Anti-B7-H3 Antibody-Drug Conjugates as Potential Therapeutics for Solid Cancer

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Abstract

Introduction: Monoclonal antibodies (mAbs) were generated via a target-unbiased approach based on intact cell immunization with cell lines, fetal progenitor cells, and cancer stem cells. An immunohistochemical (IHC) screen for cancer-specific candidates identified a panel of anti-B7-H3 (CD276) mAbs with highly differential tumor-versus-normal tissue binding. B7-H3 expression was observed in tumor epithelium as well as tumor-associated vasculature and stroma. Consistent with our findings, B7-H3 has been reported to be overexpressed in a growing number of solid cancers, including breast, lung, pancreatic, prostate, kidney, and colon cancer, as well as melanoma and glioblastoma. Furthermore, overexpression of B7-H3 has been correlated with disease severity and poor outcome in a number of these cancer types. A humanized version of an anti-B7-H3 mAb engineered with an enhanced Fc domain (enoblituzumab or MGA271[1]) and a humanized Dual-Affinity Re-Targeting (DART®) protein that recognizes both B7-H3 and CD3 and redirects T cells to kill B7-H3-expressing cells (MGD009) are being investigated in Phase 1 clinical studies. In this nonclinical study, we evaluated the therapeutic potential of anti-B7-H3 antibody-drug conjugates (ADCs) toward B7-H3-expressing solid cancers.

Methods: A panel of anti-B7-H3 mAbs was screened for internalization and a subset of mAbs that were efficiently internalized by tumor cells was identified. These mAbs were converted to ADCs via chemical conjugation; in vitro and in vivo activity studies were then conducted with a range of tumor cell lines representing human cancer types that overexpress B7-H3.

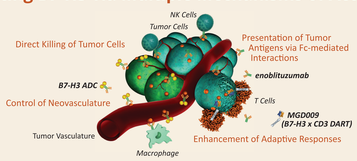
Results: The anti-B7-H3 ADCs exhibited specific, dose-dependent cytotoxicity toward B7-H3-positive tumor cell lines in vitro, including breast, lung, ovarian, pancreatic, and prostate cancer lines, with IC_{50} values generally in the sub-nM range. Cytotoxicity was not observed with cell lines lacking B7-H3 expression. The anti-B7-H3 ADCs exhibited potent antitumor activity in vivo, resulting in tumor stasis and tumor regression in mice bearing B7-H3-positive human breast, lung, and ovarian tumor xenografts.

Conclusion: Anti-B7-H3 ADCs exhibited dose-dependent cytotoxicity in vitro and potent antitumor activity in vivo toward a range of B7-H3-expressing tumor cell lines representing cancer types that overexpress B7-H3. Our findings demonstrate that ADCs targeting B7-H3 may serve as potential therapeutics for B7-H3-expressing solid cancers.

Introduction

- mAbs were generated via immunization of mice with viable human fetal progenitor cells or tumor initiating/cancer stem-like cells (CSLCs)
- An IHC screen for cancer-specific mAbs identified a panel of anti-B7-H3 (CD276) reactive mAbs with highly differential tumor-versus-normal tissue binding
- Strong B7-H3 expression was observed in tumor epithelium of a large range of solid cancers, as well as in tumor-associated vasculature and stroma
- A subset of anti-B7-H3 mAbs were efficiently internalized by tumor cells in vitro. Together with their favorable tumor-versus-normal tissue reactivity, this subset was selected for evaluation for an ADC approach
- **Objective:** Evaluate the therapeutic potential of anti-B7-H3 ADCs toward solid cancer by conducting in vitro and in vivo activity studies with anti-B7-H3 ADCs across a range of tumor cell lines representing human cancer types that overexpress B7-H3

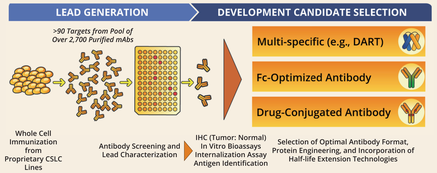
Targeting B7-H3 via Multiple Mechanisms of Action

