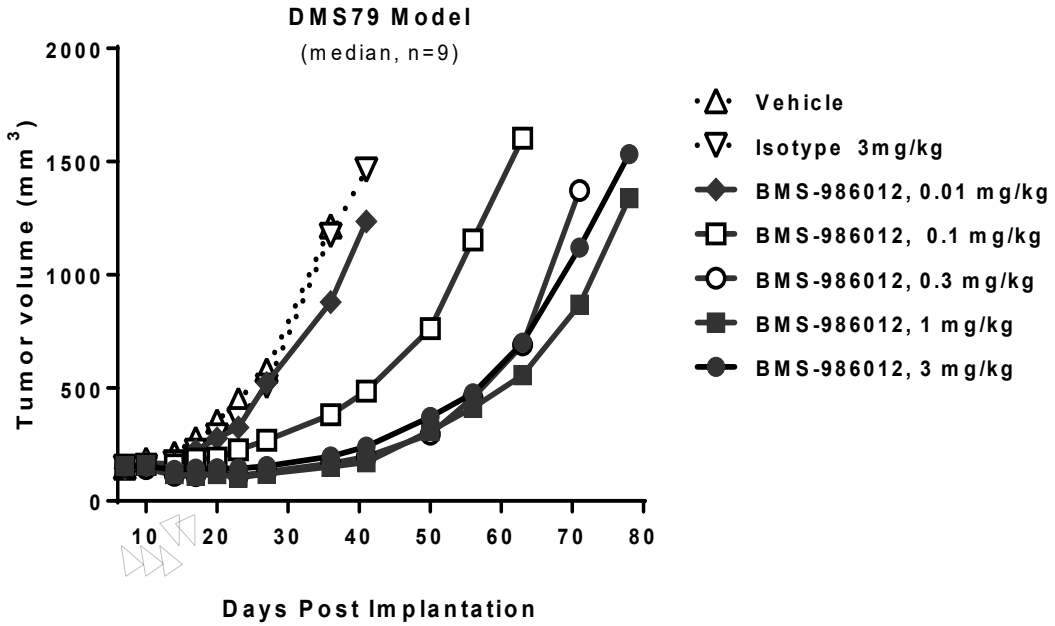
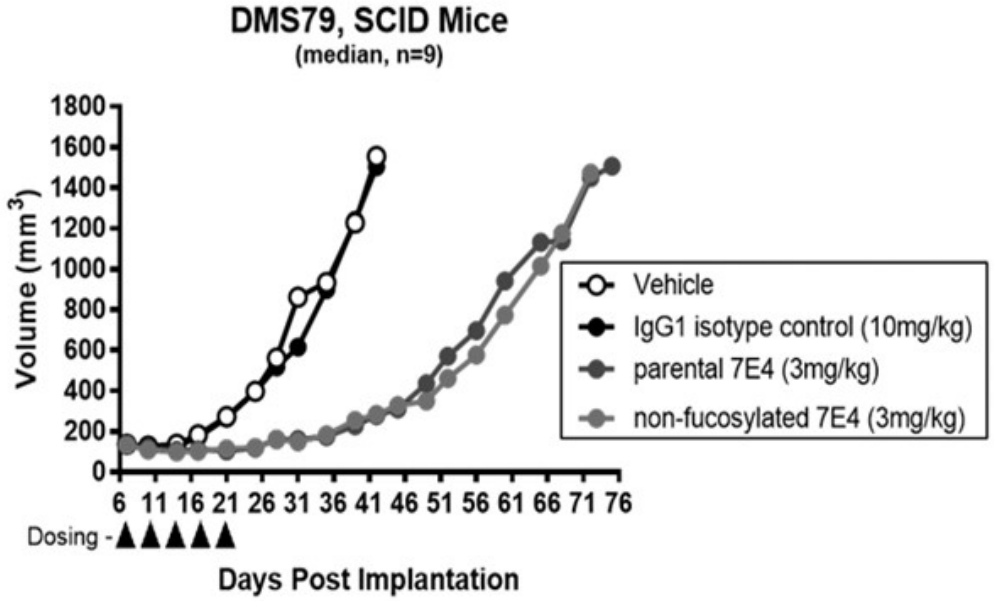
Supplemental Figure 1. BMS-986012 dose response efficacy in the DMS79 xenograft model

