

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

Juniper A. Scribner, Jennifer G. Brown, Thomas Son, Michael Chiechi, Pam Li, Sharad Sharma, Hua Li, Anushka De Costa, Ying Li, Yan Chen, Ann Easton, Nicholas C. Yee-Toy, Francine Z. Chen, Sergey Gorlatov, Bhaswati Barat, Ling Huang, Christina R. Wolff, Jeff Hooley, Tim E. Hotaling, Timur Gaynutdinov, Valentina Ciccarone, James Tamura, Scott Koenig, Paul A. Moore, Ezio Bonvini, Deryk Loo

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Additional Information:

Corresponding Author: Deryk Thomas Loo
3280 Bayshore Blvd., Suite 200
Brisbane, CA 94005
Phone: (650) 624-2736
Fax: (650) 624-2793
Email: lood@macrogenics.com

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ABSTRACT

B7-H3, also referred to as CD276, is a member of the B7 family of immune regulatory proteins. B7-H3 is overexpressed on many solid cancers, including prostate cancer, renal cell carcinoma, melanoma, squamous cell carcinoma of the head and neck, non-small cell lung cancer and breast cancer. Over-expression of B7-H3 is associated with disease severity, risk of recurrence and reduced survival. In this article, we report the preclinical development of MGC018, an antibody-drug conjugate targeted against B7-H3. MGC018 is 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. MGC018 exhibited cytotoxicity toward B7-H3-positive human tumor cell lines, and exhibited bystander killing of target-negative tumor cells when co-cultured with B7-H3-positive tumor cells. MGC018 displayed potent antitumor activity in preclinical tumor models of breast, ovarian and lung cancer, as well as melanoma. Additionally, antitumor activity was observed toward patient-derived xenograft models of breast, prostate and head and neck cancer displaying heterogeneous expression of B7-H3. Importantly, MGC018 exhibited a favorable pharmacokinetic and safety profile in cynomolgus monkeys following repeat-dose administration. The antitumor activity observed preclinically with MGC018, together with the positive safety profile, provides evidence of a potentially favorable therapeutic index and supports the continued development of MGC018 for the treatment of solid cancers.

INTRODUCTION

B7-H3, also referred to as CD276, is a member of the B7 family of immune regulatory proteins (1). B7-H3 is a type-1 transmembrane glycoprotein containing extracellular immunoglobulin-like domains. Two species-specific isoforms of B7-H3 have been reported. Mice display a 2-Ig B7-H3 form, while the primate counterpart has undergone gene duplication and is expressed as a 4-Ig-containing molecule (2, 3). Although a specific receptor or receptors that bind B7-H3 have not been identified conclusively, B7-H3 has been implicated experimentally in the delivery of both costimulatory and inhibitory signals (1, 4). Most published data, however, demonstrate an inhibitory role for B7-H3 in immune responses (5), including *in vitro* studies examining T-cell responses (2, 6), mouse knockout studies (7, 8) and structure/function analyses based on the B7-H3 crystallography data (9, 10), and supports the hypothesis that B7-H3 has a role in evading antitumor immunity.

B7-H3 is overexpressed on many cancers, including, but not limited to, prostate cancer (11-14), melanoma (13, 15), breast cancer (13, 16, 17), non-small cell lung cancer (NSCLC) (13, 18, 19) and oral squamous carcinoma (20). In renal cell carcinoma, B7-H3 is broadly expressed by both the tumor epithelium and tumor-associated vasculature (13, 21). In many cancers, overexpression of B7-H3 has been correlated with multiple adverse clinical and pathological features of disease, increased risk of recurrence, and reduced survival. Conversely, although B7-H3 mRNA expression is detectable in most normal tissues, B7-H3 protein expression is limited in normal tissues (13, 22, 23).

Notwithstanding its negative prognostic association in cancer, the biological role of B7-H3 remains unclear and controversial, a difficulty compounded by the lack of a known B7-H3 receptor. Accordingly, we have sought to develop antibody-based targeting agents that exploit the over-expression of B7-H3 by tumors. Enoblituzumab (MGA271) is an anti-B7-H3 monoclonal antibody (mAb) bearing an engineered Fc domain optimized to enhance antitumor effector-mediated function (13), while MGD009 is a CD3 x B7-H3 DART[®] molecule designed to recruit the cytolytic activity of T cell killing toward B7-H3-positive tumors.

Antibody-drug conjugates (ADCs) combine the target specificity of a mAb with cytotoxic agents, with the goal of delivering the cytotoxic agent to the tumor and improving the therapeutic index. Advances over the past couple of decades has led to development of linkers with greater serum stability and payloads with greater potency and various mechanisms of action. These advances have contributed to the approval of 7 ADCs for the treatment of cancer. The improvements in ADC technologies and clinical successes prompted us to investigate whether overexpression of B7-H3 in cancer provides an opportunity for targeted therapy with an ADC, which may complement and/or enhance B7-H3-targeting by immune-based agents.

Here, we report the preclinical development of MGC018, a duocarmycin-based humanized ADC targeting B7-H3. MGC018 exhibited cytotoxicity toward B7-H3-positive human tumor cell lines, including bystander killing, potent antitumor activity in preclinical tumor models, and a favorable pharmacokinetic (PK) and safety profile in cynomolgus monkeys.

METHODS

Humanization

To construct the humanized anti-B7-H3 mAb MGA017, humanized PRCA157 variable light chain (VL) and PRCA157 variable heavy chain (VH) amino acid sequences (Supplemental Fig. S1) were designed using the complementary-determining region (CDR) sequences from the mouse PRCA157 mAb and framework sequences from human germline V-kappa or VH segment, respectively. The humanized PRCA157 VL and VH coding sequences were synthesized de novo, and fused to the human C-Kappa or human gamma 1 constant region cDNA, respectively.

Conjugation to *vc-seco*-DUBA and hydrophobic interaction chromatography purification

Conjugation of humanized anti-B7-H3 mAb (MGA017) to *vc-seco*-duocarmycin-hydroxybenzamide azaindole (DUBA), and hydrophobic interaction chromatography (HIC) to enrich for DAR2 and DAR4 species, was performed by Byondis BV as previously described (24). A schematic representation of the MGC018 ADC structure is shown in Supplemental Figure S2. Drug distribution and DAR average by HIC for MGC018 is shown in Supplemental Figure S3. An anti-CD20 mAb conjugated to *vc-seco*-DUBA was employed as a control ADC. Analytical characteristics of MGC018 are summarized in Supplemental Table S1.

Tumor cell line culture

MDA-MB-468, A375.S2, PA-1, Calu-6, Hs700T, SW48 and LN-229 tumor cell lines were obtained from American Type Culture Collection (ATCC) and cultured in DMEM/F-12 media containing 10% fetal bovine serum (FBS). NCI-H1703 and Raji tumor cell lines were obtained from ATCC and cultured in RPMI 1640 media containing 10% FBS. Quantitative determination of cell surface antigen by quantitative flow cytometry (QFACS) is described in the Supplementary Materials and Methods. All cell lines were thawed from working cell banks, which were screened for *Mycoplasma* by PCR. Cell line identity was confirmed by short tandem repeat profiling at ATCC, and studies were performed with cultures that had undergone fewer than 25 passages.

Generation of Hs700T/B7-H3 knockout tumor cell line

The Hs700T/B7-H3 knockout (KO) line was generated using transEDIT CRISPR gRNA huCD276, pCLIP-All-EFS-Puro (TEVH-1194322) according to the manufacturer's protocol (transOMIC Technologies). Details on generation of the knockout line and flow cytometry analysis is described in the Supplementary Materials and Methods.

Cytotoxicity assays

Cells were harvested by trypsinization and plated at 5,000 cells/well with serially diluted MGC018 then incubated at 37°C. Viability was measured after 7 days using alamarBlue according to manufacturer's protocol (Thermo Fisher Scientific). Plates were read on a SpectraMax Gemini (Molecular Devices). Data was analyzed using GraphPad Prism

(4-parameter curve fit analysis; v7.02, GraphPad) to determine IC₅₀ (half-maximal inhibitory concentration) values.

Bystander killing Assay

Hs700T/B7-H3 KO cells stably expressing red fluorescent protein (Hs700T/B7-H3 KO/RFP) were generated using the IncuCyte NucLight Red Lentivirus (Sartorius) according to manufacturer's protocol. Cells were harvested by trypsinization and plated into 96-well flat bottom plates. Parental Hs700T cells were plated at densities ranging from 0 – 5,000 cells/well. Hs700T/B7-H3 KO/RFP cells were plated at 5,000 cells/well. MGC018 was added to plates at the indicated concentrations, then plates were incubated at 37°C and cell viability of Hs700T/B7-H3 KO/RFP cells was measured after 5 days using an IncuCyte Live-Cell Analysis System (Sartorius).

