MGD009, a B7-H3 x CD3 Bispecific Dual-Affinity Re-Targeting (DART®) Molecule Directing T Cells to Solid Tumors

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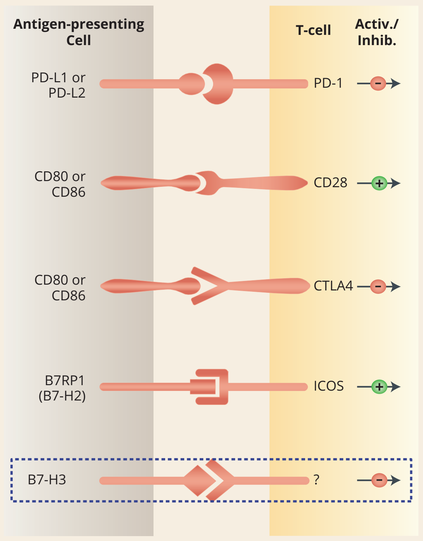
Abstract

B7-H3 (CD276) is a member of the B7 family of immune regulators that is overexpressed on solid tumors, but displays limited expression on normal human tissues. Consistent with its described T-cell inhibitory role, B7-H3 tumor expression has been associated with reduced T-cell infiltration, progressive and metastatic disease, and correlates with poor prognosis and patient survival. To promote T-cell recruitment and targeting of B7-H3-expressing tumors, we have generated MGD009, an Fc-bearing B7-H3 x CD3 bispecific DART protein capable of simultaneously binding to B7-H3 and CD3, thereby mediating redirected cytotoxic T-lymphocyte (CTL) activity against B7-H3-expressing cancer cells of various origin (including renal, breast, prostate, lung, pancreatic, pharyngeal cancer, melanoma, and glioblastoma). MGD009 activity is accompanied by T-cell activation and expansion that is strictly dependent on co-engagement of T cells with B7-H3-positive targets. Treatment with MGD009 of immune-deficient mice reconstituted with human peripheral blood mononuclear cells (PBMCs) and bearing established Detroit562 pharyngeal carcinoma showed recruitment of T cells to tumor site and dose-dependent antitumor activity with tumor regression. Evaluation of pharmacokinetics (PK) in cynomolgus monkeys, whose orthologs cross-react with MGD009, revealed linear PK with a prolonged serum half-life supporting dosing at once weekly or longer intervals in humans. A phase 1 study of MGD009 in patients with B7-H3-expressing solid tumors is currently enrolling.

Introduction

B7-H3: Member of B7 Family of Immune Regulators[1]



Background

- B7 family member mediating immunomodulatory activity[2,3]
- Negative regulator of T-cell mediated responses[4]
- Crystal structure resolved and T-cell inhibitor domain mapped[5]

Expression Profile

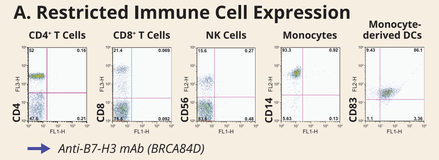
- Limited expression on adult normal tissue or resting immune cells (Panels A–C)
- Expression on tumor cells across multiple solid tumor types, including expression on tumor neovasculature (Panels C–D)
- High expression correlates with advanced disease, metastases, decreased patient survival[6,7]

Role in Tumor Biology & Immunology

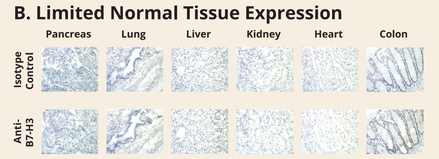
- Silencing reduces tumor migration and invasion and increases sensitivity to chemotherapy[8]
- Drives immune escape and invasiveness of glioblastoma[9]
- Suppresses T-cell mediated antitumor immune response in lung cancer[10]

