Preclinical Development of MGC018, a Duocarmycin-based Antibody-drug Conjugate Targeting B7-H3 for Solid Cancer

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820

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Abstract

Introduction: B7-H3, a member of the B7 family of immunomodulatory molecules, is overexpressed in a wide range of solid tumors; tumor overexpression has been correlated with disease severity and poor outcome in several cancer types. MGC018 is an antibody-drug conjugate (ADC) targeted against B7-H3 and comprised of the cleavable linker-duocarmycin payload, valine-citrulline-*seco* DUocarmycin hydroxyBenzamide Azaindole (vc-*seco*-DUBA), conjugated to an anti-B7-H3 humanized IgG1 kappa monoclonal antibody through reduced interchain disulfides, with an average drug-to-antibody ratio of ~2.7. Previous studies indicated MGC018 exhibited a favorable preclinical profile, with strong reactivity toward tumor cells and tumor-associated vasculature, limited normal tissue reactivity, potent cytotoxicity in vitro and antitumor activity in vivo toward a range of B7-H3-expressing tumor cell lines representing several cancer types. Based on these preliminary results, expanded preclinical development of MGC018 was undertaken to support clinical development.

Methods: vc-*seco*-DUBA conjugation to obtain MGC018 ADC was performed by Synthon Biopharmaceuticals B.V. Single- and repeat-dose in vivo efficacy studies were conducted in CD-1 nude mice with human tumor xenografts that express B7-H3 to explore the relationship between C_{max}, exposure and antitumor activity, and to define the minimal efficacious dose in these models. A GLP toxicology study was conducted in cynomolgus monkeys in which MGC018 was administered at dose levels of 1, 3, 6, and 10 mg/kg every 3 weeks for a total of 3 doses.

Results: MGC018 demonstrated specific, dose-dependent in vivo antitumor activity toward B7-H3-positive tumor xenografts representing breast, lung and ovarian cancers, and melanoma. Fractionated MGC018 dose studies were consistent with antitumor activity driven by the total exposure (AUC) rather than peak drug exposure (C_{max}). MGC018 was tolerated in cynomolgus monkeys at all dose levels tested, with 10 mg/kg, the highest dose administered, defined as the highest non-severely toxic dose (HNSTD).

Conclusion: MGC018, a preclinical candidate comprised of a humanized mAb targeting B7-H3, conjugated to the potent DNA alkylating payload DUBA via a cleavable peptide linker, exhibited a favorable preclinical profile. MGC018 demonstrated potent antitumor activity in vivo toward B7-H3-expressing tumor xenografts at clinically relevant dose levels. MGC018 was tolerated in cynomolgus monkeys, a relevant toxicology model, at exposure levels in excess of those required for antitumor activity. Our findings support the clinical development of MGC018 to evaluate its potential as an ADC therapeutic for B7-H3-expressing solid cancers.

Background

B7-H3: An Attractive Molecule for Targeted Therapy

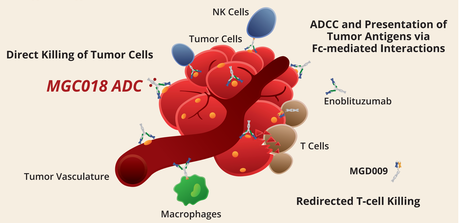
- **B7-H3: Cell-surface molecule for targeted therapy**
  - Member of the B7 family of immune regulators
  - Overexpressed on solid cancers, with high tumor-versus-normal tissue binding differential
  - Overexpression correlated with disease severity and poor outcome in multiple cancers
- **MacroGenics is targeting B7-H3 by 3 modalities**
  - Enoblituzumab (MGA271)[1]: a humanized Fc-enhanced mAb with enhanced ADCC
  - B7-H3 x CD3 DART® molecule (MGD009): a humanized Fc-bearing bispecific DART molecule for redirected T-cell killing
  - MGC018: A humanized anti-B7-H3 antibody conjugated to the vc-*seco*-DUocarmycin-hydroxyBenzamide-Azaindole (DUBA)[2] DNA alkylating payload[3]

Objectives

- **To assess antitumor activity toward B7-H3-expressing tumor xenografts at clinically relevant doses and regimens**
- **To determine safety and toxicokinetics in a repeat-dose GLP toxicology study in cynomolgus monkeys**

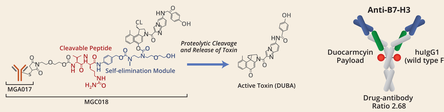
Introduction

Targeting B7-H3 via Multiple Mechanisms of Action



Duocarmycin-based Linker Payload

vc-*seco*-DUocarmycin-hydroxyBenzamide Azaindole (DUBA) Structure



Duocarmycins

- DNA alkylating agents — cell cycle independent
- Fully synthetic — picomolar activity in vitro
- Retain potency in multi-drug resistant lines
- Cleavable peptide linker — facilitates bystander effect
- Trastuzumab-vc-*seco*-DUBA (SYD985) in Phase 3 clinical trial in locally advanced or metastatic breast cancer (Synthon Biopharmaceuticals)

DUBA linker payload provided and conjugated by Synthon Biopharmaceuticals, B.V.