In vivo efficacy

Efficacy studies were carried out under protocols approved by MacroGenics, Inc. Institutional Animal Care and Use Committee.

Female CD-1 nude (homozygous) mice (Crl:CD1-*Foxn1*^{nu}) and female SCID/CES1c knockout mice (5-7 weeks old) were obtained from Charles River Laboratories and maintained under pathogen-free conditions, with food and water supplied ad libitum. Human tumor cells (5×10^6) were resuspended in 1:1 medium (DMEM/F-12) and Matrigel basement membrane matrix (Corning) and implanted subcutaneously into the flank (A375.S2, Calu-6, PA-1) or mammary fat pad (MDA-MB-468) of mice. Mice were randomized into groups of 5-7 individuals per group. ADCs or vehicle control (phosphate buffered saline; PBS) were administered intravenously (IV) by tail vein injection (10 mL/kg) following growth of established tumors (100-150 mm³). Tumors were measured twice weekly by orthogonal measurements with electronic calipers, with tumor volumes calculated as: (length x width x height)/2. Details on statistical analyses is provided in the Supplementary Materials and Methods.

Patient-derived xenograft (PDX) studies were performed at Champions Oncology (Hackensack, NJ). Female Athymic Nude-*Foxn1*^{nu} from Envigo (head and neck, triple negative breast cancer models) or male NOG mice from Taconic (prostate cancer model), 6-8 weeks of age, were used for the PDX studies. Low passage tumor fragments were implanted into stock animals. When tumors reached 1.0 - 1.5 cm³, they were re-implanted into pre-study animals unilaterally on the left flank. When tumors reached an average tumor volume of 150-300 mm³, animals were matched by tumor volume into treatment or vehicle control groups. Three animals were assigned to each group and dosed IV by tail vein injection (10 mL/kg). Tumor volumes were measured twice weekly by calipers. Tumor volume was calculated using the formula TV= (width x 2) x length x 0.52. The study endpoint was determined when the mean tumor volume of the control group reached 1500 mm³.

In vitro serum stability and in vivo pharmacokinetics

Methods assessing in vitro serum stability and in vivo pharmacokinetics in mice and cynomolgus monkeys are described in the Supplementary Materials and Methods.

Toxicology

A repeat-dose toxicology study was conducted in naïve cynomolgus monkeys. Treatment groups (5 male/5 female) were administered either the control article (0.9% sodium chloride for injection USP) or MGC018 every 3 weeks (1, 3, 6 or 10 mg/kg/dose), for a total of 3 doses, by IV infusion over 1 hour. Six animals per group (3/sex/group) were necropsied on day 57, while the remaining recovery group animals (2/sex/group) were necropsied on day 120 following an 11-week recovery period. Mortality, clinical signs, body weights, food consumption, clinical pathology and toxicokinetics were monitored prior to and throughout the study. Indirect ophthalmoscopy was performed prestudy and prior to terminal sacrifice by a veterinary ophthalmologist. Following terminal sacrifice, gross necropsy, measurement of organ weights and histopathological examinations were conducted.

RESULTS

Selection and generation of MGC018

A pool of anti-B7-H3 mAbs was generated from a series of intact cell immunizations (13). The anti-B7-H3 mAb PRCA157 was selected as an ADC candidate from the pool of anti-B7-H3 mAbs based on its characteristics, including affinity, tumor versus normal tissue cross-reactivity profile, cynomolgus monkey B7-H3 cross-reactivity, cell internalization, and antitumor activity from screens as monomethyl auristatin E-based ADCs (25). PRCA157 was humanized by CDR grafting and fused to human C-kappa and gamma 1 constant regions, yielding the precursor MGA017 mAb, which retained the B7-H3 binding affinity of the parental murine mAb. When selecting a linker-payload it is important to consider the target biology in order to maximize potential antitumor activity and safety. B7-H3 is expressed heterogeneously within the tumor epithelium of solid cancers, and is also expressed by tumor-associated vasculature (21, 26) and cancer stem-like cells (27), therefore a linker payload that possessed bystander killing activity and the ability to kill slow- or non-dividing cells was desired. The valine-citrulline-*seco* duocarmycin hydroxybenzamide-azaindole (*vc-seco*-DUBA) linker payload was selected because (1) the protease cleavable peptide linker enables bystander killing of B7-H3 negative tumor cells present in heterogeneous solid tumors, and (2) the DNA alkylation activity of the duocarmycin payload can kill both dividing and non-dividing cells, and is resistant to multidrug resistance mechanisms (28-31). The *vc-seco*-DUBA linker-payload was conjugated to MGA017 following partial reduction of the interchain disulfide bonds to generate the MGC018 ADC with an average drug-to-antibody-ratio (DAR) of ~2.7 (24). MGC018 retained the binding affinity for human and cynomolgus monkey B7-H3 observed for MGA017 (Supplemental Table S2).

In vitro profile of MGC018

MGC018-mediated in vitro cytotoxicity was evaluated across a set of tumor cell lines representing multiple cancer types expressing varying levels of B7-H3. MGC018 was highly potent toward all B7-H3-expressing tumor cell lines examined. A summary of the IC₅₀ values for MGC018 cytotoxicity across the set of human tumor cell lines, as well as the antibody-binding capacity (ABC) and flow cytometry binding EC₅₀ values of each target line, is summarized in Table 1.

To confirm specificity, sensitivity to MGC018 of the B7-H3-expressing cancer line, Hs700T, was compared to a derivative of the line in which B7-H3 was stably knocked out. Stable knockout of B7-H3 was accomplished using B7-H3-specific clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 technology, with loss of B7-H3 cell-surface protein expression confirmed by flow cytometry (Supplemental Fig. S4). The Hs700T tumor cell line lacking B7-H3 expression (Hs700T/B7-H3 KO) was insensitive to MGC018, supporting the specificity of MGC018 toward B7-H3-expressing cancer lines (Fig. 1A).

Due to the nature of the cleavable *vc-seco*-DUBA linker-payload, internalization and proteolytic processing of MGC018 can lead to the release of active DUBA metabolite from dying tumor cells. The released active DUBA has the potential to be internalized by neighboring tumor cells, irrespective of B7-H3 target expression, and mediate bystander killing. To assess the potential for bystander killing, an in vitro co-culture experiment was conducted in which Hs700T/B7-H3 KO

cells, engineered to express stabilized red fluorescent protein (Hs700T/B7-H3 KO/RFP), were cultured alone, or in the presence of progressively larger amounts of unlabeled parental Hs700T cells. The cultures were incubated with MGC018 and the number of viable RFP-labeled cells (only Hs700T/B7-H3 KO cells were visible) were monitored by time-lapse fluorescent microscopy over a period of 5 days. As shown in Figure 1B, treatment with MGC018 had no effect on the viability of Hs700T/B7-H3 KO/RFP cells when cultured alone (gray bar vs. white bar). Conversely, MGC018 treatment led to killing of greater than 90% of parental Hs700T cells. However, in the co-culture setting, bystander killing of Hs700T/B7-H3 KO/RFP cells was observed, with the magnitude of bystander killing increasing as the number of parental Hs700T cells increased (orange bars). The Supplemental Fig. S5 contains a video link of the time-lapse bystander killing. Close examination of the time-lapse microscopy revealed a temporal relationship of the bystander cytotoxicity, with killing of parental Hs700T cells commencing prior to the killing of the Hs700T/B7-H3 KO/RFP cells, consistent with payload processing and DUBA release for the bystander killing.

Serum stability and pharmacokinetics

MGC018 exhibited a high level of stability in human and cynomolgus monkey sera (Supplemental Figs. S6A and S6B). As shown in Supplemental Table S3, 111% and 85% of conjugated antibody remained following the 240-hour incubation in human or cynomolgus monkey sera, respectively. Conversely, MGC018 exhibited poor stability in wild-type CD-1 nude mouse serum. The concentration of conjugated antibody decreased rapidly following exposure to mouse serum, while the total antibody concentration remained relatively stable over the time course of exposure to serum (Supplemental Fig. S6C). Following the 240-hour incubation in mouse serum, only ~3% of the conjugated antibody remained. (Supplemental Table S3). The serum instability was due to the rodent-specific carboxylesterase CES1c, as MGC018 exhibited greater stability in serum from CES1c knockout mice (Supplemental Fig. S6D), with ~65% of the conjugated antibody still remaining following the 240-hour incubation in CES1c knockout mouse serum. As noted above, the instability of MGC018 in mouse serum is consistent with previous reports showing that the *vc-seco*-DUBA linker payload is susceptible to the carboxylesterase CES1c present in rodents (24, 28).

