# Preclinical Development of a Duocarmycin-based Antibody-Drug Conjugate Targeting B7-H3 for Solid Cancer



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http://ir.macrogenics.com/events.cfm

## Abstract

**Introduction:** B7-H3, a member of the B7 family of immunomodulatory molecules, is overexpressed in a wide range of solid cancers. B7-H3 overexpression has been correlated with disease severity and poor outcome in several cancer types. Proof-of-concept studies targeting B7-H3 demonstrated that auristatin-based B7-H3 ADCs exhibited potent cytotoxicity *in vitro* and antitumor activity *in vivo* toward a range of B7-H3-expressing tumor cell lines. Based on these preliminary results, we undertook preclinical development of a B7-H3 ADC comprised of a humanized B7-H3 mAb conjugated to a potent DNA alkylating payload.

**Methods:** Chimeric B7-H3 mAbs were conjugated to vc-*seco*-DUocarmycin-hydroxyBenzamide Azaindole (DUBA) (ADC conjugated and provided by Synthon Biopharmaceuticals B.V.). *In vitro* and *in vivo* activity studies were conducted with tumor cell lines that overexpress B7-H3. Based on the potency analysis, together with the biophysical properties and immunohistochemistry (IHC) profiles of the candidates, a lead mAb was selected for preclinical development. The mAb was humanized via CDR grafting and conjugated to DUBA to yield the development candidate MGC018. *In vitro* and *in vivo* studies were then conducted with MGC018 to confirm and extend the results with the chimeric ADCs.

**Results:** Confirming our previous data and consistent with a growing body of literature, B7-H3 mAbs exhibited strong reactivity toward carcinoma cells and the vasculature of solid cancers. Chimeric B7-H3-DUBA ADCs demonstrated specific, dose-dependent cytotoxicity toward B7-H3-positive tumor cell lines *in vitro* and potent antitumor activity *in vivo*. The humanized ADC development candidate, MGC018, retained the favorable biophysical properties and the normal tissue-versus-tumor IHC profile of the parental mAb. MGC018 displayed cytotoxicity toward B7-H3-positive tumor cell lines *in vitro*, with $IC_{50}$ values in the sub-nM range, and potent antitumor activity *in vivo*, resulting in tumor stasis and tumor regression in mice bearing B7-H3-positive human tumor xenografts, representing breast, lung and ovarian cancers.

**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, 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. Our findings support further preclinical development of MGC018 to evaluate its potential as an ADC therapeutic for B7-H3-expressing solid cancers.

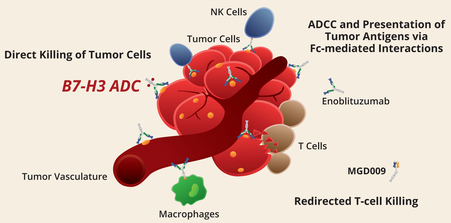
## Background

### B7-H3: An Attractive Cell-surface Molecule for Targeted Therapy

- B7-H3, a member of the B7 family of immune regulators, is overexpressed on many solid cancers and displays high tumor-versus-normal tissue binding differential
- B7-H3 overexpression has been correlated with disease severity and poor outcome in many cancer types
- MacroGenics is targeting B7-H3 by two modalities:
  - Enoblituzumab (MGA271)[1]: a humanized Fc-enhanced mAb with enhanced ADCC
  - B7-H3 x CD3 DART (MGD009): a humanized Fc-bearing bispecific DART molecule for redirected T-cell killing
- A B7-H3 ADC may provide a complementary mechanism of action:
  - Proof-of-concept studies demonstrated that auristatin-based B7-H3 ADCs exhibited potent cytotoxicity *in vitro* and antitumor activity *in vivo* (AACR 2015: Abstract# 1201[2])
- **Objective: Develop a B7-H3 ADC clinical candidate**
  - Identify a lead humanized anti-B7-H3 mAb
  - Conduct preclinical development studies with B7-H3 ADC based on the DUBA[3] DNA alkylating payload

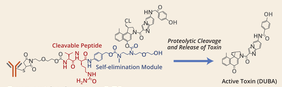
## Introduction

### Targeting B7-H3 via Multiple Mechanisms of Action




### Duocarmycin-based Linker Payload

**Structure**



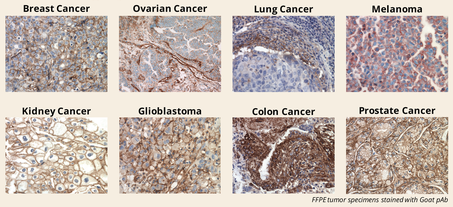

Drug-antibody ratio ~2.70

**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
- Anti-HER2-DUBA (SYD985) in Phase 1 clinical study (Synthon Biopharmaceuticals)

DUBA Linker Payload provided and conjugated by Synthon Biopharmaceuticals, B.V.