B7-H3 is Highly Expressed on a Range of Solid Cancers

Fixed Tumor MicroArray	IHC Summary of Tumor Samples Screened			
	B7-H3 Total Positive		2+ or Above	
Head and Neck Cancer	19/19	100%	19/19	100%
Kidney Cancer*	77/78	99%	75/78	96%
Glioblastoma	65/66	98%	63/66	95%
Bladder Cancer	86/88	98%	78/88	89%
Thyroid Cancer	34/35	97%	33/35	94%
Mesothelioma	45/47	96%	34/47	72%
Gastric Cancer	144/155	93%	127/155	82%
Colorectal Cancer	41/45	91%	38/45	84%
Melanoma	132/146	90%	94/146	64%
Prostate Cancer	88/89	89%	51/99	52%
Pancreatic Cancer	69/78	88%	45/78	58%
Lung Cancer	324/379	85%	300/379	79%
Breast Cancer**	189/249	76%	156/249	63%
Ovarian Cancer*	59/79	75%	36/79	46%

*Target expression on both tumor cells and tumor vasculature. **Triple negative breast cancer: 8/17 positive, 2+ or above (47%).

- B7-H3 has minimal expression on normal tissues

B7-H3-DUBA ADCs Exhibit Potent In Vitro Cytotoxicity

Summary Table of In Vitro Cytotoxicity

MGC018	Breast Cancer	Melanoma	Ovarian Cancer	Non-Small Cell Lung Cancer		Pancreatic Cancer	Glioblastoma	Colorectal Cancer
	MDA-MB-468	A375.S2	PA-1	Calu-6	NCI-H1703	Hs700T	LN-229	SW48
Antibody Binding Sites	5.7×10^4	1.5×10^5	7.4×10^4	1.4×10^5	1.2×10^5	3.1×10^5	1.4×10^5	6.0×10^4
IC_{50} (pM)	767	181	275	260	585	319	910	1447

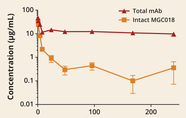
Alamar Blue cytotoxicity assay. Antibody binding sites determined by DAKO QFACS Kit.

- Cytotoxic activity observed against a range of B7-H3-positive tumor lines

Results

DUBA ADCs Exhibit Rapid Clearance in Mice Due to Rodent-specific Esterase

Concentration vs. Time for Total mAb and Intact MGC018



Summary of PK Parameters for Total mAb and Intact MGC018

mAb	IV Dose (mg/kg)	T_{max} (hr)	C_{max} (hr)	$T_{1/2}$ (hr)	AUC_{inf} (hr•µg/mL)	CL (mL/hr/kg)	MRT_{inf} (hr)
Total mAb	5	0.5	47.2	424.1	8848.0	0.6	607.5
Intact MGC018	5	0.5	33.7	39.5	189.5	26.4	48.8

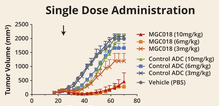
Total mAb determined with anti-human IgG capture/detection ELISA. Intact MGC018 determined with anti-hapten capture/anti-human IgG detection ELISA. PK parameters determined by noncompartmental analysis (NCA).

- Exposure to intact ADC in mice limited by rodent-specific carboxylesterase CES1c
- Modeling in this species represents a conservative estimate of MGC018 activity

MGC018: Antitumor Activity in the Mouse

PA-1 Ovarian Cancer

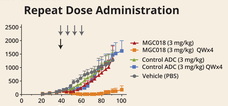
Single Dose Administration



Treatment	Dose (mg/kg)	Schedule/ IP Route	T/C-Day 59 (%)	CR
MGC018	10	Single Dose	11	3/6
MGC018	6	Single Dose	9	2/6
MGC018	3	Single Dose	57	1/6
Control ADC	10	Single Dose	84	0/6
Control ADC	6	Single Dose	89	0/6
Control ADC	3	Single Dose	111	0/6

% T/C: Percentage of mean tumor size in treatment group relative to mean tumor size of vehicle control group. CR: Complete Response defined as tumor volume ≤5 mm³ during study. Control ADC: Non-targeting DUBA ADC.