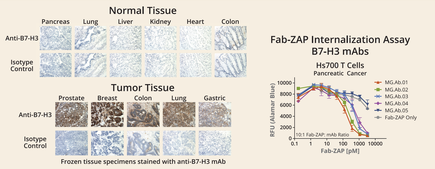


Methods

- mAb generation
  - Immunizations with intact viable fetal progenitor and tumor initiating/CSLCs were performed as previously described[2]
- Internalization assay
  - Internalization was performed in a 5-day assay using saporin-conjugated anti-mouse Fab at 1:1 or 10:1 Fab-ZAP:Test mAb ratio, as per manufacturer's protocol (Advanced Targeting Systems)
- ADC generation
  - Anti-B7-H3 mAbs (chimeric human IgG1) were converted to ADCs via cysteine-conjugation to the cleavable auristatin E linker/payload vc-MMAE to an average drug-to-antibody ratio of 4.5-4.7 (Concortis Biosystems)
- In vitro cytotoxicity
  - 7-day in vitro cytotoxicity was quantified using Alamar Blue reagent, according to manufacturer's protocol (BioRad)
- In vivo activity
  - Tumor cells (5 x 10^6) were implanted subcutaneously into the flank of CD1 nude mice. When tumors reached ~150 mm^3, mice were randomized and test articles were administered intraperitoneally. Tumors were measured twice weekly by orthogonal measurements with electronic calipers, with tumor volumes calculated as: (length x width x height)/2
- Pharmacokinetics
  - Non-tumor bearing CD1 nude mice were administered test articles intraperitoneally at a single dose of 5 mg/kg. Blood samples were collected over the course of 10 days and sandwich ELISAs were performed on sera to quantify total mAb and intact ADC concentrations

Antibody/Target Discovery Platform

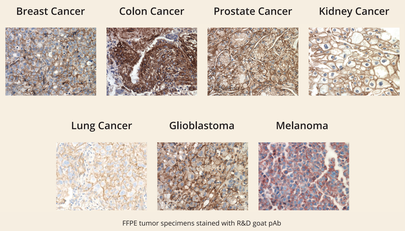




- Panel of tumor-specific B7-H3 mAbs identified
- Subset of B7-H3 mAbs that are efficiently internalized by tumor cells in vitro selected for further evaluation

Results

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



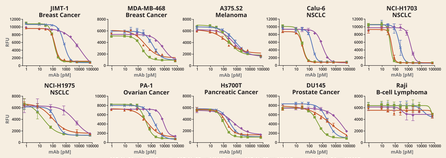
FFPE tumor specimens stained with R&D goat pAb

- Membrane expression of B7-H3 on tumor epithelium and tumor-associated vasculature

Cancer Type	IHC Summary of Tumor Samples Screened: B7-H3 Total Positive		B7-H3 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%
Melanoma	66/70	94%	32/70	46%
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%
Bladder Cancer	14/20	70%	9/20	45%

- B7-H3 is strongly expressed at a high frequency across a broad range of tumors

B7-H3 ADCs Exhibit Potent In Vitro Cytotoxicity

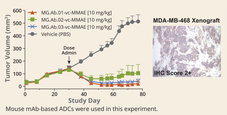


- Potency observed against a range of B7-H3-positive tumor lines

IC_{50} (pM)*	Breast Cancer: JIMT-1	Breast Cancer: MDA-MB-468	Melanoma: A375.S2	Non-Small Cell Lung Cancer: Calu-6	Non-Small Cell Lung Cancer: NCI-H1703	Non-Small Cell Lung Cancer: NCI-H1975	Ovarian Cancer: PA-1	Pancreatic Cancer: Hs700T	Prostate Cancer: DU145
Antibody Binding Sites**	1.1e6	4.2e5	7.5e5	8.5e5	8.1e5	4.8e5	6.1e5	2.1e6	2.4e5
MG.Ab.01-vc-MMAE	221	352	153	59	90	31	555	159	3770
MG.Ab.02-vc-MMAE	124	201	267	30	43	16	409	109	465
MG.Ab.03-vc-MMAE	735	1383	887	171	219	162	1795	303	2587
MG.Ab.04-vc-MMAE	9100	8095	703	995	1517	26976	8326	607	20153

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

In Vivo Activity: MDA-MB-468 Breast Cancer

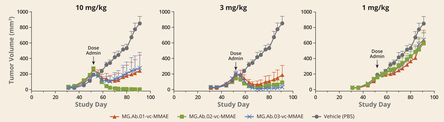


Mouse mAb-based ADCs were used in this experiment.

Treatment	IP Dose (mg/kg)	Schedule (Dosed Day 30)	T/C (%)	CR
MG.Ab.01-vc-MMAE	10	qdx1	4	6/7
MG.Ab.02-vc-MMAE	10	qdx1	20	4/7
MG.Ab.03-vc-MMAE	10	qdx1	8	1/7

T/C (%) = Percentage of tumor size relative to vehicle
CR = Tumor volume ≤5 mm^3 during study

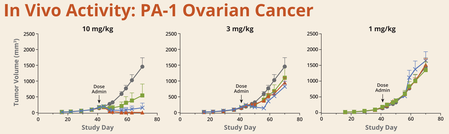
In Vivo Activity: NCI-H1703 Non-Small Cell Lung Cancer