B7-H3 Displays Favorable Tumor/Normal Differential

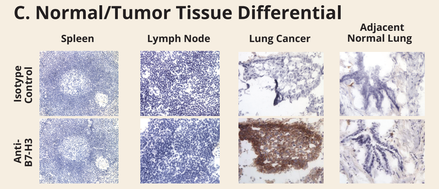
A. Restricted Immune Cell Expression



B. Limited Normal Tissue Expression



C. Normal/Tumor Tissue Differential



D. High Penetrance of B7-H3 Expression in Broad Set of Solid Tumors by IHC

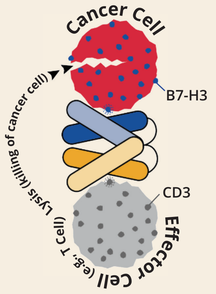
Tumor	B7-H3 Positive		2+ or Above	
Head and Neck	19/19	100%	19/19	100%
Kidney Cancer*	77/78	99%	75/78	96%
Glioblastoma	65/66	98%	63/66	95%
Thyroid Cancer	34/35	97%	33/35	94%
Mesothelioma	90/93	97%	83/93	89%
Melanoma	66/70	94%	32/70	46%
Gastric Cancer	144/155	93%	127/155	82%
Colorectal Cancer	41/45	91%	38/45	84%
Prostate Cancer	88/99	89%	51/99	52%
Pancreatic Cancer	69/78	88%	45/78	58%
Lung Cancer	226/272	83%	211/272	78%
Ovarian Cancer*	59/79	75%	36/79	46%
Breast Cancer	119/164	73%	115/164	70%

*Also expression in tumor vasculature

Results

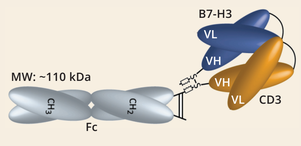
Enabling Effector Cells to Kill Tumors

Redirected T-Cell Activation

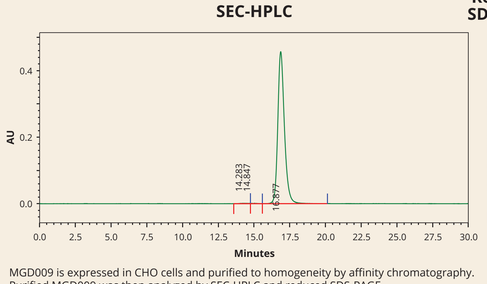


- Co-engagement of T cells (CD3) with tumor-associated antigens (e.g., B7-H3)
- Monovalent binding to CD3 to avoid target independent T-cell activation
- Strict dependence on co-engagement of both targets for T-cell activation
- T-cell receptor & MHC-independent tumor-cell recognition: virtually any T cell can kill cancer cells
- Ongoing clinical trials with DART proteins that redirect T cells to tumors:
  - CD123 x CD3 (MGD006)
  - gpA33 x CD3 (MGD007)
  - CD19 x CD3 (MGD011)

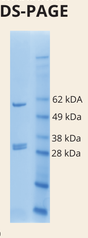
MGD009: B7-H3 x CD3 DART Protein Incorporates an Fc Domain for Enhanced PK



SEC-HPLC



Reduced SDS-PAGE



MGD009 is expressed in CHO cells and purified to homogeneity by affinity chromatography. Purified MGD009 was then analyzed by SEC-HPLC and reduced SDS-PAGE.

- MGD009 is composed of 3 polypeptide chains covalently linked by disulfide bonds
- Humanized Fv regions derived from anti-CD3 (XR32) and anti-B7-H3 (BRCA84D) mAbs that are both human and cynomolgus monkey cross-reactive were assembled in a DART configuration. BRCA84D was selected based on favorable normal/tumor differential binding profile[11]
- Fc domain is introduced at C-termini of heterodimer, modified to avoid unwanted binding to FcγR receptors, but capable of binding FcRn to prolong circulating half-life

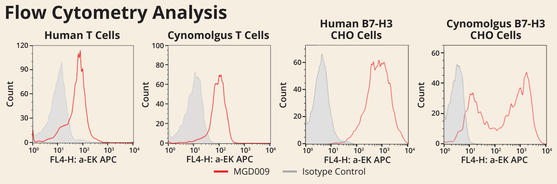
MGD009 Binds Human and Cynomolgus Monkey CD3 and B7-H3

Equilibrium Dissociation Constants

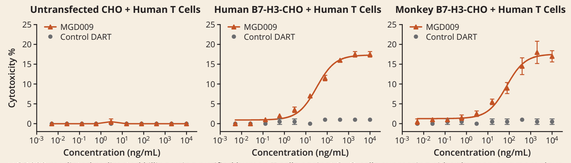
Antigen	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)
Human CD3ε/δ	1.9 (± 0.4) x 10^5	2.6 (± 0.2) x 10^{-3}	13.9 (± 1.4)
Cynomolgus CD3ε/δ	1.9 (± 0.2) x 10^5	2.8 (± 0.1) x 10^{-3}	14.7 (± 0.9)
Human B7-H3-His	2.3 (± 0.1) x 10^5	5.6 (± 0.2) x 10^{-3}	24.6 (± 1.2)
Cynomolgus B7-H3-His	1.7 (± 0.0) x 10^5	5.1 (± 0.1) x 10^{-3}	30.2 (± 0.4)

Data are mean ± SD of 3 independent experiments, each performed in duplicate.

