Pre-clinical characterization of 3A4-PL1601, a novel pyrrolobenzodiazepine (PBD) dimer-based antibody-drug conjugate (ADC) directed against KAAG1-expressing malignancies

Francesca Zammarchi[1], Francois Bertelli[2], Karin Havenith[1], Ian Kirby[1], Simon Chivers[1], Patrick H. van Berkel[1]

[1]ADC Therapeutics (UK) Ltd, London, United Kingdom, [2]Spirogen, Medimmune, London, United Kingdom

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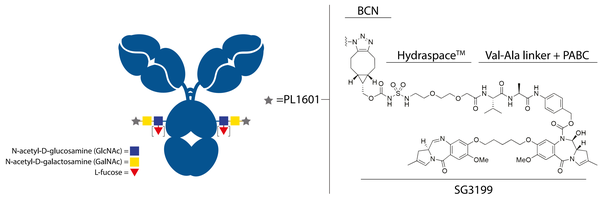
ADC THERAPEUTICS

Introduction

- Kidney-associated antigen 1 (KAAG1) is an 84 amino acid protein encoded by the reverse strand of a housekeeping gene called DCDC2 which was identified from a cDNA library derived from a histocompatibility leukocyte antigen-B7 renal carcinoma cell line as an antigenic peptide presented to cytotoxic T lymphocytes[1]. More recently, KAAG1 has been identified by a sensitive subtractive cloning technology called STAR as a novel tumor-associated antigen expressed in a high percentage of ovarian tumors, triple-negative breast cancers (TNBCs) and castration-resistant prostate cancer, while it has restricted normal tissue expression[2]. All these features make KAAG1 an attractive target for the development of an ADC to treat KAAG1-expressing cancers.
- 3A4-PL1601 is an ADC composed of a 3A4, humanized IgG1 antibody against human KAAG1, site-specifically conjugated using Glycoconnect™ technology[3] to PL1601, which contains Hydraspace™, a valine-alanine cleavable linker and the PBD dimer cytotoxin SG3199. The drug to antibody ratio (DAR) is ~2 (Figure 1).

Figure 1: 3A4-PL1601



Aim of this study

The purpose of this study was to characterize the *in vitro* and *in vivo* anti-tumor activity of 3A4-PL1601 in human cancer cell lines and xenografts models and to determine its pharmacokinetics (PK) and tolerability in the rat and cynomolgus monkey.

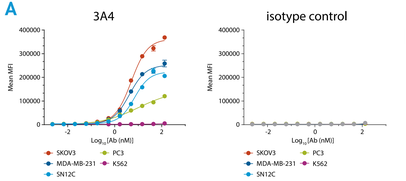
Material & Methods

- Antibodies titration cell binding experiments were carried out by standard flow cytometry. Cytotoxicity of 3A4-PL1601 and an isotype-control ADC (DAR 1.9; obtained using the same Glycoconnect™ technology and PL1601 payload) was determined by the CellTiterGlo® assays (Promega).
- *In vivo*, 3A4-PL1601 was administered intravenously (i.v.) as single dose to athymic nude mice containing MDA-MB-231 and to CB.17 SCID mice containing SN12C xenograft. The activity of 3A4-PL1601 was compared to that of an isotype control PBD-ADC.
- PK analysis of 3A4-PL1601 was performed in male Sprague-Dawley Crl:CD(SD) rats as well as in Mauritian cynomolgus monkeys. Serum samples were collected for each time point after a single dose administration (1 mg/kg and 0.8 mg/kg in rat and cynomolgus monkeys, respectively). For both PK studies, quantitation of total antibody was performed by ECLIA assay using a biotinylated anti-human IgG-Fc antibody as a capture and an anti-human IgG-Fc-sulfotag conjugated antibody for detection.

Results

Figure 2: Cell binding and targeted *in vitro* cytotoxicity

A



	3A4 -Top MFI	3A4 - EC_{50} (nM)
SKOV3	361,709	5.1
MDA-MB-231	252,337	4.3
SN12C	223,642	6.9
PC3	145,382	10.2
K562	N/A	N/A

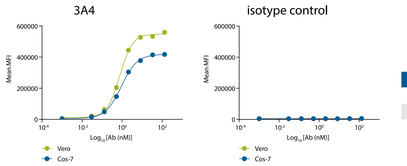
B

	SKOV3	MDA-MB-231	SN12C	PC3	K562
KAAG1 status	+++	++	++	+	-
tumor type	ovarian	TNBC	renal	prostate	T-NHL
Mean 3A4-PL1601 IC_{50} (µg/mL)	0.52	0.44	10	2.4	21
Mean isotype-PL1601 IC_{50} (µg/mL)	3.6	7.4	21,485	1,108	3.1

A. Binding of 3A4 and isotype control antibodies to a panel of cancer cell lines,

B. *In vitro* cytotoxicity of 3A4-PL1601 and isotype-control ADC on same panel of cell lines tested above. TNBC, triple-negative breast cancer; T-NHL, T-non-Hodgkin lymphoma. Data are presented as mean calculated from 2 independent experiments. MFI, mean fluorescence intensity.