Repeat Dose Administration

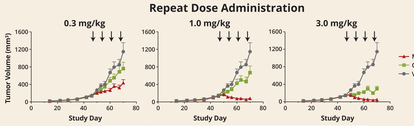


Treatment	Dose (mg/kg)	Schedule/ IV Route	T/C-Day 77 (%)	CR
MGC018	3	Single Dose	71	0/6
MGC018	3	QWx4	3	4/6
Control ADC	3	Single Dose	87	0/6
Control ADC	3	QWx4	92	1/6

% T/C: Percentage of mean tumor size in treatment group relative to mean tumor size of vehicle control group. CR: Complete Response defined as tumor volume ≤5 mm³ during study. Control ADC: Non-targeting DUBA ADC.

PA-1 Ovarian Cancer

Repeat Dose Administration

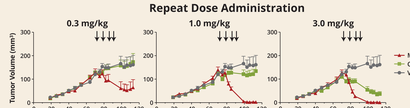


Treatment	Dose (mg/kg)	Schedule/IV Route	T/C-Day 70 (%)	CR
MGC018	3	QWx4	4	0/7
MGC018	1	QWx4	7	2/7
MGC018	0.3	QWx4	38	0/7
Control ADC	3	QWx4	26	0/7
Control ADC	1	QWx4	58	0/7
Control ADC	0.3	QWx4	66	0/7

% T/C: Percentage of mean tumor size in treatment group relative to mean tumor size of vehicle control group. CR: Complete Response defined as tumor volume ≤5 mm³ during study. Control ADC: Non-targeting DUBA ADC.

MDA-MB-468 Breast Cancer

Repeat Dose Administration



Treatment	Dose (mg/kg)	Schedule/IV Route	T/C-Day 113 (%)	CR
MGC018	3	QWx4	0	7/7
MGC018	1	QWx4	2	7/7
MGC018	0.3	QWx4	40	3/7
Control ADC	3	QWx4	23	2/7
Control ADC	1	QWx4	83	0/7
Control ADC	0.3	QWx4	107	0/7

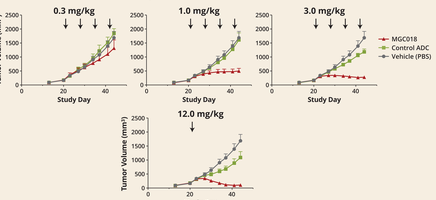
% T/C: Percentage of mean tumor size in treatment group relative to mean tumor size of vehicle control group. CR: Complete Response defined as tumor volume ≤5 mm³ during study. Control ADC: Non-targeting DUBA ADC.

- MGC018 exhibits anti-tumor activity as single dose administration
- Repeat-dose administration of MGC018 leads to enhanced antitumor response

MGC018: Antitumor Activity Following Repeat-dose Administration

Calu-6 Lung Cancer

Repeat Dose Administration

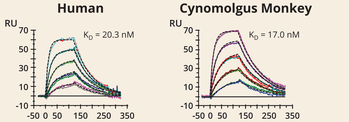


Treatment	Dose (mg/kg)	Schedule/IV Route	T/C-Day 44 (%)	CR
MGC018	12	Single Dose	6	0/7
MGC018	3	QWx4	17	0/7
MGC018	1	QWx4	30	0/7
MGC018	0.3	QWx4	78	0/7
Control ADC	12	Single Dose	65	0/7
Control ADC	3	QWx4	70	0/7
Control ADC	1	QWx4	96	0/7
Control ADC	0.3	QWx4	100	0/7

% T/C: Percentage of mean tumor size in treatment group relative to mean tumor size of vehicle control group. CR: Complete Response defined as tumor volume ≤5 mm³ during study. Control ADC: Non-targeting DUBA ADC.

- Dose-dependent antitumor activity observed with repeat-dose administration
  - Nearly equivalent antitumor activity with fractionated dosing

MGC018 Precursor Antibody (MGA017) is Cross-reactive with Cynomolgus Monkey B7-H3



SPR Analysis

Antibody	Human B7-H3			Cyno B7-H3		
	k_a (1/Ms)	k_d (1/s)	K_D (nM)	k_a (1/Ms)	k_d (1/s)	K_D (nM)
MGA017	6.9×10^5	1.4×10^{-2}	20.3	5.9×10^5	1.0×10^{-2}	17.0

Surface plasmon resonance (SPR) analysis was conducted to measure binding of human and cynomolgus monkey B7-H3 V1-C1(6.25-100 nM) to mAbs captured on Fab52 Goat Anti-Human Fc surface (normalized: 1:1 binding fit).