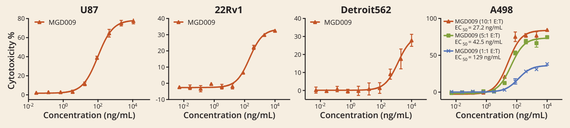
Flow Cytometry Analysis



MGD009 Mediates Redirected Killing of Multiple B7-H3-expressing Tumor Lines



MGD009 mediated redirected killing using purified human T cells against CHO cells expressing either human or cynomolgus monkey B7-H3. The LDH release assay was performed at an effector:target (E:T) cell ratio of 5:1 for 24 hours; no cell killing was observed with untransfected CHO cells lacking B7-H3.

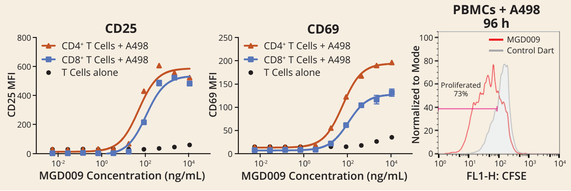


MGD009 EC_{50} Concentrations

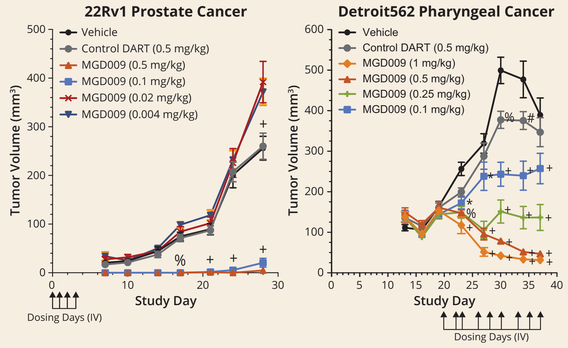
Target Cell Line	Tumor Type	MFI	EC_{50} (ng/mL)
JIMT-1	Breast	65	47
A498	Kidney	150	64
DU145	Prostate	24	84
A375	Melanoma	101	97
U87	Glioblastoma	11	99
BxPC-3	Pancreatic	12	196
22Rv1	Prostate	35	212
SKMES-1	Lung	12	319
Detroit562	Pharyngeal	20	1275
Raji	B-lymphoma	Negative	No activity
CHO	Normal Chinese hamster ovary	Negative	No activity

Redirected cell killing by MGD009 against B7-H3-positive human cancer target cells. MGD009 was incubated with purified human T cells and target cells at E:T cell ratio of 5:1 (or as otherwise indicated) for 24 hours (LDH release assay). Representative cells lines are illustrated in figures with corresponding EC_{50} values for all target cell lines evaluated in table.

MGD009-mediated T-cell Activation and Proliferation Is Target Antigen Dependent



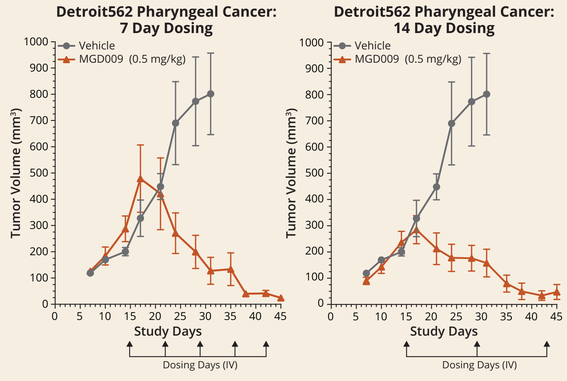
MGD009 Mediates Antitumor Activity in Multiple In Vivo Models



Co-mix mouse xenograft model:
- NOD/SCID mice (n = 8/group)
- Co-mix: activated human T cells and tumor cells implanted subcutaneously (SC) at E:T cell ratio of 1:5 (Day 0)

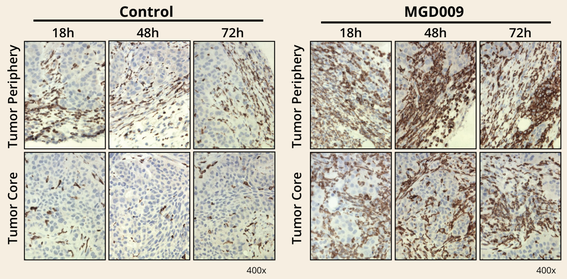
Established tumor model in human T-cell reconstituted mice:
- NSG β2m$^{-/-}$ mice (n = 7-8/group)
- Reconstitution: 1 x 10^7 human PBMCs intraperitoneally (Day -1)
- Tumor implantation: 5 x 10^6 tumor cells intradermally (Day 0)