The pharmacokinetics of MGC018 was investigated in cynomolgus monkeys following IV administration of MGC018 at 1, 3, 6 and 10 mg/kg. The maximum concentration (C_{max}) and total exposure (AUC_{inf}) increased with increasing dose for both total and conjugated antibody (Supplemental Fig. S7A-D). Consistent with the stability of MGC018 in cynomolgus serum, PK parameters including C_{max} , total exposure and clearance of conjugated and total antibody were similar at each dose level, and the detectable amount of unconjugated DUBA was very low (Supplemental Fig. S7A-D). PK parameters derived from noncompartmental analysis for conjugated antibody, total antibody, and DUBA are summarized in Supplemental Tables S4-S6.

For conjugated antibody and total antibody at the dose range of 1-6 mg/kg, the mean AUC_{inf} increased more than proportionally to dose level and the drug clearance (CL) decreased with increasing dose level of MGC018, suggesting a non-linear PK. However, at doses above 6 mg/kg, AUC_{inf} increased proportionally to dose level and the CL was essentially unchanged. These data suggest PK is linear at doses higher than 6 mg/kg, but non-linear at the lower doses.

This may be due to target-mediated drug disposition at lower doses and/or saturation of target specific clearance mechanisms with increasing doses.

The PK properties of MGC018 following a single dose via IV bolus of 5 mg/kg was assessed in CD-1 nude mice, the primary species employed in the tumor xenograft studies, and in SCID/CES1c knockout mice (32). Consistent with the observed instability of MGC018 in wild-type mouse serum, conjugated antibody degraded rapidly following IV administration of MGC018 in wild-type mice (Supplemental Fig. S7E). CL_F was considerably faster for conjugated antibody compared to total antibody (26.38 vs. 0.57 mL/hr/kg). In contrast, MGC018 exhibited favorable stability following IV administration in SCID/CES1c knockout mice (Supplemental Fig. S7F). CL_F was very similar for conjugated antibody compared to total antibody (0.66 vs. 0.75 mL/hr/kg), respectively. PK parameters derived from noncompartmental analysis for conjugated antibody and total antibody are summarized in Supplemental Table S7.

In vivo antitumor activity of MGC018

Single dose administration

Single dose administration of MGC018 led to a dose-dependent decrease in tumor growth of MDA-MB-468 triple negative breast cancer xenografts (Fig. 2A). A rapid decrease in tumor volume was observed following treatment with MGC018 at 6 mg/kg, with a 98% reduction in tumor volume compared to the vehicle control, and 4/5 complete regressions, whereas the control ADC reduced tumor volume by 57% with no complete regressions. Treatment at 3 mg/kg with MGC018 resulted in a rapid reduction in tumor volume for 3/5 tumors, leading to 1 complete regression and an overall 44% reduction in tumor volume compared to the vehicle control. Treatment at 3 mg/kg with the control ADC resulted in tumor stasis, with an overall 58% reduction in tumor volume over the course of the experiment.

In the PA-1 ovarian cancer xenograft, antitumor activity was observed at all 3 dose levels of MGC018 (Fig. 2B). A reduction in tumor volume of 89%, 91%, and 43%, with 3/6, 2/6, and 1/6 complete regressions, was observed following treatment with MGC018 at 10, 6, and 3 mg/kg, respectively. The control ADC exhibited minimal antitumor activity at these dose levels.

Antitumor activity was also observed against A375.S2 melanoma xenografts following a single dose administration of MGC018 (Fig. 2C). At the 3 mg/kg dose level, MGC018 treatment resulted in a 99% reduction in tumor volume and 6/7 complete regressions, compared to a 67% reduction with no complete responses for the control ADC. At the 1 mg/kg dose level, MGC018 treatment resulted in an 84% reduction in tumor volume compared to a 30% reduction for the control ADC.

In a fourth model, antitumor activity was observed against Calu-6 lung cancer xenografts following administration of a single dose of MGC018 (Fig. 2D). A reduction in tumor volume of 91%, 84% and 72% was observed following treatment with MGC018 at 10, 6 and 3 mg/kg, respectively. By comparison, treatment with the control ADC led to a 48% and 0% reduction in tumor volume at 10 and 3 mg/kg, respectively.

Based on the poor stability of MGC018 in wild-type mouse serum with ensuing limited exposure following administration in CD-1 nude mice, an *in vivo* efficacy study was conducted to assess the antitumor activity of MGC018 in SCID/CES1c knockout mice. SCID/CES1c knockout mice bearing Calu-6 lung cancer xenografts were administered a single dose of MGC018 or the control ADC at 10, 6 or 3 mg/kg. As shown in Fig. 2E, treatment with MGC018 led to a dose-dependent antitumor response, with a reduction in tumor volume of 100%, 97% and 79% for the 10, 6 and 3 mg/kg dose, respectively, and 5/6 animals treated at 10 mg/kg had complete regressions.

Repeat-dose administration

In the clinical setting, the majority of ADCs are administered via a repeat-dose regimen, with intervals ranging from once weekly to once every 6 weeks. To evaluate the antitumor activity following repeat-dose administration, a study was performed in PA-1 ovarian cancer xenografts comparing single dose administration to weekly times 4 (QWx4) and every other week times 4 (Q2Wx4) administration. As shown in Fig. 3A, repeat-dose administration of MGC018 led to greater antitumor response than administration of a single dose. A single dose of 3 mg/kg MGC018 achieved short-term tumor stasis of approximately 10 days, followed by tumor regrowth. Repeat-dose administration of 3 mg/kg MGC018 led to tumor regression, resulting in a 97% and 92% reduction in tumor volume (4/6 and 2/6 complete regressions) for QWx4 and Q2Wx4 treatment, respectively. The control ADC exhibited minimal antitumor activity under all dose regimens. Based on these results, repeat-dose administration studies (QWx4) were extended to four xenograft models with lower dose levels of MGC018.

Repeat-dose administration of MDA-MB-468 triple negative breast cancer tumor xenografts with MGC018 resulted in a rapid decrease in tumor volume and strong overall antitumor response (Fig. 3B). Repeat-dose administration (QWx4) resulted in a reduction in tumor volume of 98% and 60%, and complete regressions in 7/7 and 3/7 animals were observed at 1 and 0.3 mg/kg MGC018, respectively. The control ADC exhibited reduced antitumor activity compared to MGC018, with 17% and 7% reduction in tumor volume for 1 and 0.3 mg/kg, respectively. No complete regressions were observed in the control ADC treatment groups.

Dose-dependent antitumor activity was also observed toward PA-1 ovarian cancer xenografts following repeat-dose administration of MGC018 (Fig. 3C). Repeat-dose administration (QWx4) resulted in a reduction in tumor volume of 93% and 62% for 1 and 0.3 mg/kg MGC018, respectively. Complete regressions were observed in 2/7 animals at the 1 mg/kg MGC018. The control ADC exhibited reduced antitumor activity compared to MGC018, with 42% and 34% reduction in tumor volume for 1 and 0.3 mg/kg, respectively, with no complete regressions observed in any control ADC treatment group animals.

Antitumor activity was observed toward A375.S2 melanoma xenografts following repeat-dose administration of MGC018 at both of the doses tested (Fig. 3D). Repeat-dose administration (QWx4) resulted in a reduction in tumor volume of 100% and 75% for 1 and 0.3 mg/kg MGC018, respectively. Complete regressions were observed in 5/7 animals at the 1 mg/kg MGC018. The control ADC exhibited reduced antitumor activity compared to MGC018, with 68% and 44% reduction in tumor volume for 1 and 0.3 mg/kg, respectively, with one complete regression observed in the 1 mg/kg control ADC treatment group.

As shown in Fig. 3E, antitumor activity was also observed toward Calu-6 lung cancer xenografts following repeat-dose administration of MGC018. Repeat-dose administration (QWx4) resulted in a reduction in tumor volume of 83%, 70%, and 22% for 3, 1, and 0.3 mg/kg MGC018, respectively. Minimal activity was observed for the control ADC under the same dosing regimens.

Although antitumor activity was observed in some models with the control ADC, which is conjugated with *vc-seco*-DUBA at a similar DAR, the antitumor activity was substantially less than that observed with MGC018, consistent with target-mediated activity of MGC018. Background antitumor activity with control ADCs, including the DUBA-based control ADC employed in our studies (24, 33), has been observed by other investigators, and may result from extracellular cleavage of the linker-payload or macropinocytosis (34). To address the limitations of the control ADC, and confirm the requirement for targeting the DUBA payload to target-positive cancer cells, we conducted a study in Calu-6 lung cancer xenografts, comparing MGC018, with MGA017 (unconjugated antibody) in combination with SYD978 (free payload). In the study, we observed that MGC018 had potent antitumor activity, while the combination of MGA017 plus SYD978 had very little antitumor activity (Supplemental Fig. S8).