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



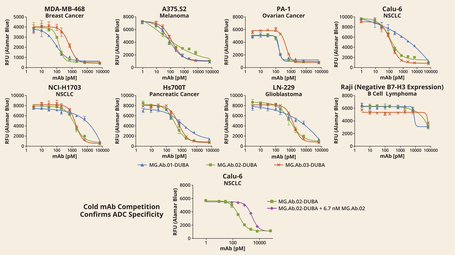

*FFPE tumor specimens stained with Goat pAb*

| Fixed Tumor MicroArray | IHC Summary of Samples Screened | | | |
|---|---|---|---|---|
| | B7-H3 Positive | | 2+ or Above | |
| ***Potential Indications:*** | | | | |
| Head and Neck | 19/19 | 100% | 19/19 | 100% |
| Kidney Cancer | 77/78 | 99% | 75/78 | 96% |
| Glioblastoma | 65/66 | 98% | 63/66 | 95% |
| Thyroid Cancer | 34/35 | 97% | 33/35 | 94% |
| Mesothelioma | 41/44 | 93% | 39/44 | 89% |
| Melanoma | 132/146 | 90% | 94/146 | 64% |
| Prostate Cancer | 88/99 | 89% | 51/99 | 52% |
| Pancreas Cancer | 69/78 | 88% | 45/78 | 58% |
| Bladder | 134/156 | 86% | 123/156 | 79% |
| Lung Cancer | 324/379 | 85% | 300/379 | 79% |
| Breast Cancer | 189/249 | 76% | 156/249 | 63% |
| Ovarian Cancer | 59/79 | 75% | 36/79 | 46% |

- Membrane expression on tumor epithelium and tumor-associated vasculature

## Results

### B7-H3-DUBA ADCs Exhibit Potent In Vitro Cytotoxicity




**Relative Potency Across Multiple Tumor Types**

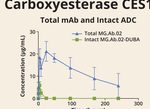
| $IC_{50}$ (pM) * | Breast Cancer | Melanoma | Ovarian Cancer | Non-Small Cell Lung Cancer | | Pancreatic Cancer | Glioblastoma |
|---|---|---|---|---|---|---|---|
| | MDA-MB-468 | A375.S2 | PA-1 | Calu-6 | NCI-H1703 | Hs700T | LN-229 |
| Antibody Binding Sites ** | 4.20E+05 | 7.50E+05 | 6.10E+05 | 8.50E+05 | 8.10E+05 | 2.10E+05 | 8.12E+05 |
| MG.Ab.01-DUBA | 87 | 86 | 161 | ND | ND | 1187 | ND |
| MG.Ab.02-DUBA | 140 | 27 | 160 | 116 | 1523 | 373 | 920 |
| MG.Ab.03-DUBA | 647 | 130 | 471 | 132 | 1763 | 661 | 1412 |

*Alamar Blue cytotoxicity assay; **Antibody Binding Sites determined by Bangs QFACS Kit

- Activity observed against a range of B7-H3-positive tumor lines

### DUBA ADCs Exhibit Fast PK in Rodents

**Exposure to Intact ADC in Mice Limited by Rodent-specific Carboxyesterase CES1c**



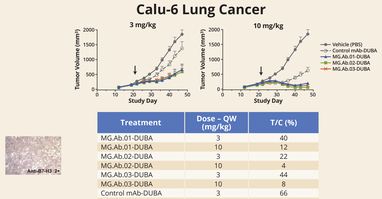

| mAb | Dose (mg/kg) | Route | $T_{½}$ (hr) | $C_{max}$ (µg/mL) | AUC Last (hr*µg/mL) |
|---|---|---|---|---|---|
| Total IgG | 5 | IP | 78.0 | 21.2 | 2635 |
| Intact ADC | 5 | IP | ND | 5.9 | 45 |

Total IgG determined with anti-human IgG capture/detection ELISA. Intact ADC determined with anti-hapten capture/anti-human IgG detection ELISA.

