ADCT-701, a novel pyrrolobenzodiazepine (PBD) dimer-based antibody-drug conjugate (ADC) targeting DLK1-expressing tumors

no. 744

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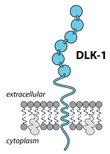
Introduction

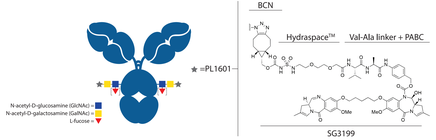
- Delta-like 1 homolog protein (DLK-1) is an EGF-like membrane bound protein consisting of six tandem EGF-like repeats, a juxtamembrane region with a TACE (ADAM17)-mediated cleavage site, a transmembrane domain, and a short intracellular tail[1] (Figure 1).

Figure 1: DLK-1 structure



- DLK-1 is strongly expressed during fetal development, while its expression is highly restricted in adults[2]. Conversely, DLK-1 gets re-expressed in several tumors, such as neuroblastoma, hepatocellular carcinoma (HCC), rhabdomyosarcoma, small cell lung cancer, myelodysplastic syndrome and acute myeloid leukemia[3,4]. Interestingly, in HCC DLK-1 has been shown to be a marker of cancer stem cells, a subpopulation of cells responsible for tumor initiation, growth, metastasis, and recurrence[5].
- Altogether, DLK-1 represents an attractive target for an antibody-drug conjugate (ADC) approach based on its selective expression in a wide range of malignancies and restricted expression in healthy organs, as well as its expression on HCC cancer stem cells.
- ADCT-701 is an ADC composed of a humanized IgG1 antibody against human DLK-1, site-specifically conjugated using GlycoConnect™ technology[6] to PL1601, which contains Hydraspace™, a valine-alanine cleavable linker and the PBD dimer cytotoxin SG3199. The drug to antibody ratio (DAR) is 1.9 (Figure 2).

Figure 2: ADCT-701



Aim of this study

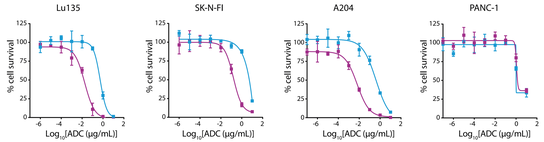
The purpose of this study was to characterize the *in vitro* and *in vivo* anti-tumor activity of ADCT-701 in human cancer cell lines and patient-derived xenograft (PDX) models and to determine its safety and tolerability in the rat.

Material & Methods

- *In vitro* cytotoxicity of ADCT-701 and isotype-control ADC (DAR 1.9; obtained using the same GlycoConnect™ technology and PL1601 payload) was determined by the CellTiterGlo® assays (Promega).
- *In vivo*, ADCT-701 was administered intravenously (i.v.) as single dose to NOD-SCID mice containing SK-N-FI and to BALB/c nude mice containing LI1097 patient-derived xenografts (PDX). The activity of ADCT-701 was compared to that an isotype control PBD ADC.
- PK analysis of ADCT-701 was performed in female Wistar Han IGS: Crl: WI (Han) rats. Serum samples were collected for each time point after a single dose administration (5 mg/kg in rat). Quantitation of total antibody, DLK1 antigen binding antibody and PBD-conjugated antibody was performed by ECLIA using a biotinylated anti-human IgG-Fc antibody, a DLK1 antigen and a biotinylated anti-PBD mouse antibody as a capture, respectively. For both the total and DLK1 antigen 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 DLK1-expression on FFPE tumor section from LI1097 PDX was performed by immunohistochemistry (IHC) using a proprietary mouse monoclonal anti-human DLK-1 antibody.

Results

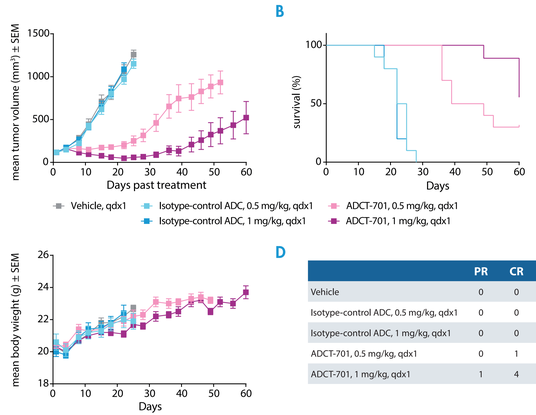
Figure 3: *In vitro* targeted cytotoxicity



	Lu135	SK-N-FI	A204	PANC-1
DLK-1 status	+	+	+	-
Tumor type	SCLC	NB	Rhab	PaC
Mean ADCT-701 (IC_{50} [µg/mL])	0.018	0.16	0.0064	N/A
Mean Isotype-control ADC (IC_{50} [µg/mL])	0.53	N/A	0.41	N/A

In vitro cytotoxicity (IC_{50}) of ADCT-701 and isotype-control ADC on a panel of four cell lines. *SCLC*, small cell lung cancer; *NB*, neuroblastoma; *Rhab*, Rhabdomyosarcoma; *PaC*, pancreatic cancer.