Patient-derived xenograft studies

The antitumor activity of MGC018 was also evaluated in a set of PDX models which exhibit heterogeneous expression of B7-H3 (Fig. 4A). Treatment of a triple negative breast cancer PDX model (H-score 180) with MGC018 (3 mg/kg; QWx2) led to a rapid tumor regression, with a 98% reduction in tumor volume compared to the vehicle control (Fig. 4B). Minimal activity was observed for the control ADC. In a prostate cancer PDX model (H-score 130), rapid tumor regression, with a reduction of 95% compared to the vehicle control, was observed following treatment with MGC018 (3 mg/kg; QWx3) (Fig. 4C). Treatment with the control ADC did not lead to tumor regression, and tumor volume was reduced by 43% compared to the vehicle control. Similarly, treatment of a squamous cell carcinoma of the head and neck PDX model (H-score 240) with MGC018 (3 mg/kg; Q2Wx2) led to tumor regression and a 98% reduction in tumor volume compared to the vehicle control (Fig. 4D). Treatment with the control ADC did not lead to tumor regression with only 52% tumor reduction compared to the vehicle control.

MGC018 was well tolerated in all the mouse models tested. No significant weight loss or clinical signs of toxicity were observed.

Toxicology

Cynomolgus monkey was selected as a representative toxicology species for humans. The binding affinity between human and cynomolgus monkey is nearly identical for B7-H3 ($K_D = 22.7$ nM and 22.1 nM for human and cynomolgus monkey, respectively; Supplemental Table S2) and the normal tissue expression pattern of B7-H3 in cynomolgus monkey is comparable with that observed in human (13).

MGC018 administered as a 1-hour IV infusion every three weeks (total of 3 doses) was tolerated in cynomolgus monkeys up to 10 mg/kg/dose, the highest dose tested. All findings were minimal to mild in nature, and reversible (Table 2). MGC018-related clinical signs that were

predominantly dose dependent included skin findings (hyperpigmentation, dry skin, erythema) and sparse hair noted, particularly at $\geq 6\text{mg/kg/dose}$. Slight changes in body weight gain were noted but not considered adverse.

No MGC018-related life-threatening toxicities, irreversible findings or mortality was observed in the study. MGC018-related changes in clinical pathology parameters included a mild acute inflammatory response (increases in C-reactive protein and fibrinogen), transient decreases in neutrophils, mild to moderate decrease in reticulocyte counts, decreases in lymphocytes, transient minimal to mild increases in aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) and minimal decreases in alkaline phosphatase (ALP), all of which were dose responsive in nature, reversed prior to or during the recovery phase, and did not have microscopic correlates.

Based on the limited and reversible nature of the findings in the study, the highest non-severely toxic dose (HNSTD) was determined to be the highest dose administered, 10 mg/kg/dose, which corresponds to peak and systemic exposure (gender combined mean) of 310.9 $\mu\text{g/mL}$ (C_{max}) and 15,075 $\text{hr}^*\mu\text{g/mL}$ (AUC_{inf}) for the conjugated antibody, 250.5 $\mu\text{g/mL}$ (C_{max}) and 14,053 $\text{hr}^*\mu\text{g/mL}$ (AUC_{inf}) for the total antibody and 118.0 pg/mL (C_{max}) and 7,412 $\text{hr}^*\text{pg/mL}$ (AUC_{last}) for the free DUBA payload, respectively.

Due to the instability of MGC018 in mouse serum, rapid clearance in wild-type mice, and relative stability in cynomolgus monkey, pharmacokinetic/pharmacodynamic assessments and estimates of therapeutic index (TI) are difficult to perform with confidence. The exposure of conjugated antibody in the cynomolgus monkey at the HNSTD of 10 mg/kg was 15,075 $\text{hr}^*\mu\text{g/mL}$ (AUC_{inf}), while the exposure in the CD-1 nude mouse was 190 $\text{hr}^*\mu\text{g/mL}$ (AUC_{inf}) at a dose of 5 mg/kg, a dose considerably higher than the efficacious dose in the repeat-dose xenograft studies in the CD-1 nude mouse. Recognizing the limitations, the preclinical data is consistent with a large therapeutic index, and provides evidence for a favorable clinical therapeutic window.

Discussion

We previously described a target-unbiased approach to generating mAbs to overexpressed cell-surface cancer antigens with therapeutic potential (35). Using this approach, we identified B7-H3 as a cell surface protein with limited expression on normal tissues but overexpressed on the epithelium and tumor-associated vasculature in solid cancers. Validation studies showed that ligation of B7-H3 by several mAbs in our panel led to mAb internalization and antitumor activity toward B7-H3-expressing tumor cells when the mAbs were conjugated to auristatin (25). Based on these results, we selected a lead candidate mAb and sought to develop an ADC targeting B7-H3 for the treatment of cancer.

In this article, we described the preclinical profile of MGC018, a duocarmycin-based ADC directed against B7-H3. In vitro studies showed that MGC018 mediated specific killing across a range of B7-H3-expressing solid tumor cell types. Owing to the hydrophobic nature of the DUBA payload, together with its conjugation via a cleavable linker, MGC018 mediated bystander killing of B7-H3-negative tumor cells in the presence of B7-H3-positive tumor cells. MGC018 exhibited potent antitumor activity in murine xenograft studies, which in general

correlated with the in vitro cytotoxicity observed for the four models. MGC018 was active toward lung, breast and ovarian cancer, as well as melanoma cell line-derived xenografts following a single dose administration; furthermore, the antitumor activity was enhanced following repeat-dose administration at dose levels predicted to be achievable in the clinic based on the cynomolgus monkey toxicology profile. Importantly, MGC018 was also active toward triple negative breast, prostate and head and neck patient-derived xenografts that exhibit heterogeneous expression of B7-H3, consistent with the bystander killing observed in co-culture experiments.

In the cynomolgus monkey, MGC018 was tolerated up to 10 mg/kg/dose, the highest dose tested, when administered once every three weeks for a total of 3 doses. The toxicology profile of MGC018 was consistent with the toxicity profile reported for SYD985 (24, 36), an anti-Her2 ADC bearing the same *vc-seco*-DUBA linker-payload as MGC018, that is currently in Phase III clinical testing. Several toxicities are consistent with those reported for various linker/payload platforms (i.e. transient decrease in white blood cell populations and red blood cell mass, transient increases in transaminases) (37-40), but also findings such as minimal skin hyperpigmentation and epidermal hyperplasia appear to be associated with duocarmycin payloads (41). Low level expression of both B7-H3 and Her2 has been observed in the basal layer of epithelium and sweat glands of the skin in cynomolgus monkeys; therefore, a contribution of target expression toward the skin findings cannot be ruled out. Weak expression of B7-H3 has also been observed in liver sinusoid lining cells and hepatocytes, therefore the potential for target-mediated liver toxicity was of particular interest. Administration of MGC018 in the cynomolgus monkey led to only transient, minimal to mild increases in AST and ALT levels. While it is possible that target expression contributed to the transient increases, the AST and ALT levels remained within the historical normal range for most animals and were not associated with microscopic correlates in the liver or muscle. Neutropenia is a commonly observed toxicity with ADCs, particularly ADCs conjugated to MMAE via a cleavable valine-citrulline linker, and is proposed to represent a non-target mediated toxicity driven by multiple mechanisms (40, 42). As we have not observed expression of B7-H3 in neutrophils nor their precursor cells, we hypothesize that the transient decreases observed in neutrophil counts in the cynomolgus monkey following administration of MGC018 were driven by non-target-mediated mechanisms. Other toxicities associated with tubulin modifying agents, including thrombocytopenia and peripheral sensory neuropathy, were not observed with MGC018.

The *vc-seco*-DUBA linker-payload is susceptible to the rodent-specific carboxylesterase CES1c, which leads to poor stability in wild-type mouse plasma and poor exposure in wild-type mice (24, 28, 32). As the in vivo efficacy of MGC018 correlates with exposure, based on dose fractionation xenograft studies, the antitumor activity of MGC018 was likely underrepresented in xenograft studies in wild-type mice. Data from our studies employing SCID/CES1c knockout mice is consistent with this notion. The antitumor activity of MGC018 toward tumor xenografts in SCID/CES1c knockout mice showed that greater targeted activity of MGC018 was achieved, in terms of reduction in tumor volume and rate of response, in the SCID/CES1c knockout mice compared to wild-type CD-1 nude mice.