- DUBA ADC activity in rodents may under-predict activity in humans

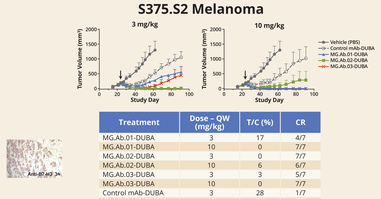
### chB7-H3-DUBA ADCs Exhibit Anti-Tumor Activity

**Calu-6 Lung Cancer**




| Treatment | Dose – QW (mg/kg) | T/C (%) |
|---|---|---|
| MG.Ab.01-DUBA | 3 | 40 |
| MG.Ab.01-DUBA | 10 | 12 |
| MG.Ab.02-DUBA | 3 | 22 |
| MG.Ab.02-DUBA | 10 | 4 |
| MG.Ab.03-DUBA | 3 | 44 |
| MG.Ab.03-DUBA | 10 | 8 |
| Control mAb-DUBA | 3 | 66 |
| Control mAb-DUBA | 10 | 37 |

**S375.S2 Melanoma**




| Treatment | Dose – QW (mg/kg) | T/C (%) | CR |
|---|---|---|---|
| MG.Ab.01-DUBA | 3 | 17 | 4/7 |
| MG.Ab.01-DUBA | 10 | 0 | 7/7 |
| MG.Ab.02-DUBA | 3 | 0 | 7/7 |
| MG.Ab.02-DUBA | 10 | 6 | 6/7 |
| MG.Ab.03-DUBA | 3 | 3 | 5/7 |
| MG.Ab.03-DUBA | 10 | 0 | 7/7 |
| Control mAb-DUBA | 3 | 28 | 1/7 |
| Control mAb-DUBA | 10 | 21 | 2/7 |

### Lead Candidate Selection and Humanization

- MG.Ab.02 selected as lead candidate based on:
  - Tumor v. Normal tissue reactivity
    - Reduced liver reactivity
  - *In vitro* potency
  - *In vivo* anti-tumor activity
  - Favorable CMC properties
- The murine mAb was humanized by replacing mouse variable region heavy chain (VH) and mouse variable region light chain (VL) framework sequences with human germline framework sequences

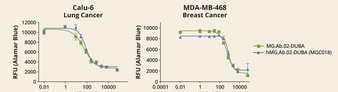
**hMG.Ab.02-DUBA = MGC018**

**SPR Analysis**

| Antibody | Human B7-H3 | | | Cyno B7-H3 | | |
|---|---|---|---|---|---|---|
| | $K_D$ | On Rate | Off Rate | $K_D$ | On Rate | Off Rate |
| MG.Ab.02 | 18.2 nM | 5.5E+05 | 1.0E-02 | 11.8 nM | 5.7E+05 | 6.7E-03 |
| hMG.Ab.02 | 20.3 nM | 6.9E+05 | 1.4E-02 | 17.0 nM | 5.9E+05 | 1.0E-02 |

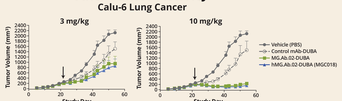
Binding of human and B7-H3 V1-C1(6.25-100 nM) to mAbs captured on F(ab)2 GAH Fc surface (normalized; 1:1 binding fit).

**In Vitro Cytotoxicity**




**Anti-Tumor Activity**

**Calu-6 Lung Cancer**

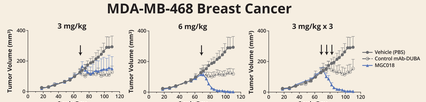



| Treatment | Dose (mg/kg) | T/C (%) |
|---|---|---|
| MG.Ab.02-DUBA | 3 | 37 |
| MG.Ab.02-DUBA | 10 | 10 |
| hMG.Ab.02-DUBA (MGC018) | 3 | 27 |
| hMG.Ab.02-DUBA (MGC018) | 10 | 7 |
| Control mAb-DUBA | 3 | 67 |
| Control mAb-DUBA | 10 | 67 |

