Preclinical development of ADCT-211, a novel pyrrolobenzodiazepine dimer-based antibody-drug conjugate targeting solid tumors expressing IL13RA2

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INTRODUCTION

Interleukin 13 receptor subunit alpha 2 (IL13RA2) is one of the two major receptors for the cytokine interleukin 13 (IL-13).

It contains an unusual top-mounted S-type Ig fold, followed by two fibronectin type III-like domains and a WSXWS box that make up the prototypical "cytokine-binding homology region" (CHR) [1] (**Figure 1**).

While IL13RA1, the other main IL-13 receptor, has low affinity for IL-13 and it is expressed ubiquitously in humans, IL13RA2 has high binding affinity to IL-13 and its expression in normal tissues is mainly restricted to the testes.

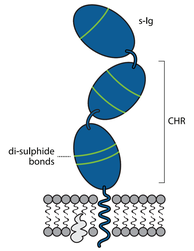


Figure 1. IL13RA2 subunit.

IL13RA2 is reported to be expressed at a high frequency in glioblastoma multiforme (GBM) as well as in other solid tumors including malignant melanoma, renal cell carcinoma and adrenocortical carcinoma and is correlated with poor prognosis [2].

ADCT-211 is an antibody-drug conjugate (ADC) composed of HuCl47, a humanized IgG1 antibody of clone 47 [3], directed against human IL13RA2, site-specifically conjugated using GlycoConnect™ technology [4] to PL1801, which contains Hydraspace™, a valine-alanine cleavable linker and the PBD dimer cytotoxin SG2000 (drug to antibody ratio ~ 1.8) (**Figure 2**).

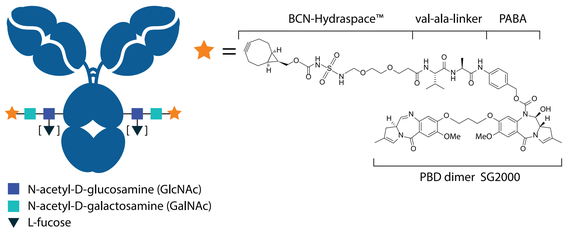


Figure 2. ADCT-211.

AIM OF THE STUDY

The aim of this study was to characterize the *in vitro* and *in vivo* anti-tumor activity of ADCT-211 in human cancer cell lines and xenograft models, to determine its safety, tolerability and pharmacokinetics (PK) in the rat and to measure IL13RA2 expression by immunohistochemistry (IHC) in human tumor specimens of GBM and malignant melanoma.

MATERIALS AND METHODS

Cytotoxicity of ADCT-211 and an isotype-control ADC was determined by the CellTiterGlo® assays (Promega).

Binding of ADCT-211 and HuCl47 to human recombinant IL13RA1 and IL13RA2 was tested by ELISA.

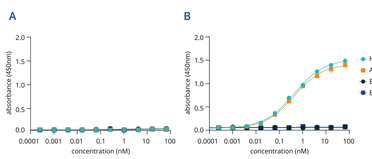
In vivo, ADCT-211 was administered intravenously (i.v.) as single dose to athymic nude mice containing A375 or U251 s.c. xenografts.

PK analysis of ADCT-211 was performed in male Crl:CD(SD) rats. Quantitation of total antibody or PBD-conjugated antibody was performed by ECLIA.

Analysis of IL13RA2 expression was performed by IHC using a commercial monoclonal anti-human IL13RA2 antibody.

RESULTS

Figure 3. HuCl47 and ADCT-211 bind specifically to IL13RA2



	IL13RA2 EC50 nM (SD)
HuCl47	0.487 (0.0422)
ADCT-211	0.432 (0.138)

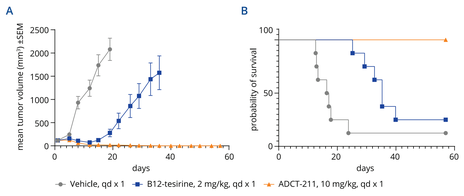
Binding of HuCl47 and ADCT-211 to recombinant human IL13RA1 (**A**) and IL13RA2 (**B**).

Table 1. ADCT-211 *in vitro* cytotoxicity

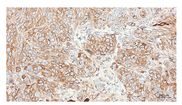
	RPMI-7951	Daoy	SK-N-AS	B-CPAP	UM-UC-3	A375
IL13RA2 status	+/-	+	++	++	+++	+++
Tumor type	Melanoma	Medulloblastoma	Neuroblastoma	Thyroid carcinoma	Bladder carcinoma	Melanoma
ADCT-211 IC50 pM	17,158	34.85	23.8	27.01	35	80
B12-PL1801 IC50 pM	45,198	27,097	33,400	100,717	37,068	ND

In vitro cytotoxicity (IC50) of ADCT-211 and isotype-control ADC (B12-PL1801) on a panel of six cancer cell lines.

