MGC018, a Duocarmycin-based Antibody-drug Conjugate Targeting B7-H3, Exhibits Immunomodulatory Activity and Enhanced Antitumor Activity in Combination with Checkpoint Inhibitors

Juniper A. Scribner, Michael Chiechi, Pam Li, Thomas Son, Jeff Hooley, Ying Li, Anushka De Costa, Peter Lung, Nicholas Yee-Toy, Francine Chen, Zhuangyu Pan, Bhaswati Barat, Christina Wolff, Valentina Ciccarone, James Tamura, Scott Koenig, Chet Bohac, Jon Wigginton, Paul A. Moore, Ezio Bonvini, Deryk Loo

MacroGenics, Inc., Brisbane, CA and Rockville, MD

Presenter Contact Information: scribnerj@macrogenics.com

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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. B7-H3 tumor overexpression has been correlated with disease severity and poor outcome. MGC018 is a duocarmycin-based antibody-drug conjugate (ADC) targeting B7-H3. MGC018 exhibits a favorable preclinical profile, with strong reactivity toward tumor cells and tumor-associated vasculature, limited normal tissue reactivity, and potent antitumor activity toward B7-H3-expressing tumor xenografts. With the emergence of immune-checkpoint blockade as a promising treatment for cancer, interest has grown in understanding the potential of cytotoxic agents to promote immune surveillance or stimulate immune responses to dying cancer cells, leading to immunological memory. ADCs bearing tubulin and DNA modifying cytotoxic payloads have been reported to induce immunogenic cell death (ICD), mediate antitumor immunity in immunocompetent mouse models, and synergistically combine with checkpoint inhibitors to deliver enhanced antitumor responses. Based on those results, we investigated the immunomodulatory potential of MGC018 and the prospect to combine with checkpoint blockade to enhance antitumor responses.

Methods: Syngeneic mouse models expressing human B7-H3 were employed to investigate the antitumor activity of MGC018 in an immune competent setting. Studies were conducted to assess the role of the immune system in the MGC018-mediated antitumor responses, whether MGC018 could impart antitumor memory responses in vivo, and the potential to enhance antitumor responses by combining MGC018 with PD-1 blockade.

Results: MGC018 demonstrated specific, dose-dependent in vivo antitumor activity toward human B7-H3-bearing tumors in immunocompetent syngeneic mouse models. Depletion of CD8$^+$ T cells led to reduced antitumor responses, indicating that CD8$^+$ T cells contributed to MGC018-mediated antitumor activity. Antitumor activity in these models was enhanced when MGC018 was combined with anti-PD-1. Treatment with MGC018 alone, or in combination with anti-PD-1, led to complete antitumor responses, and the majority of mice rejected subsequent tumor rechallenge.

Conclusion: MGC018, a clinical-stage therapeutic comprised of a humanized antibody targeting B7-H3, conjugated to a duocarmycin-based DNA alkylating payload, exhibits a favorable preclinical profile. Results from these syngeneic model studies support the hypothesis that the antitumor activity of the duocarmycin-based MGC018 ADC (1) mediates immunomodulatory activity, (2) is enhanced by combination with checkpoint blockade, and (3) induces immunological memory. Our findings support a clinical strategy that combines MGC018 with checkpoint blockade for the treatment of B7-H3-expressing solid cancers.

Background

MGC018 a Clinical-stage Anti-B7-H3 ADC Therapeutic

B7-H3

- 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

MGC018

- Comprised of a humanized antibody targeting B7-H3 (MGA017)
- Conjugated to a duocarmycin-based DNA alkylating payload via native cysteines
- Potent antitumor activity in mouse models toward B7-H3-expressing human tumor xenografts at clinically relevant dose levels, despite model limitations
  - Rapid clearance (half-life of ADC ~40 hours) due to degradation by rodent-specific carboxylesterase CES1c — *not present in primates or humans*
- Favorable safety and toxicokinetics in a repeat-dose GLP toxicology study in cynomolgus monkeys, a validated toxicology species for MGC018
- Phase I/II clinical study in advanced solid cancers in progress (NCT03729596)

Immunomodulatory Activity of ADC's

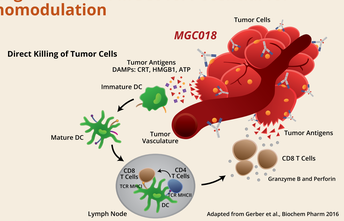
- Have been reported to induce immunogenic cell death (Gerber et al., *Biochem Pharm* 2016)
- Synergistically combine with checkpoint inhibitors to deliver enhanced antitumor responses
- Mediate antitumor immunity in immunocompetent mouse models

Objectives

- Evaluate MGC018 to mediate immunomodulatory activity
- Determine whether treatment with MGC018 is enhanced by combination with checkpoint blockade
- Assess whether MGC018 induces immunological memory

Introduction

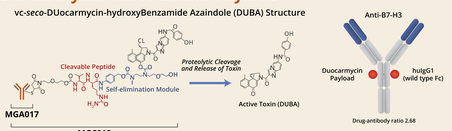
Targeting B7-H3 with MGC018: Potential for Immunomodulation



DAMPs: Damage-associated molecular patterns.