A



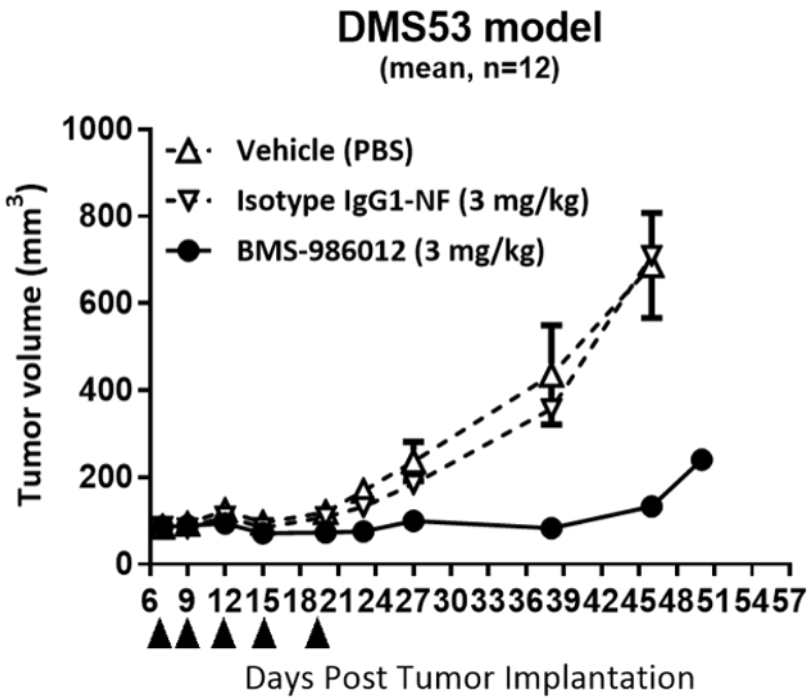
B



A. Mice were dosed i.p. on days 7, 10, 14, 17, and 20 post tumor implantation with vehicle (PBS), 3 mg/kg of irrelevant IgG1 isotype control, or BMS-986012 at 0.01, 0.1, 0.3, 1 and 3 mg/kg. BMS-986012 induced dose-dependent tumor growth inhibition (TGI) on DMS79 xenografts while the IgG1 isotype control at 3 mg/kg had no anti-tumor efficacy. Mean tumor volumes were calculated on day 36 post tumor implantation. BMS-986012 at 0.01 mg/kg showed tumor growth inhibition (TGI) of 27%, but this was not statistically significant (one-way ANOVA). BMS-986012 at 0.1 mg/kg produced a statistically significant TGI of 78% ($P<0.05$ vs. vehicle). Maximal activity of tumor stasis occurred at doses from 0.3 - 3 mg/kg (TGI > 95 – 98 %, $P<0.001$).