Figure 3: Binding to monkey KAAG1



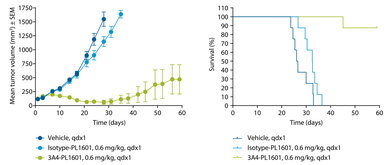
	3A4 -Top MFI	3A4 - EC_{50} (nM)
Vero	548,759	0.8
Cos-7	418,500	0.9

Binding of 3A4 and isotype control antibodies to monkey cell lines Cos-7 and Vero.

MFI, mean fluorescence intensity.

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-PL1601, 0.6 mg/kg, qd x1	0	0	0
3A4-PL1601, 0.6 mg/kg, qd x1	4	3	2

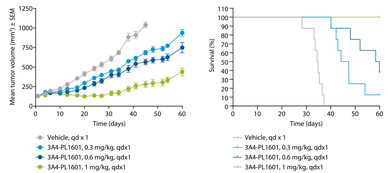
A. 3A4-PL1601 and isotype-control ADC were administered i.v. (day 1) to treatment groups of 8 mice. A vehicle-treated group served as control.

B. Kaplan-Meier analysis of survival.

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

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

A B



C

	PR	CR	TFS
Vehicle	0	0	0
3A4-PL1601, 0.3 mg/kg, qd x1	0	0	0
3A4-PL1601, 0.6 mg/kg, qd x1	0	0	0
3A4-PL1601, 0.3 mg/kg, qd x1	2	0	0

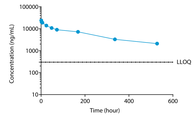
A. 3A4-PL1601 was administered i.v. (day 1) to treatment groups of 8 mice. A vehicle-treated group served as control.

B. Kaplan-Meier analysis of survival.

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

Figure 6: PK analysis in rat at 1 mg/kg (MTD dose)

A



B

NCA parameters - total antibody						
	Cmax ng/mL	Tmax hours	Clast ng/mL	Tlast hours	half life hours	half life days
Mean total Ab	23821	1	2100	504.0	192.9	8

A. Quantification of total conjugated and unconjugated Ab after administration of a single dose of 3A4-PL1601 (1 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).

Figure 7: PK analysis in cynomolgus monkey at 0.8 mg/kg (MTD dose)

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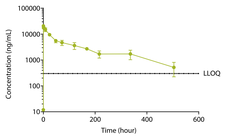

B

NCA parameters - total antibody						
	Cmax ng/mL	Tmax hours	Clast ng/mL	Tlast hours	half life hours	half life days
Mean total Ab	20685	0.5	517.6	504.0	138.2	5.8

A. Quantification of total conjugated and unconjugated Ab after administration of a single dose of 3A4-PL1601 (0.8 mg/kg). The graph shows the mean ± SD (n=3/group) up to 504 hours.

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

Conclusions

- 3A4-PL1601 showed potent and highly targeted *in vitro* cytotoxicity in a panel of KAAG1-expressing solid cancer cell lines while its activity was reduced in a KAAG1-negative cell line.
- *In vivo*, single, low-doses of 3A4-PL1601 demonstrated potent and durable anti-tumor efficacy in breast and renal cancer derived xenografts.
- In the rat (non cross-reactive species), 3A4-PL1601 had a maximum tolerated dose (MTD) of 1 mg/kg, with a 'normal' IgG1 ADC pharmacokinetic profile (t1/2 ~ 8 days).
- In the cynomolgus monkey (cross-reactive species), 3A4-PL1601 had a MTD of 0.8 mg/kg and it showed a favorable pharmacokinetic profile (t1/2 ~ 6 days).
- Together, these data demonstrate that
  - 3A4-PL1601 has a favorable therapeutic index in pre-clinical models
  - KAAG1 is a suitable target for the development of a PBD-based ADC to treat KAAG1-expressing tumors.

Acknowledgements

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In vivo studies: Charles River Discovery Research Services (USA). PK assays: ADC Therapeutics PK team (London, UK).

References

1. Van Den Eynde BJ, Gaugler B, Probst-Kepper M, et al. *A new antigen recognized by cytolytic T lymphocytes on a human kidney tumor results from reverse strand transcription.* J Exp Med. 1999;190(12):1793-1800.
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