Duocarmycin-based Linker Payload

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

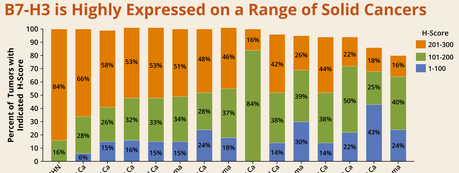


Duocarmycins

- 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 (Byondis, B.V.)

DUBA Linker Payload provided and conjugated by Byondis, B.V.

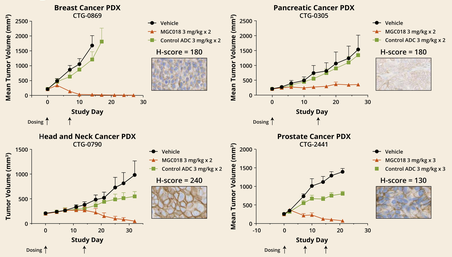
B7-H3 is Highly Expressed on a Range of Solid Cancers



- B7-H3 is expressed on tumor epithelium and tumor-associated vasculature

FFPE staining with goat polyclonal antibody (R&D Systems). Independent specimens ranged from 19-189 for each indication.

MGC018 Antitumor Activity Toward Patient-derived Xenograft (PDX) Models



- MGC018 displays antitumor activity toward heterogeneous B7-H3 expressing PDX models representing a range of solid cancers

Results

Syngeneic Mouse Model System

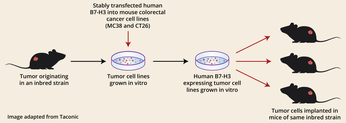
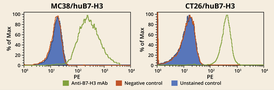


Image adapted from Taconic

- Tumor cells are originally derived from the same genetic background as the implanted mouse
- Mice have an intact and fully functioning immune system
- Syngeneic models provide an effective approach for studying how cancer therapies perform in the presence of a functional immune system
- While transduced human B7-H3 may be immunogenic in the mouse, the model provides proof-of-concept for the ADC's ability to enhance adaptive responses

Murine Colorectal Cancer Cell Lines Engineered to Overexpress Human B7-H3

Relative Expression of Human B7-H3 on the Cell Surface

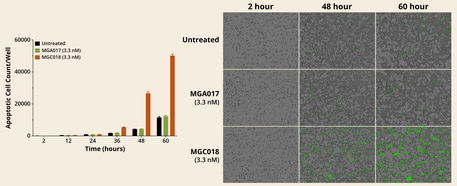


QFACS		
	MC38/huB7-H3 Murine Colorectal Cancer	CT26/huB7-H3 Murine Colorectal Cancer
Antibody Binding Capacity	4.08E+04	5.37E+04
Syngeneic Mouse Model	C57BL/6	BALB/c

Antibody Binding Capacity determined by Bangs QFACS Kit.

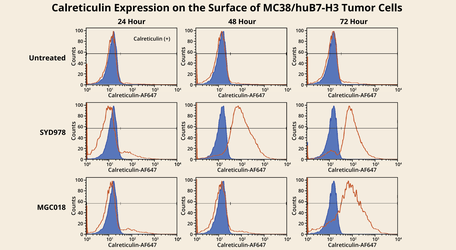
MGC018 Mediates Apoptosis of Murine Tumor Cells

Caspase 3/7 Activation of MC38/huB7-H3 Tumor Cells



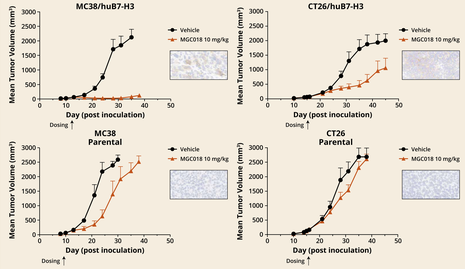
MGC018 Modulates Damage-associated Molecular Patterns