- Humanized MG.Ab.02-DUBA (MGC018):
  - Retains binding affinity for human and cyno B7-H3
  - Retains potency toward B7-H3-positive tumor cells *in vitro* and *in vivo*

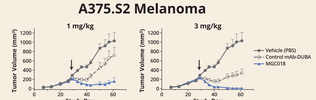
### MGC018 Exhibits Potent Anti-Tumor Activity

**MDA-MB-468 Breast Cancer**




| Treatment | Dose (mg/kg) | T/C (%) | CR |
|---|---|---|---|
| MGC018 | 3 | 51 | 1/5 |
| MGC018 | 6 | 1 | 4/5 |
| MGC018 | 3 x 3 | 2 | 3/5 |
| Control mAb-DUBA | 3 | 43 | 0/5 |
| Control mAb-DUBA | 6 | 41 | 0/5 |
| Control mAb-DUBA | 3 x 3 | 53 | 0/5 |

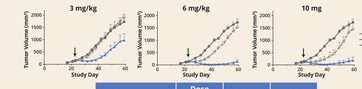
**A375.S2 Melanoma**




| Treatment | Dose (mg/kg) | T/C (%) | CR |
|---|---|---|---|
| MGC018 | 1 | 16 | 1/7 |
| MGC018 | 3 | 1 | 5/7 |
| Control mAb-DUBA | 1 | 70 | 1/7 |
| Control mAb-DUBA | 3 | 33 | 0/7 |

### MGC018 Exhibits Potent Anti-Tumor Activity

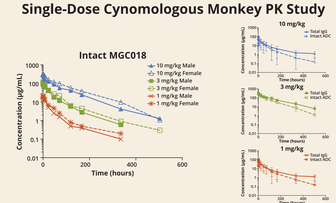
**PA-1 Ovarian Cancer**




| Treatment | Dose (mg/kg) | T/C (%) | CR |
|---|---|---|---|
| MGC018 | 3 | 57 | 1/6 |
| MGC018 | 6 | 9 | 2/6 |
| MGC018 | 10 | 11 | 3/6 |
| Control mAb-DUBA | 3 | 111 | 0/6 |
| Control mAb-DUBA | 6 | 89 | 0/6 |
| Control mAb-DUBA | 10 | 84 | 0/6 |

### MGC018 Exhibits Favorable PK Profile

**Single-Dose Cynomologous Monkey PK Study**




**PK Parameters for Intact MGC018**

| Species | Dose (mg/kg) | Route | $T_{½}$ (hr) | $C_{max}$ (µg/mL) | AUC Last (hr*µg/mL) |
|---|---|---|---|---|---|
| Cyno | 1 | IV | 46.5 | 20.5 | 561 |
| Cyno | 3 | IV | 62.7 | 113.5 | 3798 |
| Cyno | 10 | IV | 57.3 | 331.0 | 17978 |

### MGC018: Observations in Cynomolgus Monkeys

**A Single Intravenous Infusion of 1, 3 & 10 mg/kg of MGC018 was Well Tolerated in Male Cynomolgus Monkeys**

- Body weight: no change
- Red blood cell parameters & platelets: no change
- White blood cell populations
  - Transient mild-to-moderate increases in neutrophil counts in individual animals at 10 mg/kg
- Coagulation
  - Mild to moderate increases in fibrinogen concentration at 10 mg/kg
- Liver
  - Mild, transient increases in ALT/AST at doses of ≥3 mg/kg in ~ half of animals
  - No correlative histopathological findings in liver

## Conclusions

- MGC018 (hMG.Ab.02–DUBA ADC):
  - Favorable tumor-versus-normal tissue IHC profile
  - Potent *in vitro* cytotoxicity and *in vivo* antitumor activity toward B7-H3-positive tumor cell lines representing a range of solid cancers
  - Cross-reactive with cynomolgus monkey B7-H3 with equivalent $K_D$ values
  - Favorable pharmacokinetic properties in cynomolgus monkeys
  - Well tolerated in cynomolgus monkeys at a single dose administration up to 10 mg/kg

**The preclinical profile supports continued 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.** Loo D, Scribner J, Son T et al., AACR Annual Meeting Abstract(1201) 2015. **3.** Dokter W, Ubink R et al., Mol Cancer Ther 13(11) 2014

## Acknowledgment

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