Figure 4: *In vivo* anti-tumor activity in the SK-N-FI neuroblastoma xenograft



D

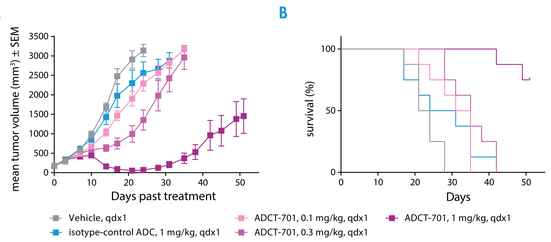
	PR	CR	TFS
Vehicle	0	0	0
Isotype-control ADC, 0.5 mg/kg, qdx1	0	0	0
Isotype-control ADC, 1 mg/kg, qdx1	0	0	0
ADCT-701, 0.5 mg/kg, qdx1	0	1	0
ADCT-701, 1 mg/kg, qdx1	1	4	1

A. ADCT-701 and isotype-control ADC were administered i.v. (day 1) to treatment groups of 9 or 10 mice. A vehicle-treated group served as control.

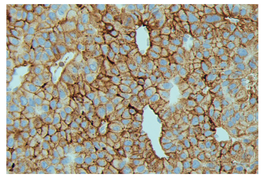
B. Kaplan-Meier analysis of survival.

C. Mean body-weights. D. Response summary. *PR*, partial response; *CR*, complete response; *TFS*, tumor-free survivors.

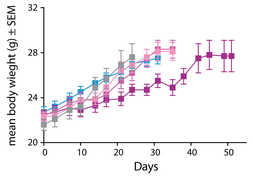
Figure 5: *In vivo* anti-tumor activity in LI1097 hepatocellular cancer PDX



C



D



E

	PR	CR	TFS
Vehicle	0	0	0
Isotype-control ADC, 1 mg/kg, qdx1	0	0	0
ADCT-701, 0.1 mg/kg, qdx1	0	0	0
ADCT-701, 0.3 mg/kg, qdx1	0	0	0
ADCT-701, 1 mg/kg, qdx1	3	2	0

A. ADCT-701 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.

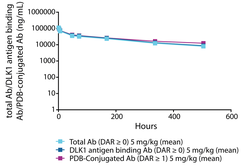
C. Representative scan of FFPE LI1097 tumor section stained for DLK-1 by IHC.

D. Mean body-weights.

E. Response summary. *PR*, partial response; *CR*, complete response; *TFS*, tumor-free survivors.

Figure 6: PK analysis in the rat

A



B

	NCA parameters					
	Cmax ng/mL	Tmax hours	Clast ng/mL	Tlast hours	half life hours	half life days
total Ab	112075.2	1.0	7980.9	504.0	208.9	8.7
PBD-conjugated Ab	104255.6	1.0	12515.8	504.0	279.1	11.6
DLK1 antigen binding Ab	111714.0	1.0	8806.3	504.0	209.4	8.7

A. Quantification of total (conjugated and unconjugated) Ab, DLK1 antigen binding Ab and PBD-conjugated Ab obtained via an anti-human IgG-Fc antibody, antigen DLK1 or anti-PBD antibody, respectively. The graph shows the mean ± SD (n=3/group) for the whole duration of the study (504 hours).

B. Table with PK parameters according to a non-compartmental PK analysis (NCA).

Conclusions

1. ADCT-701 showed potent and targeted *in vitro* cytotoxicity in a panel of solid cancer cell lines.
2. *In vivo*, single low-doses of ADCT-701 (0.5 – 1.0 mg/kg) demonstrated potent anti-tumor efficacy in the DLK1-expressing, neuroblastoma-derived SK-N-FI xenograft and in the HCC PDX model LI1097.
3. A single low dose of ADCT-701 at 5 mg/kg was well tolerated in rats. PK analysis of ADCT-701 in non-tumor bearing rats showed that ADCT-701 has excellent stability, with a half-life of 11 days.
4. Together, these data demonstrate that ADCT-701 has a favorable therapeutic index and this warrants further development of ADCT-701 for the treatment of DLK1-expressing tumors.

Acknowledgements

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