Calreticulin Expression on the Surface of MC38/huB7-H3 Tumor Cells



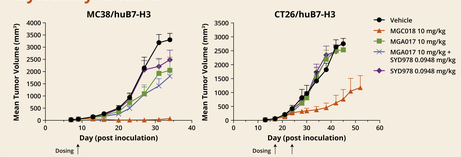
- MGC018 and SYD978 (*seco*-DUBA Payload) induced calreticulin translocation to the surface of MC38/huB7-H3 murine tumor cells
- Analysis of other DAMPs are currently underway

Antitumor Activity of MGC018 is Dependent on Human B7-H3 Expression



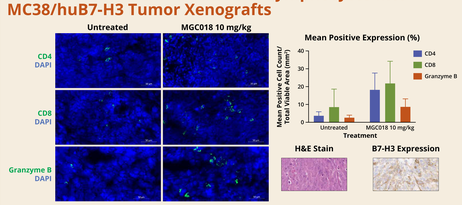
- Parental xenografts lacking human B7-H3 expression are insensitive to MGC018

Antitumor Activity is Dependent on Targeted Delivery of Payload by MGC018



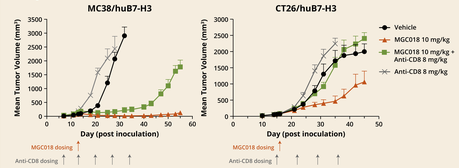
- No antitumor activity with MGA017, SYD978 (*seco*-DUBA payload) or combination of MGA017 + SYD978
- Antitumor activity of SYD978 requires conjugation to B7-H3-targeting mAb

MGC018 Increases Infiltration of Lymphocytes into MC38/huB7-H3 Tumor Xenograft



- MGC018 treatment increases CD4 and CD8 infiltration and Granzyme B expression

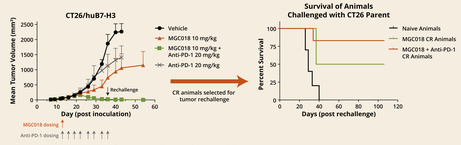
CD8$^+$ T Cells Contribute to MGC018-mediated Antitumor Response



- Anti-CD8 depletes CD8$^+$ T cells and attenuates MGC018 antitumor activity

Anti-CD8 antibody from BioXCell: Clone 53-6.7.

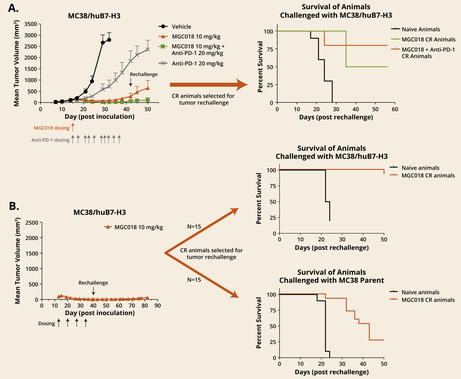
Antitumor Activity of MGC018 in Combination with Anti-PD-1 in CT26 Model: Induction of Immunological Memory



- Antitumor activity of MGC018 is enhanced by anti-PD-1
- MGC018 and MGC018 + anti-PD-1 induces immunological memory

CR defined as tumors < 24 mm³. Anti-PD-1 antibody from BioXCell: Clone RMP1-14.

Antitumor Activity of MGC018 in Combination with Anti-PD-1 in MC38 Model: Induction of Immunological Memory



- Antitumor activity of MGC018 is enhanced by anti-PD-1
- MGC018 and MGC018 + anti-PD-1 induces immunological memory

CR defined as tumors < 16 mm³. Anti-PD-1 antibody from BioXCell: RMP1-14.

Conclusions

- MGC018 demonstrated in vivo antitumor activity toward heterogeneous B7-H3-expressing PDX models
  - TNBC, Pancreatic, Prostate, Head and Neck Squamous Cell Carcinoma
- MGC018 demonstrated specific, dose-dependent in vivo antitumor activity toward human B7-H3-expressing tumors in immunocompetent syngeneic mouse models
  - MGC018 induced translocation of calreticulin, a component of damage-associated molecular patterns (DAMPs)
  - MGC018 treatment increased infiltration of CD4$^+$ and CD8$^+$ lymphocytes into the tumor
- Depletion of CD8$^+$ T cells led to reduced antitumor responses, indicating that CD8$^+$ T cells contributed to MGC018-mediated antitumor activity
- Antitumor activity was enhanced when MGC018 was combined with anti-PD-1 leading to more complete antitumor responses
- MGC018 alone or in combination with anti-PD-1 led to complete responses and subsequent tumor rejection, indicative of immunological memory

Our findings support a clinical strategy that combines MGC018 with checkpoint blockade for the treatment of B7-H3-expressing solid cancers

Acknowledgements

DUBA linker payload provided and conjugated by Byondis, B.V.

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