Figure 3: *In vivo* anti-tumor activity in the A375 melanoma xenograft model

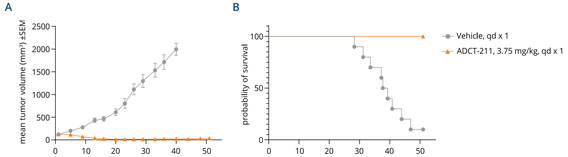


	PR	CR	TFS
Vehicle	0/8	1/8	1/8
B12-tesirine, 2 mg/kg, qdx1	0/8	2/8	2/8
ADCT-211, 10 mg/kg, qdx1	0/8	8/8	8/8

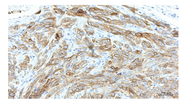


A. ADCT-211 and isotype-control ADC (B12-tesirine) were administered i.v. (day 1) to treatment groups of 8 mice. A vehicle-treated group served as control. **B.** Kaplan-Meier analysis of survival. **C.** Response summary. PR, partial response; CR, complete response; TFS, tumor-free survivors. **D.** Representative scan of FFPE A375 tumor section stained for IL13RA2 by IHC.

Figure 4: *In vivo* anti-tumor activity in the U251 glioblastoma

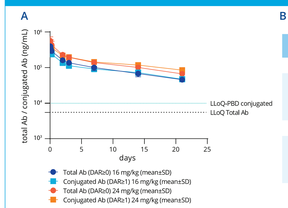


	PR	CR	TFS
Vehicle	0/10	1/10	1/10
ADCT-211, 3.75 mg/kg, qdx1	5/10	5/10	2/10



A. ADCT-211 was administered i.v. (day 1) to treatment groups of 10 mice. A vehicle-treated group served as control. An isotype-control ADC tested in the same model in a separate study resulted in only marginal anti-tumor activity (data not shown). **B.** Kaplan-Meier analysis of survival. **C.** Response summary. PR, partial response; CR, complete response; TFS, tumor-free survivors. **D.** Representative scan of FFPE U251 tumor section stained for IL13RA2 by IHC.

Figure 5: PK analysis in rat

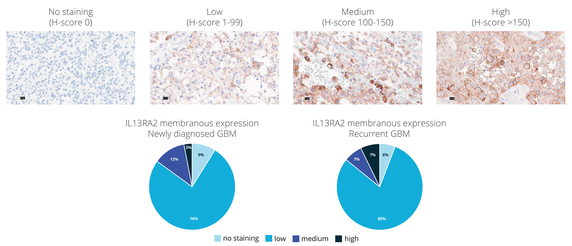


B

	Cmax ng/mL	Tmax hours	Clast hours	Tlast hours	half life hours	half life days
NCA parameters @ 16 mg/kg						
mean total antibody	395630	1	45625	504	302	12.6
mean PBD-conjugated Ab	344223	1	46997	504	378	15.7
NCA parameters @ 24 mg/kg						
mean total antibody	564216	1	66286	504	323	13.4
mean PBD-conjugated Ab	490473	1	84753	504	445	18.5

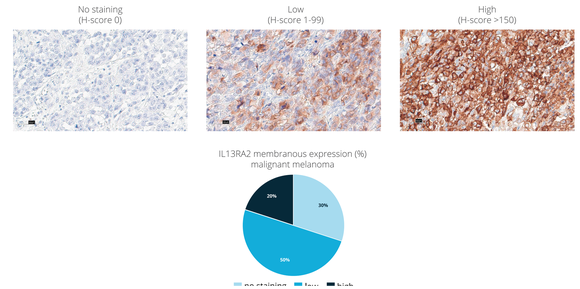
PK following a single IV dose of ADCT-211 to rats (n=3/group). **A.** Serum exposure to total (conjugated and unconjugated) Ab and PBD-conjugated Ab determined by ECLIA using anti-human IgG-Fc and/or anti-PBD antibodies (mean ± SD is shown). **B.** PK parameters determined by non-compartmental analysis (NCA).

Figure 6A. IL13RA2 membranous expression in a panel of primary and refractory GBM patient samples



Representative images of IL13RA2 IHC staining across the four H-scores measured. Pie charts indicate % positivity for each H-score across 34 primary GBM and 15 recurrent GBM patients.

Figure 6B. IL13RA2 membranous expression in a panel of malignant melanoma patient samples



Representative images of IL13RA2 IHC staining across the three H-score measured. Pie chart indicates % positivity for each H-score across 10 malignant melanoma patients.

CONCLUSIONS

ADCT-211 showed potent *in vitro* cytotoxicity in a panel of IL13RA2-expressing solid cancer cell lines.

In vivo, single doses of ADCT-211 demonstrated potent and durable anti-tumor efficacy in melanoma and GBM derived xenografts.

In an MTD study in male rats, ADCT-211 was stable and tolerated up to 24 mg/kg single dose, with exposure data being indicative of a stable ADC and linear PK profile with a half-life of 12-19 days.

Membranous expression of IL13RA2 was confirmed by IHC in a high proportion of primary and refractory GBM samples as well as malignant melanoma.

In conclusion, ADCT-211 demonstrated potent and specific *in vitro* and *in vivo* anti-tumor activity and it was stable and well tolerated in the rat, warranting further development of ADCT-211 into the clinic in IL13RA2-expressing cancers, like GBM and malignant melanoma.

ACKNOWLEDGEMENTS

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