Treatment	IP Dose (mg/kg)	Schedule (Dosed Day 52)	T/C (%)	CR
MG.Ab.01-vc-MMAE	10	qdx1	28	5/7
MG.Ab.01-vc-MMAE	3	qdx1	22	3/7
MG.Ab.01-vc-MMAE	1	qdx1	74	0/7
MG.Ab.02-vc-MMAE	10	qdx1	0	6/7
MG.Ab.02-vc-MMAE	3	qdx1	11	5/7
MG.Ab.02-vc-MMAE	1	qdx1	70	0/7
MG.Ab.03-vc-MMAE	10	qdx1	32	5/7
MG.Ab.03-vc-MMAE	3	qdx1	4	6/7
MG.Ab.03-vc-MMAE	1	qdx1	76	0/7

T/C (%) = Percentage of tumor size relative to vehicle
CR = Tumor volume ≤5 mm^3 during study

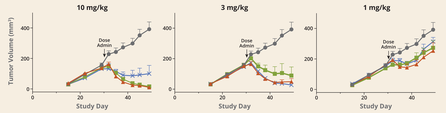
In Vivo Activity: PA-1 Ovarian Cancer



Treatment	IP Dose (mg/kg)	Schedule (Dosed Day 42)	T/C (%)	CR
MG.Ab.01-vc-MMAE	10	qdx1	0	6/7
MG.Ab.01-vc-MMAE	3	qdx1	65	0/7
MG.Ab.01-vc-MMAE	1	qdx1	105	0/7
MG.Ab.02-vc-MMAE	10	qdx1	37	3/7
MG.Ab.02-vc-MMAE	3	qdx1	76	1/7
MG.Ab.02-vc-MMAE	1	qdx1	93	0/7
MG.Ab.03-vc-MMAE	10	qdx1	11	5/7
MG.Ab.03-vc-MMAE	3	qdx1	57	1/7
MG.Ab.03-vc-MMAE	1	qdx1	113	0/7

T/C (%) = Percentage of tumor size relative to vehicle
CR = Tumor volume ≤5 mm^3 during study

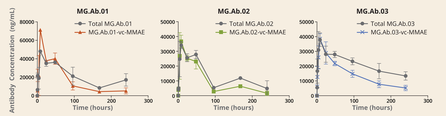
In Vivo Activity: A375.S2 Melanoma



Treatment	IP Dose (mg/kg)	Schedule (Dosed Day 30)	T/C (%)	CR
MG.Ab.01-vc-MMAE	10	qdx1	3	5/7
MG.Ab.01-vc-MMAE	3	qdx1	13	1/7
MG.Ab.01-vc-MMAE	1	qdx1	65	0/7
MG.Ab.02-vc-MMAE	10	qdx1	4	1/7
MG.Ab.02-vc-MMAE	3	qdx1	23	0/7
MG.Ab.02-vc-MMAE	1	qdx1	70	0/7
MG.Ab.03-vc-MMAE	10	qdx1	26	2/7
MG.Ab.03-vc-MMAE	3	qdx1	7	0/7
MG.Ab.03-vc-MMAE	1	qdx1	80	0/7

T/C (%) = Percentage of tumor size relative to vehicle
CR = Tumor volume ≤5 mm^3 during study

Pharmacokinetics of B7-H3 ADCs



Antibody	Total Antibody: $t_{1/2}$ (h)	Total Antibody: AUC (h*µg/mL)	Intact ADC: $t_{1/2}$ (h)	Intact ADC: AUC (h*µg/mL)
MG.Ab.01-vc-MMAE	114.1	4,796	58.9	4,033
MG.Ab.02-vc-MMAE	75.9	2,699	52.6	2,202
MG.Ab.03-vc-MMAE	177.2	5,162	87.3	3,502

- Auristatin E-conjugated B7-H3 ADCs are highly stable in mice

Conclusions

- B7-H3 is overexpressed in a wide range of solid cancers, including breast, lung, pancreatic, head and neck, prostate, kidney, and colon cancer, as well as in glioblastoma and melanoma
- Anti-B7-H3 auristatin E ADCs:
  - Exhibited specific, dose-dependent cytotoxicity toward a wide range of B7-H3-positive tumor cell lines in vitro
  - Demonstrated significant antitumor activity toward B7-H3-positive mouse tumor xenograft models of breast, lung, and ovarian cancers, as well as melanoma
  - Showed typical pharmacokinetics in mice, with a half-life of ~2.2-3.6 days for the intact ADC

ADCs targeting B7-H3 may serve as potential therapeutics for the treatment of B7-H3-expressing malignancies

References

1. Loo D, et al. Clin Cancer Res. 2012 Jul 15;18(14):3834-45. **2.** Loo D, et al. Mol Cancer Ther. 2007 Mar;6(3):856-65.

Acknowledgements

ADCs were provided by Concortis Biosystems, San Diego, CA