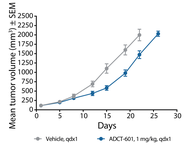
D

	PR	CR	TFS
Vehicle	0	0	0
Isotype-control ADC, 1 mg/kg, qdx1	0	0	0
ADCT-601, 0.3 mg/kg, qdx1	0	8	8
ADCT-601, 0.6 mg/kg, qdx1	0	8	8

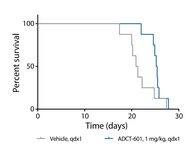
A. ADCT-601 and isotype-control ADC were administered i.v. (day 1) to treatment groups of either 7 or 8 mice. A vehicle-treated group served as control. **B.** Kaplan-Meier analysis of survival. **C.** Representative scan of FFPE PAXF1657 tumor section stained for AXL by IHC. **D.** Response summary. *PR*, partial response; *CR*, complete response; *TFS*, tumor-free survivors.

Figure 7: *In vivo* anti-tumor activity in the AXL-negative Karpas299 ALCL xenograft

A



B



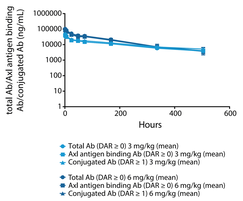
C

	PR	CR	TFS
Vehicle	0	0	0
ADCT-601, 1 mg/kg, qdx1	0	0	0

A. ADCT-601 was administered i.v. (day 1) to treatment groups of 8 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. *ALCL:* anaplastic large cell lymphoma.

Figure 8: PK analysis in rat

A



B

NCA parameters @ 3 mg/kg	Cmax ng/mL	Tmax hours	Clast ng/mL	Tlast hours	half life hours	half life days
Mean total Ab	45857.8	1.0	3832.2	504.0	210.5	8.8
Mean AXL antigen binding Ab	40555.4	1.7	4068.3	504.0	220.4	9.2
Mean conjugated Ab	49445.9	1.0	5237.5	504.0	228.5	9.5

NCA parameters @ 6 mg/kg	Cmax ng/mL	Tmax hours	Clast ng/mL	Tlast hours	half life hours	half life days
Mean total Ab	103072.0	1.0	4185.3	504.0	136.3	5.7
Mean AXL antigen binding Ab	91849.0	3.3	4343.0	504.0	139.8	5.8
Mean conjugated Ab	93354.9	1.0	3771.4	504.0	137.5	5.7

A. Quantification of total conjugated and unconjugated Ab, AXL antigen binding Ab and PBD-conjugated Ab after administration of a single dose of ADCT-601 (3 or 6 mg/kg). The graph shows the mean ± SD (n=3/group) for the whole duration of the study (504 hours). **B.** Tables with PK parameters according to a non-compartmental PK analysis (NCA) for both treatment groups (3 and 6 mg/kg).

Conclusions

1. ADCT-601 showed potent and highly targeted *in vitro* cytotoxicity in a panel of AXL-expressing solid cancer cell lines.
2. *In vivo*, single, low doses of ADCT-601 demonstrated potent and durable anti-tumor efficacy in breast and renal cancer-derived xenografts, while ADCT-601 did not have a significant anti-tumor activity in an AXL-negative xenograft model.
3. A single, low dose of 0.3 mg/kg ADCT-601 provided 8 out of 8 TFS in an AXL-expressing pancreatic PDX model.
4. PK analysis of ADCT-601 in non-tumor bearing rats showed that ADCT-601 has excellent stability *in vivo*, with a half-life of about 9.5 days at 3 mg/kg and of about 6 days at the maximum tolerated dose of 6 mg/kg.
5. Together, these data demonstrate that ADCT-601 has a favorable therapeutic index and this warrants further development of ADCT-601 for the treatment of AXL-expressing tumors.

Acknowledgements

Chemistry and manufacturing: Synaffix BV (The Netherlands) and ADC Biotechnology (UK). *In vivo* studies: Charles River Discovery Research Services (USA and Germany GmbH). IHC: Narinder Janghra (UCL, UK).

References

1. Li Y, Ye X, Tan C, et al. Axl as a potential therapeutic target in cancer: role of Axl in tumor growth, metastasis and angiogenesis. Oncogene. 2009;28(39):3442-3455.
2. Ben-Batalla I, Schultze A, Wroblewski M, et al. Axl, a prognostic and therapeutic target in acute myeloid leukemia mediates paracrine crosstalk of leukemia cells with bone marrow stroma. Blood. 2013;122(14):2443-2452.
3. Wu X, Liu X, Koul S, Lee CY, Zhang Z, Halmos B. AXL kinase as a novel target for cancer therapy. Oncotarget. 2014;5(20):9546-9563.
4. van Geel R, Wijdeven MA, Heesbeen R, et al. Chemoenzymatic Conjugation of Toxic Payloads to the Globally Conserved N-Glycan of Native mAbs Provides Homogeneous and Highly Efficacious Antibody-Drug Conjugates. Bioconjug Chem. 2015;26(11):2233-2242.