- MGA017 exhibits equal affinity for human and cynomolgus monkey B7-H3

Cynomolgus Monkey Repeat-dose GLP Toxicology Study

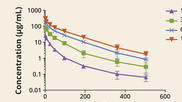
- Repeat-dose administration of 1, 3, 6, and 10 mg/kg of MGC018 was well tolerated in cynomolgus monkeys
  - MGC018 administered by IV infusion Q3Wx3 (5/sex/group)
  - Terminal sacrifice 2 weeks following the 3rd dose administration
  - Two animals/sex/group placed into 11-week recovery period

Summary of Findings

- Clinical Observations
  - Dose-dependent hyperpigmentation and dry skin
- Clinical Pathology
  - Transient decrease in neutrophil counts, mild/moderate decrease in reticulocytes, minimal/mild decrease in lymphocytes. Lacked microscopic correlates, resolved during recovery
  - Increased C-reactive protein and fibrinogen. Resolved at subsequent collections
  - Minimal/mild increases in AST and/or ALT. Lacked correlative microscopic findings in liver, resolved at subsequent collections
- Postmortem Evaluation
  - Spectrum of skin findings
    - Minimal/mild increase in pigment, lymphocytic infiltrate, minimal epidermal hyperplasia, single cell necrosis. Resolved or ongoing reversibility during recovery
- **No life threatening toxicities, irreversible findings or mortality**
  - **HNSTD: 10 mg/kg/dose at Q3Wx3 — the highest dose administered**

MGC018 Exhibits Favorable PK Profile in Cynomolgus Monkeys

First Dose Concentration vs. Time Profile for Intact MGC018

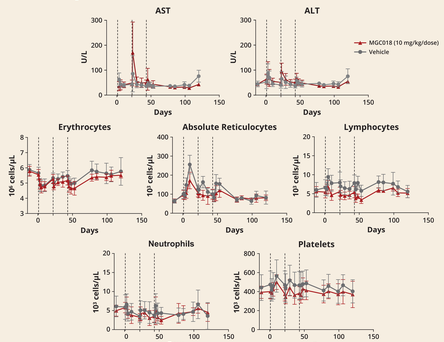


First Dose PK Parameters for Intact MGC018

Dose (mg/kg)	T_{max} (hr)	C_{max} (µg/mL)	$T_{½}$ (hr)	AUC_{inf} (hr•µg/mL)	CL (mL/hr/kg)	V_{ss} (mL/kg)	MRT_{inf} (hr)
1	1.2	26.4	78.1	663	1.5	81.4	53.9
3	1.8	92.0	84.5	3210	1.0	66.3	69.7
6	1.9	181.5	67.9	9032	0.7	56.8	84.8
10	1.2	310.9	74.1	15075	0.7	64.6	97.1

Intact MGC018 determined with anti-hapten capture/anti-human IgG detection ELISA. PK parameters determined by NCA.

Clinical Chemistries/Hematology from Repeat-dose GLP Study



Vertical lines represent days of dose administration (Day 1, 22, 43).

- Limited clinical chemistry and hematology findings
  - No microscopic correlates, resolved during recovery

Conclusions

- Potent antitumor activity in mouse models toward B7-H3-expressing human tumor xenografts at clinically relevant dose levels, despite model limitations (rapid ADC clearance)
- Equal affinity to human and cynomolgus monkey B7-H3 protein
- Favorable safety and toxicokinetics in a repeat-dose GLP toxicology study in cynomolgus monkeys, a validated toxicology species for MGC018
  - The HNSTD was determined to be 10 mg/kg/dose which corresponds to a systemic exposure of 310.9 µg/mL (C_{max}) and 15075 hr•µg/mL (AUC_{inf})
- Favorable therapeutic window for MGC018

The preclinical profile supports clinical development of MGC018 as a therapeutic ADC for the treatment of B7-H3-positive cancers

References

1. Loo D, Alderson R, Chen F et al., Clin Cancer Res 18(14) 2012. **2.** Dokter W, Ubink R et al., Mol Cancer Ther 13(11) 2014. **3.** Son T, Scribner J, Hooley J et al., AACR Annual Meeting Abstract (42) 2017.

Acknowledgment

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