B7-H3 is an attractive target for cancer therapy due to its overexpression in cancer and association with disease progression and survival. We have developed two clinical-stage antibody-based therapeutics targeting B7-H3 for cancer: enoblituzumab, an Fc-optimized mAb with enhanced effector function, and MGD009, a B7-H3 x CD3 redirecting T cell DART[®] molecule. Interim results from an ongoing Phase 1 study in patients with refractory B7-H3-expressing tumors and/or tumor associated vasculature indicate enoblituzumab was well tolerated as a single agent, showed antitumor activity across several tumor types, and increased T-cell repertoire clonality in the peripheral blood of patients following treatment. The anti-B7-H3 mAb 8H9 has been investigated preclinically as both an 8H9 single-chain Fv conjugated to *Pseudomonas* exotoxin (PE38) (43, 44) and as a radiolabeled mAb (10, 22, 45). ¹³¹I-8H9 and ¹²⁴I-8H9 mAbs are currently under evaluation in pediatric central nervous system-associated cancers, where the mAbs are administered via compartmental intrathecal administration and convection-enhanced delivery (45). Beyond the published clinical studies, a B7-H3 ADC bearing an exetecan-derived payload (46), a novel afucosylated B7-H3 mAb (47) and chimeric antigen receptor T cells targeting B7-H3 (48, 49) have shown encouraging antitumor activity in preclinical studies.

Preclinical studies performed with a monomethyl-auristatin E- and pyrrolobenzodiazepine-conjugated B7-H3 ADC are particularly noteworthy (50). In several cancer types, including renal cell carcinoma and ovarian cancer, B7-H3 has been observed to be expressed not only in the tumor epithelium, but also in the tumor-associated vasculature, and provides an opportunity to target not only the tumor epithelial component, but also the tumor-associated stroma. Indeed, Seaman et al. (50) showed that an anti-B7-H3 ADC can destroy both the tumor epithelium and tumor vasculature, eradicating established tumors and metastasis, resulting in improved long-term overall survival. Importantly, this study showed that sensitivity of the tumor vasculature was dependent on the nature of the payload. While a pyrrolobenzodiazepine-conjugated ADC was effective at killing both tumor epithelium and tumor-associated vasculature, the auristatin-conjugated ADC was only effective in killing tumor epithelium. The resistance of tumor-associated vasculature to the auristatin-conjugated ADC was linked in part to expression of the ATP-binding cassette transporter P-glycoprotein. Additionally, differences in the growth rate of tumor epithelium compared to tumor-associated vasculature may contribute to resistance to tubulin modifying agents, since those agents are only cytotoxic to actively dividing cells. Although we were unable to test MGC018 in this context due to its lack of cross-reactivity with mouse B7-H3, duocarmycin is a DNA damaging agent that is cytotoxic toward both dividing and non-dividing tumor cells and would be anticipated to be cytotoxic toward both tumor epithelium and tumor-associated vasculature. Furthermore, duocarmycin is also a poor substrate for P-glycoprotein (28, 29).

In conclusion, we have developed an anti-B7-H3 ADC, MGC018, that incorporates a duocarmycin-based DNA alkylating payload via a cleavable valine-citrulline linker. MGC018 exhibits potent antitumor activity across a range of human tumor xenografts, mediates bystander killing and shows a favorable safety profile in cynomolgus monkey. The antitumor activity observed preclinically with MGC018, together with the positive safety profile, provides evidence

of a potentially favorable therapeutic index and supports the continued development of MGC018 for the treatment of solid cancers.

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References

1. Zang X, Allison JP. The B7 family and cancer therapy: costimulation and coinhibition. *Clin Cancer Res.* 2007;13(18 Pt 1):5271-9.
2. Ling V, Wu PW, Spaulding V, Kieleczawa J, Luxenberg D, Carreno BM, et al. Duplication of primate and rodent B7-H3 immunoglobulin V- and C-like domains: divergent history of functional redundancy and exon loss. *Genomics.* 2003;82(3):365-77.
3. Steinberger P, Majdic O, Derdak SV, Pfistershammer K, Kirchberger S, Klauser C, et al. Molecular characterization of human 4Ig-B7-H3, a member of the B7 family with four Ig-like domains. *J Immunol;* 2004;2004. p. 2352-9.
4. Hofmeyer KA, Ray A, Zang X. The contrasting role of B7-H3. *Proc Natl Acad Sci U S A.* 2008;105(30):10277-8.
5. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer.* 2012;12(4):252-64.
6. Prasad DV, Nguyen T, Li Z, Yang Y, Duong J, Wang Y, et al. Murine B7-H3 is a negative regulator of T cells. *J Immunol.* 2004;173(4):2500-6.
7. Suh WK, Gajewska BU, Okada H, Gronski MA, Bertram EM, Dawicki W, et al. The B7 family member B7-H3 preferentially down-regulates T helper type 1-mediated immune responses. *Nat Immunol.* 2003;4(9):899-906.
8. Wang L, Fraser CC, Kikly K, Wells AD, Han R, Coyle AJ, et al. B7-H3 promotes acute and chronic allograft rejection. *Eur J Immunol.* 2005;35(2):428-38.
9. Vigdorovich V, Ramagopal UA, Lazar-Molnar E, Sylvestre E, Lee JS, Hofmeyer KA, et al. Structure and T Cell Inhibition Properties of B7 Family Member, B7-H3. *Structure.* 2013.
10. Ahmed M, Cheng M, Zhao Q, Goldgur Y, Cheal SM, Guo HF, et al. Humanized Affinity-matured Monoclonal Antibody 8H9 Has Potent Antitumor Activity and Binds to FG Loop of Tumor Antigen B7-H3. *J Biol Chem.* 2015;290(50):30018-29.
11. Zang X, Thompson RH, Al-Ahmadie HA, Serio AM, Reuter VE, Eastham JA, et al. B7-H3 and B7x are highly expressed in human prostate cancer and associated with disease spread and poor outcome. *Proc Natl Acad Sci U S A.* 2007;104(49):19458-63.
12. Chavin G, Sheinin Y, Crispen PL, Boorjian SA, Roth TJ, Rangel L, et al. Expression of immunosuppressive B7-H3 ligand by hormone-treated prostate cancer tumors and metastases. *Clin Cancer Res.* 2009;15(6):2174-80.
13. Loo D, Alderson RF, Chen FZ, Huang L, Zhang W, Gorlatov S, et al. Development of an Fc-enhanced Anti-B7-H3 Monoclonal Antibody with Potent Antitumor Activity. *Clin Cancer Res.* 2012;18(14):3834-45.
14. Roth TJ, Sheinin Y, Lohse CM, Kuntz SM, Frigola X, Inman BA, et al. B7-H3 ligand expression by prostate cancer: a novel marker of prognosis and potential target for therapy. *Cancer Res.* 2007;67(16):7893-900.
15. Wang J, Chong KK, Nakamura Y, Nguyen L, Huang SK, Kuo C, et al. B7-H3 associated with tumor progression and epigenetic regulatory activity in cutaneous melanoma. *J Invest Dermatol.* 2013;133(8):2050-8.
16. Liu C, Liu J, Wang J, Liu Y, Zhang F, Lin W, et al. B7-H3 expression in ductal and lobular breast cancer and its association with IL-10. *Mol Med Rep.* 2013;7(1):134-8.
17. Maeda N, Yoshimura K, Yamamoto S, Kuramasu A, Inoue M, Suzuki N, et al. Expression of B7-H3, a potential factor of tumor immune evasion in combination with the number of regulatory T cells, affects against recurrence-free survival in breast cancer patients. *Ann Surg Oncol.* 2014;21 Suppl 4:S546-S54.