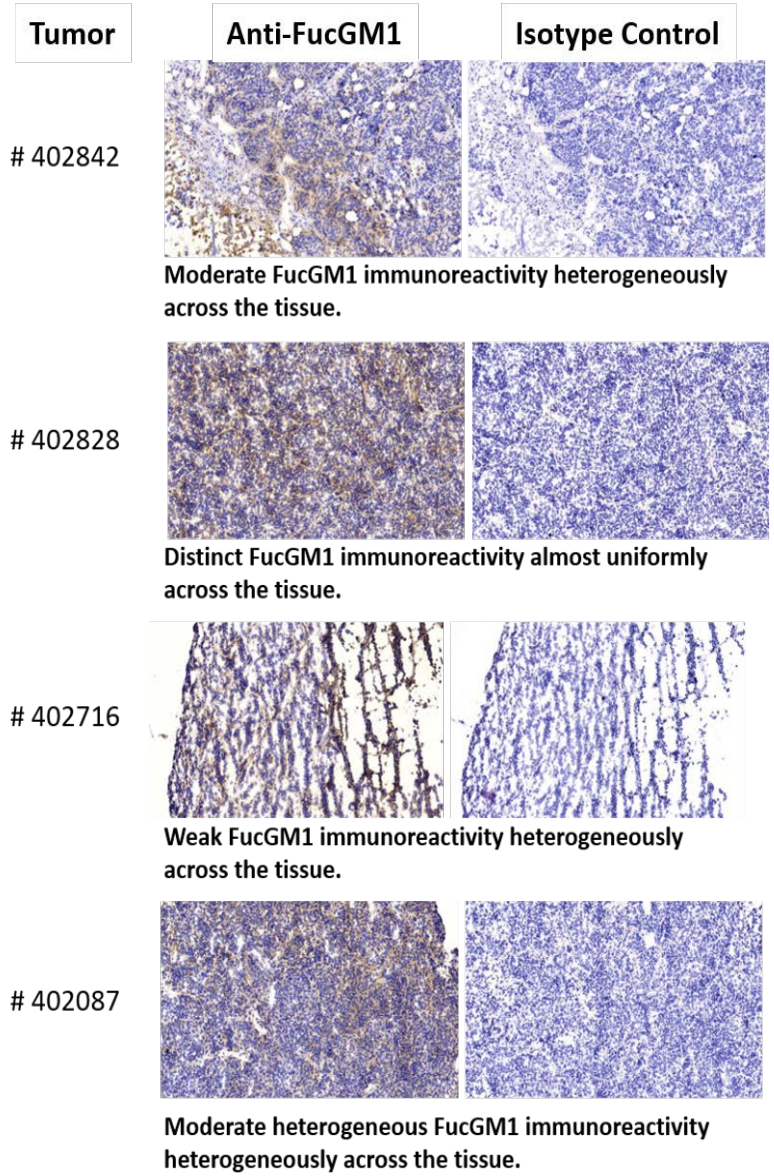
B. Parental 7E4 antibody and nonfucosylated BMS-986012 were similarly efficacious in vivo.

Supplemental Figure 2. BMS-986012 efficacy in the DMS53 xenograft model



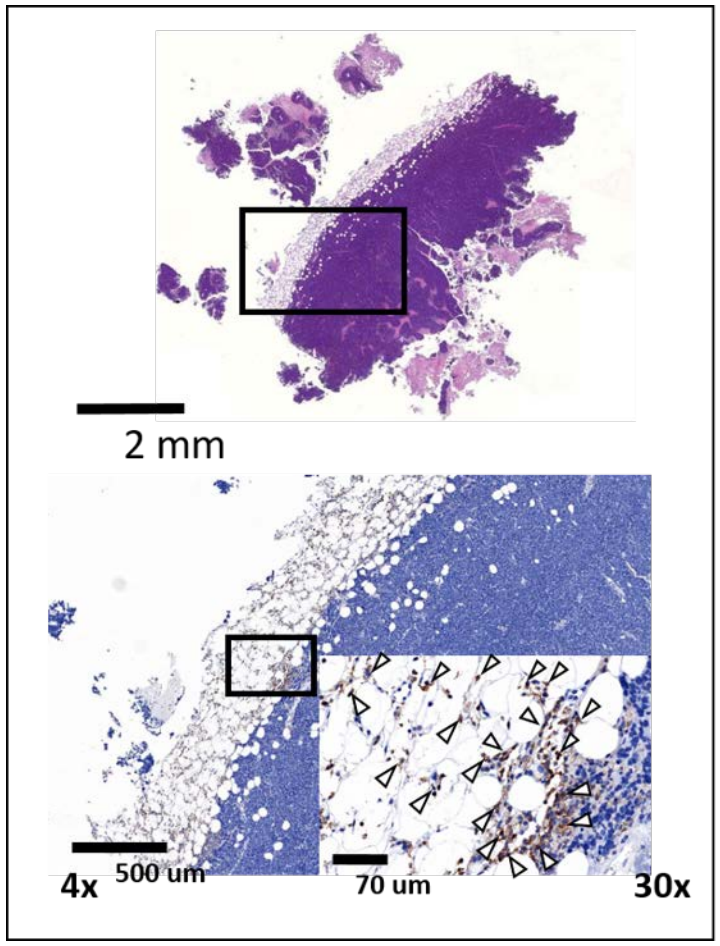
Mice were dosed i.p. on days 7, 10, 14, 17, and 20 post tumor implantation with vehicle (PBS), 3 mg/kg of irrelevant IgG1-NF isotype control, or 3 mg/kg BMS-986012. BMS-986012 induced tumor growth inhibition of DMS53 xenografts while the IgG1-NF isotype control at 3 mg/kg had no anti-tumor efficacy. In addition to the DMS79 and DMS53 models expressing high levels of FucGM1, four additional models with lower expression of FucGM1 were also evaluated for efficacy in xenograft models. H4IIE and H187 with moderate FucGM1 expression by IHC showed TGI by BMS-986012 of 98% and 66%, respectively. Minimal efficacy was observed in H128 and H209 xenograft models (TGI of 20% and 0%, respectively) expressing low levels of FucGM1.