Mean ± SEM; two-way ANOVA with Bonferroni post-test: * p < 0.05; # p < 0.01; % p < 0.001; + p < 0.0001



Established tumor model in human T-cell reconstituted mice:
- MHC1$^{-/-}$ mice (n = 5-7/group)
- Reconstitution: 1 x 10^7 human PBMCs intraperitoneally (Day 0)
- Tumor implantation: 5 x 10^6 tumor cells intradermally (Day 0)

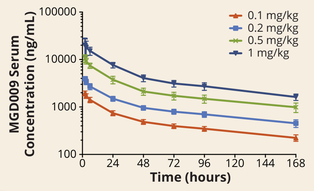
Treatment with MGD009 is Associated with T-cell Recruitment to Tumor Xenografts



IHC staining of CD3-positive T cells in NSG/β2m$^{-/-}$ mice engrafted SC with Detroit562 cancer cells, reconstituted with human PBMCs, and treated IV with MGD009 (0.5 mg/kg) or vehicle control.

MGD009 Displays Prolonged PK in Cynomolgus Monkeys

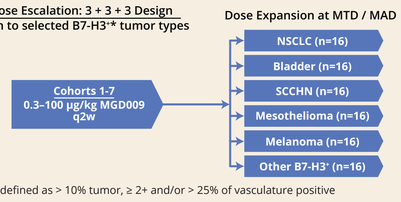
- MGD009 PK were linear over 0.1 to 1 mg/kg dose range with C_{max} and AUC increasing in proportion to dose
- Mean clearance was 0.9 mL/h/kg
- Mean beta half-life ($t_{1/2,\beta}$) ranged from 114-136 hours
- Mean resonance time (MRT) ranged from 144-160 hours



MGD009 serum concentrations (mean ± SD) by dose group following first dose administered by IV infusion over 2 hours in cynomolgus monkeys (n = 5 animals/sex/group).

MGD009 Phase 1 Study Ongoing — Currently Enrolling Patients

Dose Escalation: 3 + 3 + 3 Design
Open to selected B7-H3+* tumor types



*B7-H3 positivity defined as > 10% tumor, ≥ 2+ and/or > 25% of vasculature positive

Conclusions

- MGD009 mediates in vitro redirected T-cell killing of B7-H3-expressing human cancer cell lines originated from a wide range of tumor types
- MGD009 mediated T-cell activation and proliferation is strictly dependent upon co-engagement of B7-H3-expressing target cells with T cells
- MGD009 demonstrated inhibition of growth and tumor regression of B7-H3-expressing tumor xenografts in human T cell or PBMC-reconstituted mice
- MGD009 demonstrated prolonged half-life (5-6 days) in cynomolgus monkeys, supporting dosing at biweekly intervals in humans

These data support evaluation of MGD009 in patients with B7-H3-positive tumors.

A Phase 1 study of unresectable or metastatic B7-H3-expressing tumors, including non-small cell lung cancer, bladder cancer, squamous cell carcinoma of the head and neck, mesothelioma, and melanoma, is currently recruiting patients. (ClinicalTrials.gov Identifier: NCT02628535)

References

1. Pardoll DM. Nat Rev Cancer. 2012 Mar 22;12(4):252-64. **2.** Suh WK, et al. Nat Immunol. 2003 Sep;4(9):899-906. **3.** Leitner J, et al. Eur J Immunol. 2009 Jul;39(7):1754-64. **4.** Veenstra RG, et al. Blood. 2015 May 21;125(21):3335-46. **5.** Vigdorovich V, et al. Structure. 2013 May 7;21(5):707-17. **6.** Zang X, et al. Proc Natl Acad Sci USA. 2007 Dec 4;104(49):19458-63. **7.** Chen JT, et al. Proc Natl Acad Sci USA. 2015 Oct 20;112(42):13057-62. **8.** Liu H, et al. Mol Cancer Ther. 2011 Jun;10(6):960-71. **9.** Lemke D, et al. Clin Cancer Res. 2012 Jan 1;18(1):105-17. **10.** Chen C, et al. Exp Cell Res. 2013 Jan 1;319(1):96-102. **11.** Loo D, et al. Clin Cancer Res. 2012 Jul 15;18(14):3834-45.

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