18. Sun Y, Wang Y, Zhao J, Gu M, Giscombe R, Lefvert AK, et al. B7-H3 and B7-H4 expression in non-small-cell lung cancer. *Lung Cancer*. 2006;53(2):143-51.
19. Mao Y, Li W, Chen K, Xie Y, Liu Q, Yao M, et al. B7-H1 and B7-H3 are independent predictors of poor prognosis in patients with non-small cell lung cancer. *Oncotarget*. 2015;6(5):3452-61.
20. Chen JT, Chen CH, Ku KL, Hsiao M, Chiang CP, Hsu TL, et al. Glycoprotein B7-H3 overexpression and aberrant glycosylation in oral cancer and immune response. *Proc Natl Acad Sci U S A*. 2015;112(42):13057-62.
21. Crispen PL, Sheinin Y, Roth TJ, Lohse CM, Kuntz SM, Frigola X, et al. Tumor cell and tumor vasculature expression of B7-H3 predict survival in clear cell renal cell carcinoma. *Clin Cancer Res*. 2008;14(16):5150-7.
22. Modak S, Kramer K, Gultekin SH, Guo HF, Cheung NK. Monoclonal antibody 8H9 targets a novel cell surface antigen expressed by a wide spectrum of human solid tumors. *Cancer Res*. 2001;61(10):4048-54.
23. Xu H, Cheung IY, Guo HF, Cheung NK. MicroRNA miR-29 modulates expression of immunoinhibitory molecule B7-H3: potential implications for immune based therapy of human solid tumors. *Cancer Res*. 2009;69(15):6275-81.
24. Dokter W, Ubink R, van der Lee M, van der Vleuten M, van Achterberg T, Jacobs D, et al. Preclinical profile of the HER2-targeting ADC SYD983/SYD985: introduction of a new duocarmycin-based linker-drug platform. *Mol Cancer Ther*. 2014;13(11):2618-29.
25. Loo D, Scribner J, Son T, Hooley J, Hotaling T, Chiechi M, et al. Anti-B7-H3 antibody-drug conjugates as potential therapeutics for solid cancer. *Cancer Research*. 2016;76(14 Suppl). Abstract 1201.
26. Zang X, Sullivan PS, Soslow RA, Waitz R, Reuter VE, Wilton A, et al. Tumor associated endothelial expression of B7-H3 predicts survival in ovarian carcinomas. *Mod Pathol*. 2010.
27. Bin Z, Guangbo Z, Yan G, Huan Z, Desheng L, Xueguang Z. Overexpression of B7-H3 in CD133+ colorectal cancer cells is associated with cancer progression and survival in human patients. *J Surg Res*. 2014;188(2):396-403.
28. Elgersma RC, Coumans RG, Huijbregts T, Menge WM, Joosten JA, Spijker HJ, et al. Design, Synthesis, and Evaluation of Linker-Duocarmycin Payloads: Toward Selection of HER2-Targeting Antibody-Drug Conjugate SYD985. *Mol Pharm*. 2015;12(6):1813-35.
29. Giddens AC, Lee HH, Lu GL, Miller CK, Guo J, Lewis Phillips GD, et al. Analogues of DNA minor groove cross-linking agents incorporating aminoCBI, an amino derivative of the duocarmycins: Synthesis, cytotoxicity, and potential as payloads for antibody-drug conjugates. *Bioorg Med Chem*. 2016;24(22):6075-81.
30. Kobayashi E, Okamoto A, Asada M, Okabe M, Nagamura S, Asai A, et al. Characteristics of antitumor activity of KW-2189, a novel water-soluble derivative of duocarmycin, against murine and human tumors. *Cancer Res*. 1994;54(9):2404-10.
31. Gomi K, Kobayashi E, Miyoshi K, Ashizawa T, Okamoto A, Ogawa T, et al. Anticellular and antitumor activity of duocarmycins, novel antitumor antibiotics. *Jpn J Cancer Res*. 1992;83(1):113-20.
32. Ubink R, Dirksen EHC, Rouwette M, Bos ES, Janssen I, Egging DF, et al. Unraveling the Interaction between Carboxylesterase 1c and the Antibody-Drug Conjugate SYD985: Improved Translational PK/PD by Using Ces1c Knockout Mice. *Mol Cancer Ther*. 2018;17(11):2389-98.
33. van der Lee MM, Groothuis PG, Ubink R, van der Vleuten MA, van Achterberg TA, Loosveld EM, et al. The Preclinical Profile of the Duocarmycin-Based HER2-Targeting ADC SYD985 Predicts for Clinical Benefit in Low HER2-Expressing Breast Cancers. *Mol Cancer Ther*. 2015;14(3):692-703.
34. Ha KD, Bidlingmaier SM, Liu B. Macropinocytosis Exploitation by Cancers and Cancer Therapeutics. *Front Physiol*. 2016;7:381.

35. Loo DT, Mather JP. Antibody-based identification of cell surface antigens: targets for cancer therapy. *Curr Opin Pharmacol*. 2008;8(5):627-31.
36. Banerji U, van Herpen CML, Saura C, Thistlethwaite F, Lord S, Moreno V, et al. Trastuzumab duocarmazine in locally advanced and metastatic solid tumours and HER2-expressing breast cancer: a phase 1 dose-escalation and dose-expansion study. *Lancet Oncol*. 2019.
37. Hinrichs MJ, Dixit R. Antibody Drug Conjugates: Nonclinical Safety Considerations. *AAPS J*. 2015;17(5):1055-64.
38. Masters JC, Nickens DJ, Xuan D, Shazer RL, Amantea M. Clinical toxicity of antibody drug conjugates: a meta-analysis of payloads. *Invest New Drugs*. 2018;36(1):121-35.
39. Saber H, Leighton JK. An FDA oncology analysis of antibody-drug conjugates. *Regul Toxicol Pharmacol*. 2015;71(3):444-52.
40. Donaghy H. Effects of antibody, drug and linker on the preclinical and clinical toxicities of antibody-drug conjugates. *MAbs*. 2016;8(4):659-71.
41. Owonikoko TK, Hussain A, Stadler WM, Smith DC, Kluger H, Molina AM, et al. First-in-human multicenter phase I study of BMS-936561 (MDX-1203), an antibody-drug conjugate targeting CD70. *Cancer Chemother Pharmacol*. 2016;77(1):155-62.
42. Zhao H, Gulesserian S, Malinao MC, Ganesan SK, Song J, Chang MS, et al. A Potential Mechanism for ADC-Induced Neutropenia: Role of Neutrophils in Their Own Demise. *Mol Cancer Ther*. 2017;16(9):1866-76.
43. Onda M, Wang QC, Guo HF, Cheung NK, Pastan I. In vitro and in vivo cytotoxic activities of recombinant immunotoxin 8H9(Fv)-PE38 against breast cancer, osteosarcoma, and neuroblastoma. *Cancer Res*. 2004;64(4):1419-24.
44. Luther N, Cheung NK, Souliopoulos EP, Karampelas I, Bassiri D, Edgar MA, et al. Interstitial infusion of glioma-targeted recombinant immunotoxin 8H9scFv-PE38. *Mol Cancer Ther*. 2010;9(4):1039-46.
45. Kramer K, Kushner BH, Modak S, Pandit-Taskar N, Smith-Jones P, Zanzonico P, et al. Compartmental intrathecal radioimmunotherapy: results for treatment for metastatic CNS neuroblastoma. *J Neurooncol*. 2010;97(3):409-18.
46. Ogitani Y, Abe Y, Iguchi T, Yamaguchi J, Terauchi T, Kitamura M, et al. Wide application of a novel topoisomerase I inhibitor-based drug conjugation technology. *Bioorg Med Chem Lett*. 2016;26(20):5069-72.
47. Nagase-Zembutsu A, Hirotsu K, Yamato M, Yamaguchi J, Takata T, Yoshida M, et al. Development of DS-5573a: A novel afucosylated mAb directed at B7-H3 with potent antitumor activity. *Cancer Sci*. 2016;107(5):674-81.
48. Du H, Hirabayashi K, Ahn S, Kren NP, Montgomery SA, Wang X, et al. Antitumor Responses in the Absence of Toxicity in Solid Tumors by Targeting B7-H3 via Chimeric Antigen Receptor T Cells. *Cancer Cell*. 2019;35(2):221-37 e8.
49. Majzner RG, Theruvath JL, Nellan A, Heitzeneder S, Cui Y, Mount CW, et al. CAR T Cells Targeting B7-H3, a Pan-Cancer Antigen, Demonstrate Potent Preclinical Activity Against Pediatric Solid Tumors and Brain Tumors. *Clin Cancer Res*. 2019;25(8):2560-74.
50. Seaman S, Zhu Z, Saha S, Zhang XM, Yang MY, Hilton MB, et al. Eradication of Tumors through Simultaneous Ablation of CD276/B7-H3-Positive Tumor Cells and Tumor Vasculature. *Cancer Cell*. 2017;31(4):501-15 e8.

Tables

Table 1: Summary of B7-H3 expression and MGC018 in vitro cytotoxicity against the indicated human tumor cell lines

| Indication | Breast Cancer | Melanoma | Ovarian Cancer | Lung Cancer | | Pancreatic Cancer | Glio-blastoma | Colorectal Cancer | B-Cell Lymphoma |
|---|---------------|----------|----------------|-------------|-----------|-------------------|---------------|-------------------|-----------------|
| Cancer Cell Line | MDA-MB-468 | A375.S2 | PA-1 | Calu-6 | NCI-H1703 | Hs700T | LN-229 | SW48 | Raji |
| QFACS (ABC) | 57,000 | 153,000 | 73,700 | 138,000 | 122,000 | 310,000 | 139,000 | 59,800 | Negative |
| MGC018 FLOW Binding EC ₅₀ (nM) | 460 | 456 | 323 | 406 | 354 | 797 | 420 | 253 | Negative |
| MGC018 Cytotoxicity IC ₅₀ (pM) | 767 | 181 | 275 | 260 | 585 | 319 | 910 | 1447 | >10,000 |

Abbreviations: ABC: antibody-binding capacity; IC₅₀: half-maximal inhibitory concentration; EC₅₀: half-maximal effective concentration; QFACS: quantitative flow cytometry.