Supplemental Figure 3. FucGM1 expression is heterogeneous in established DMS79 xenografts



Established DMS79 tumor xenografts were harvested on day 48 post implantation and stained with anti-FucGM1 or isotype control antibody as described in Materials and Methods. Representative examples of high (#402828), moderate (#402842, #402087), and weak (#402716) anti-FucGM1 staining are shown. Staining in #402842, #402087, and #402716 was heterogeneous, with stained and unstained areas of the tissue. Staining in #402828 was homogeneous, with uniform staining across the tissue. No immunoreactivity was observed with isotype control.

Supplemental Figure 4. KP3 syngeneic model presents with a low immune cell infiltrate



KP3 tumors were harvested from mice 41 days after implantation. Left Panel: Tumors were assessed for the presence immune cells by IHC. Top Left Panel shows tumor staining by H&E. Bottom Left Panel shows staining for CD45+ cells. Few CD45+ cells are observed in KP3 tumors and are predominately scattered around the tumor periphery (White arrowheads Bottom Left Panel inset). Right Panel: Tumor infiltrate was also characterized by flow cytometry (N=12). CD45+ cells represented 0.5% - 1.9% of total cells. CD45+ cells were comprised of: 5-8% CD4+ T cells, 10% CD8+ T cells, 0.2% Treg cells, 1-4% CD19+ B cells, 31-51 % CD11b+, 1-6% MDSC, and 4-8% NK cells.

Supplemental Table 1A. Prevalence of FucGM1 expression in lung cancer patient populations by immunohistochemistry (IHC)

Tumor Type	Number Tested	Percent FucGM1+
Small Cell Lung Carcinoma (SCLC)	24	50
Non-Small Cell Lung Carcinoma	103	20
– Large cell or Mixed	5	0
– Adenocarcinoma	49	29
– Squamous Cell	49	14

Immunohistochemistry analyses of FucGM1 expression were performed in 128 human lung cancer samples, including 24 SCLC, 104 NSCLC and xenograft tumor samples obtained from Analytical Biological Services (Wilmington, DE), Asterand Bioservices (Detroit, MI), Cooperative Human Tissue Network (NCI), Cureline (Brisbane, CA), Impath-BCP (New York, NY), Indivumed (Lewisburg, PA), and Tissue Solutions (Glasgow, UK). Cryostat sections at 5 μm were fixed with acetone for 10 minutes at room temperature, and stored at –80ºC prior to use. For staining slides were warmed to room temperature and washed twice with PBS. Endogenous peroxidase activity was blocked by incubation for 10 minutes with peroxidase block supplied with the EnVision+ System (Dako, Carpinteria, CA). After two washes with PBS, slides were incubated with Dako protein block supplemented with 1% human gamma globulins. Subsequently, primary F12 antibody (mouse anti-FucGM1 IgG3 antibody, Fujirebio Diagnostic, Malvern, PA) at 1, 2, 5, or 20 μg/mL) or isotype control (mouse IgG3 at 5 or 20 μg/mL, Serotec, Raleigh, NC) was applied to tissue sections and incubated for 1 hour. Following three washes with PBS, slides were incubated with the peroxidase- conjugated anti-mouse IgG polymer supplied in with the Dako EnVision+ System for 30 minutes. Slides were then washed and reacted with a 3,3'-diaminobenzidine (DAB) substrate-chromogen solution for 6 minutes, washed again with deionized water, and counterstained with Mayer's hematoxylin (Dako). Stained slides were digitalized for qualitative or qualitative assessment of immunostaining.

Supplemental Table 1B. Prevalence and cut off data for FucGM1 in SCLC from this report combined with data from other published studies

Sample Set	Number of samples	*Cut Off	Number positive	¶Number positive at indicated cut offs	Reference
1	24	≥ 10	12 (48%)	12/24 ≥ 10%	This report
2	8	≥ 35	5 (62%)	4 /8 = 75% 1/8 = 26-75%	Brezicka 2000 lung cancer 28:29
3	21	Not specified	19 (90%)	10/21 = 100% 5/21 > 50% 4/21 < 50%	Brezicka 1989 Cancer Res 49:1300
4	19	Not specified	11 (58%)	11/19 > 50%	Brezicka 1991 APMIS 99:797
5	28	Not specified	21 (75%)	7/28 < 25% 14/28 > 25%	Brezicka 1992 Tumor Biol 13:308
6	6	≥ 50	4 (67%)	4/6 > 50%	Zhang 1997 Int J Cancer 73:42
All	**106**		**72 (68%)**		

Prevalence data for FucGM1 expression in SCLC. Data from this report (Sample Set 1) combined with data from five published studies (Sample Sets 2 – 6) reporting FucGM1 expression in SCLC frozen tissue specimens stained with the same antibody.

*Cut Off or percent of tumors cells stained to be reported as a FucGM1 positive specimen if specified in the referenced report.

¶ Number of tumors with the indicated percent of tumor cells stained.

Supplemental Table 1C. Comparison of FucGM1 expression in human SCLC cell lines with BMS-986012 efficacy in xenografts

Xenograft Model	FucGM1 Expression by IHC	In Vivo Efficacy (TGI)
H740	High	40%
DMS79	Intermediate	95%
DMS53	Intermediate	92%
H4IIE	Low	98%
H187	Very Low	66%
H128	Negligible	20%
H209	Negligible/Absent	0%

IHC, immunohistochemistry; TGI, tumor growth inhibition

Supplemental Table 2. Binding kinetics of BMS-986012

Temp	$K_{on} \times 10^{4}$ (1/Ms)	$K_{off} \times 10^{-4}$ (1/Ms)	K_D (nM)
25 °C	9.4	8.3	8.8
37 °C	7.3	6.7	9.3

Supplemental Table 3. BMS-986012 equilibrium dissociation constant (Kd) values for human Fc receptors by SPR

Receptor	Kd (nM)
FcγRI	9
FcγRIIa	122
FcγRIIb	92
FcγRIIIa/V158	21
FcγRIIIa/F158	389
FcRn*	222

* pH=6.0

Supplemental Table 4. Summary of single-dose pharmacokinetics of BMS-986012 in mice

Model	Route	Dose (mg/kg)	C0 or Cmax (μg/mL)	Tmax (h)	AUC(INF) (μg•h/mL)	T1/2 (h)	CLT (mL/h/kg)	Vss (mL/kg)	F (%)
No tumor	IV	30	490	-	69,183	189	0.43	120	NA
	IP	0.3	1.9	24	594	241	NA	NA	85
	IP	30	237	24	89,173	249	NA	NA	129
Tumor-bearing	IV	30	623	-	62,318	153	0.48	102	NA
	IP	0.3	1.63	24	386	148	NA	NA	63
	IP	30	267	6	56,531	133	NA	NA	91

NA: not applicable. Abbreviations: AUC(INF): area under the concentration time curve from time zero to infinity. CLT: Total serum clearance. Cmax: maximum concentration. F: absolute bioavailability. IP: intraperitoneal. IV: intravenous. MRT: mean residence time. T-HALF: apparent elimination half-life. Tmax: time of maximum concentration. Vss: volume of distribution at steady state.