Table 2: Summary of findings in the GLP repeat dose toxicology study in cynomolgus monkey

| Parameter | Findings |
|-----------------------|---|
| Clinical Observations | <ul style="list-style-type: none"> • Mean body weight increased in all dose groups; however, minimal decrease in overall body weight gain at 10 mg/kg • Dose dependent hyperpigmentation and dry skin. Erythema at locations of dry skin at ≥ 6 mg/kg. Resolved during recovery |
| Clinical Pathology | <ul style="list-style-type: none"> • Transient decrease in neutrophil counts at ≥ 6 mg/kg, minimal/mild decrease in lymphocytes at 10 mg/kg. Lacked microscopic correlates, resolved during recovery • Decrease in red cell mass associated with increase in reticulocytes; less pronounced increase in reticulocytes at ≥ 6 mg/kg. Fully resolved during recovery • Increased C-reactive protein and fibrinogen. Resolved at subsequent collections • Transient minimal/mild increases in AST and/or ALT. Lacked correlative microscopic findings in the liver or muscle, resolved at subsequent collections |
| Gross Pathology | <ul style="list-style-type: none"> • Skin hyperpigmentation most prominent on limbs and head at ≥ 1 mg/kg. Generally dose dependent in incidence and severity. Ongoing reversibility during recovery |
| Histopathology | <ul style="list-style-type: none"> • Skin epidermal hyperplasia, lymphocytic infiltrate, single cell necrosis and increased pigment most prominent at ≥ 6 mg/kg. Ongoing resolution during recovery |

Abbreviations: AST: aspartate aminotransferase; ALT: alanine aminotransferase.

Figures Legends

Figure 1. MGC018 specificity and bystander activity. **A**, evaluation of the in vitro cytotoxicity of MGC018 on Hs700T parental line (B7-H3 positive; closed circles) compared to a derivative of Hs700T in which B7-H3 was stably knocked out (Hs700T/B7-H3 KO; open square). Cell viability was measured by the alamarBlue cytotoxicity assay. **B**, evaluation of bystander activity of MGC018. The indicated number of Hs700T parental cells (unlabeled, antigen positive) and Hs700T/B7-H3 KO/RFP cells (expressing red fluorescent protein, antigen negative) were mixed and incubated with 6.7 nM (1 μ g/ml) MGC018 or left untreated. The number of red fluorescent Hs700T/B7-H3 KO/RFP cells remaining following a 5-day incubation was counted with an IncuCyte Live-Cell Analysis System.

Figure 2. MGC018 exhibits antitumor activity in multiple xenograft models. **A**, MDA-MB-468 triple negative breast cancer orthotopic xenografts were treated with MGC018 or control ADC at 6 and 3 mg/kg. **B**, PA-1 ovarian cancer subcutaneous xenografts were treated with MGC018 or control ADC at 10, 6 and 3 mg/kg. **C**, A375.S2 melanoma subcutaneous xenografts were treated with MGC018 or control ADC at 3 and 1 mg/kg. **D**, Calu-6 lung cancer subcutaneous xenografts were treated with MGC018 or control ADC at 10, 6 and 3 mg/kg. **E**, Calu-6 lung cancer subcutaneous xenografts were treated with MGC018 ADC at 10, 6 and 3 mg/kg. Studies performed in CD-1 nude mice (**A-D**) or SCID/CES1c knockout mice (**E**). Treatment day(s) are indicated by the arrows. Tumor volume is shown as group mean \pm SEM. Representative images and H-scores are shown for each xenograft model.

Figure 3. MGC018 exhibits antitumor activity in multiple xenograft models following repeat-dose administration. **A**, PA-1 ovarian cancer subcutaneous xenografts were treated with MGC018 or control ADC at 3 mg/kg as a single dose (QWx1), QWx4, or Q2Wx4. **B**, MDA-MB-468 triple negative breast cancer orthotopic xenografts were treated with MGC018 or control ADC at 1 and 0.3 mg/kg QWx4. **C**, PA-1 ovarian cancer subcutaneous xenografts were treated with MGC018 or control ADC at 1 and 0.3 mg/kg QWx4. **D**, A375.S2 melanoma subcutaneous xenografts were treated with MGC018 or control ADC at 1 and 0.3 mg/kg QWx4. **E**, Calu-6 lung cancer subcutaneous xenografts were treated with MGC018 or control ADC at 3, 1 and 0.3 mg/kg QWx4. All studies were performed in CD-1 nude mice. Treatment days are indicated by the arrows. Tumor volume is shown as group mean \pm SEM.

Figure 4. MGC018 exhibits antitumor activity in multiple PDX models. **A**, representative examples of B7-H3 FFPE staining in the PDX models of triple negative breast cancer, prostate cancer and head and neck cancer, as well as IgG1 isotype control staining. **B**, triple negative breast cancer subcutaneous PDX model treated with MGC018 or control ADC at 3 mg/kg

(QWx2). **C**, prostate cancer subcutaneous PDX model treated with MGC018 or control ADC at 3 mg/kg (QWx3). **D**, head and neck cancer subcutaneous PDX model treated with MGC018 or control ADC at 3 mg/kg (QWx2). Treatment days are indicated by the arrows. Tumor volume is shown as group mean \pm SEM.