Plasma PK was conducted in naïve or DMS79 (mean = 400mm3) tumor-bearing CB.17 SCID mice following a single IV tail vein or IP injection at indicated doses. N = 3 male SCID mice / time point. Mean values are shown. After an IV bolus dose, the serum BMS-986012 concentration exhibited a bi-exponential decline (not shown). Serum clearance of BMS-986012 was similar in tumor-bearing mice and non-tumor-bearing mice (0.48 and 0.43 mL/h/kg, respectively). The Vss was also similar in tumor-bearing and non-tumor-bearing mice (102 and 120 mL/kg, respectively). The t1/2 of BMS-986012 was longer in non-tumor-bearing mice (189 h) than in mice bearing DMS79 tumors (153 h). Following IP administration, BMS-986012 appeared to be well absorbed. At doses of 0.3 mg/kg and 30 mg/kg IP, absolute bioavailability was similar in tumor-bearing mice (63% and 91%, respectively) and non-tumor bearing mice (85% and 129%, respectively). The t1/2 of BMS-986012 was longer in non-tumor-bearing mice than in tumor-bearing mice (189 h vs 153 h, respectively collectively suggesting that FucGM1 expressed on the tumor rather than normal tissue expression of FucGM1 affects antibody distribution and clearance in vivo.

Supplemental Method: BMS-986012 pharmacokinetics in mice

The concentration of BMS-986012 in mouse serum samples was measured by electrochemiluminescence (ECL) using the Meso Scale Discovery (MSD) platform (Gaithersburg, MD). All reagents were prepared in-house, unless otherwise specified. A BMS-986012 specific biotinylated mouse anti-idiotype (a-ID) antibody was immobilized on

a MSD streptavidin plate to capture the analyte from test samples. Samples, standards, and QC samples were brought up to a final matrix of 5% serum and incubated on the plates. Samples were analyzed at 5% minimum required dilution in 1% bovine serum albumin (BSA)/phosphate buffered saline (PBS)/0.05% Tween 20 buffer with additional dilutions of 1-fold to 1000-fold in 5% serum, if needed. The plates were washed with 0.05% Tween 20/PBS and the captured BMS-986012 was detected using Sulfo-TAG-labeled goat a-ID pAb (Lot 17Feb2012Tag) as the detection antibody. Following addition of MSD read buffer, concentration of BMS-986012 in serum samples was calculated from luminescence intensity as measured using a MSD reader with a 5-parameter logistic calibration curve generated from BMS-986012 standards. The calibration curve ranged from 20 to 10,000 ng/mL in undiluted serum. Quality control samples were prepared at 1900, 225, and 60 ng/mL in neat sera and analyzed on each plate to ensure acceptable assay performance.

The PK parameters of BMS-986012 were obtained by non-compartmental analysis of serum concentration vs time data (WinNonlin, Version 5.3, Pharsight Corporation, Mountain View, CA). Studies conducted in mice were composite-design studies and the values are based upon the composite time-concentration curve. The peak concentration (Cmax) and time for Cmax (Tmax) were recorded directly from experimental observations. The area under the curve from time zero to infinity [AUC(INF)] was calculated using a combination of linear and log trapezoidal summations. The total serum clearance (CLTs), steady-state volume of distribution (Vss), apparent elimination half-life ($t_{1/2}$), and mean residence time (MRT) were estimated after IV administration. Estimations of $t_{1/2}$ were made by regression of the terminal log-linear portion of the serum concentration vs time profile. The absolute bioavailability (F) was estimated as the ratio of dose-normalized AUC values following IP and IV doses.