Figure 1

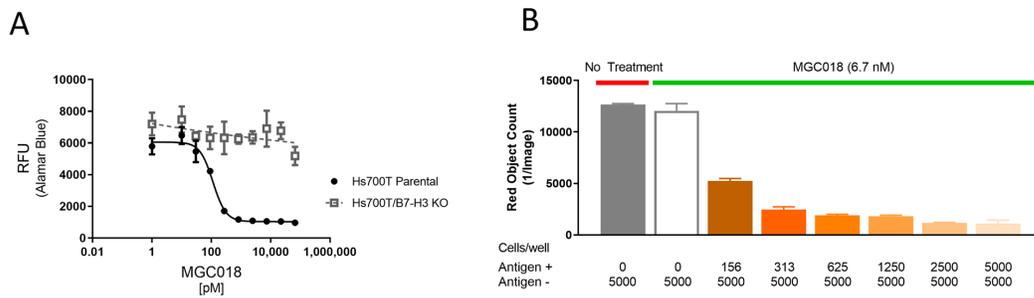


Figure 2

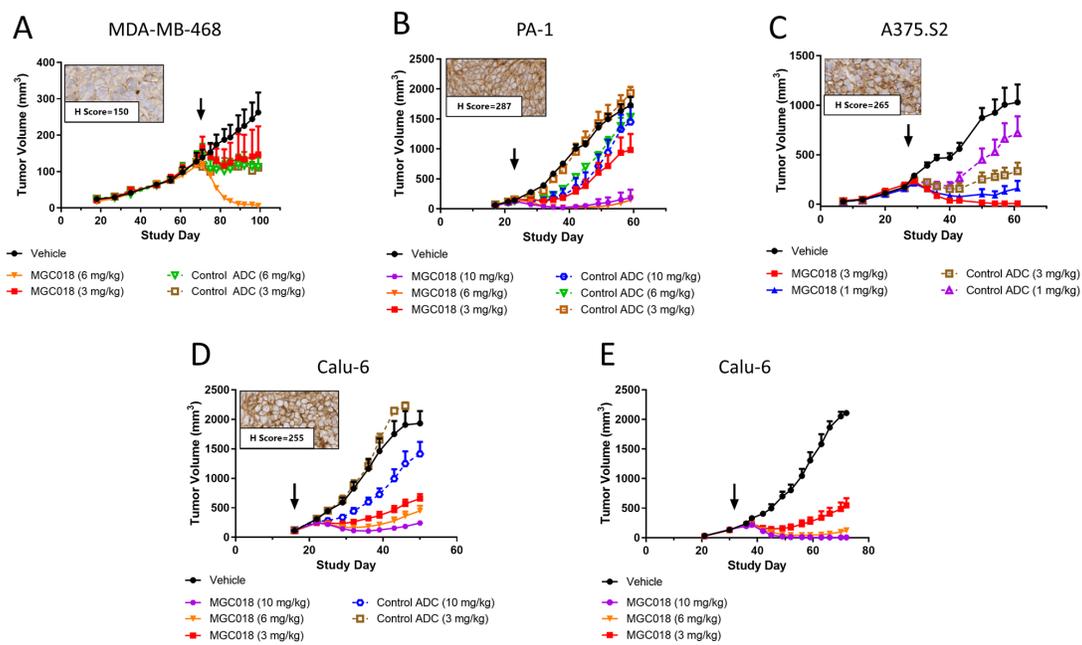


Figure 3

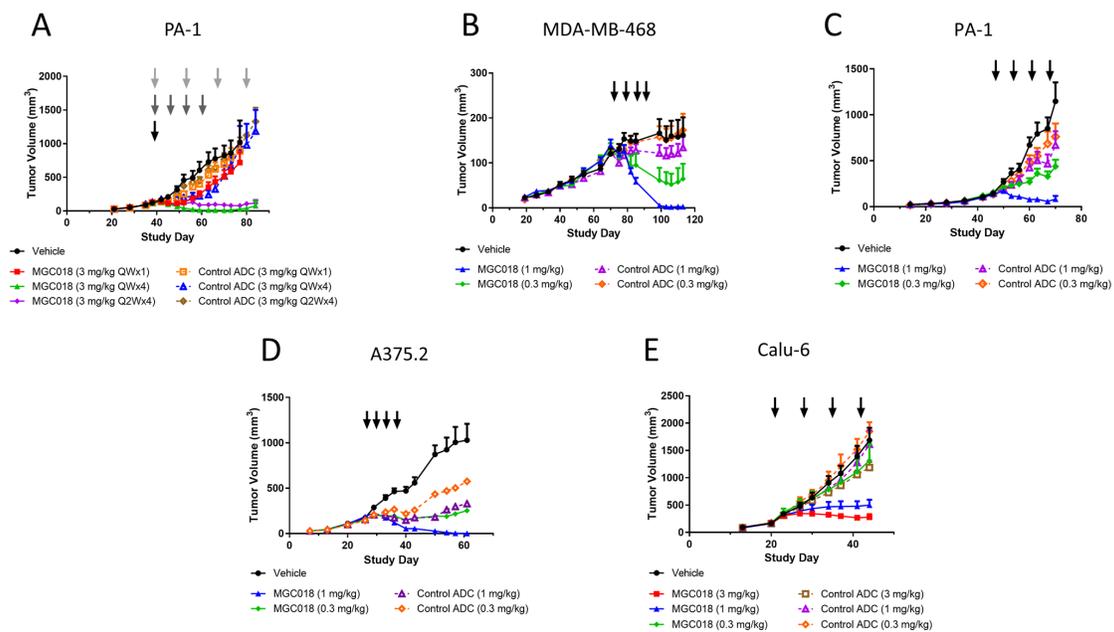
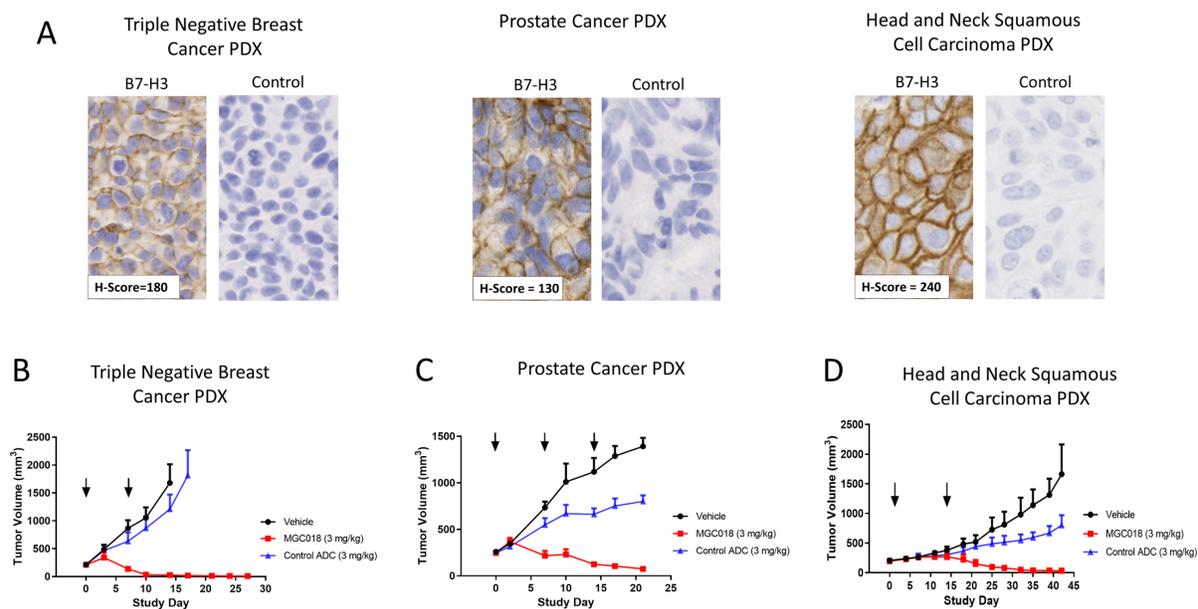
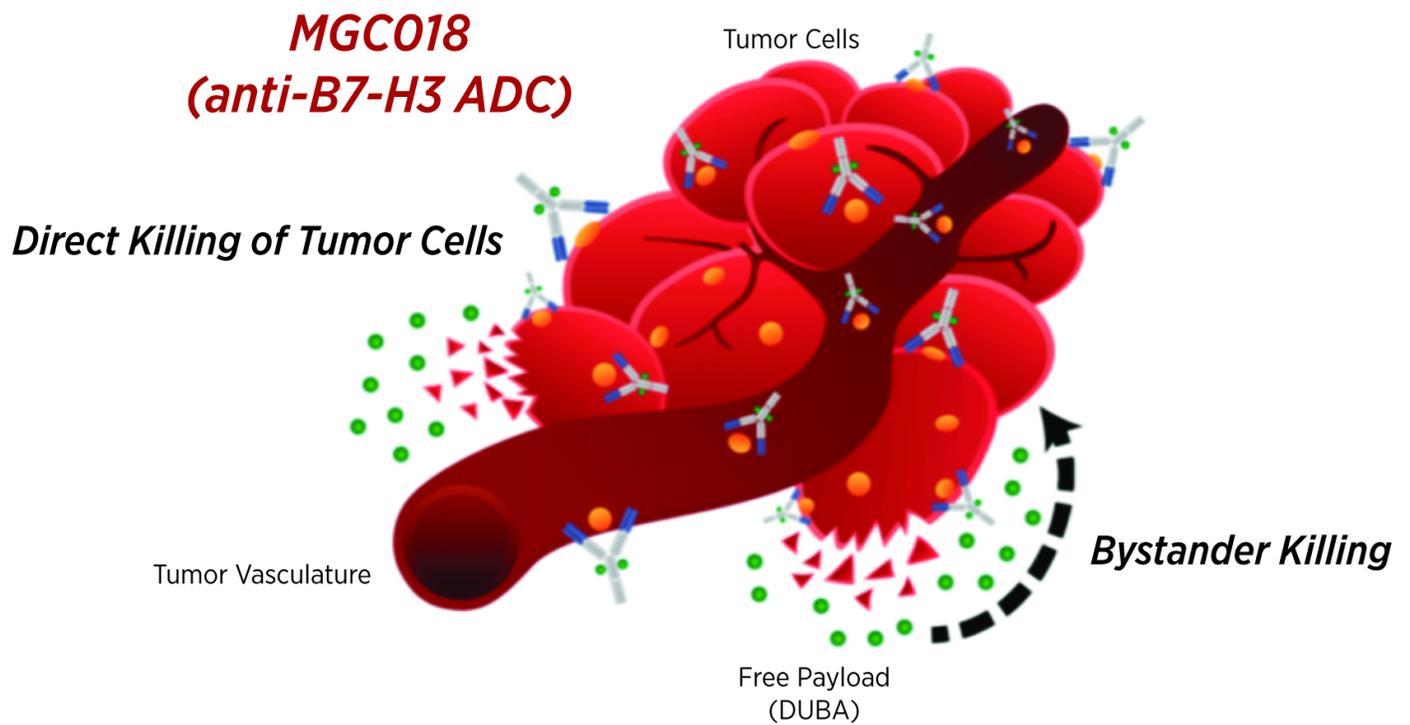


Figure 4



Graphical Abstract



MGC018 ADC targets B7-H3 on tumor cells and tumor-associated vasculature and mediates bystander killing

Molecular Cancer Therapeutics

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

Juniper A Scribner, Jennifer G Brown, Thomas Son, et al.

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