Preclinical Evaluation of HuMab-1B7 and HuMab-31F9 Monoclonal Antibodies in GD2 Positive Sarcoma Xenografts.

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Elizabeth R. Butch[1], Amy L. Vavere[1], Ritsuko Sawada[2], Scott E. Snyder[1], and Wolfgang W. Scholz[2]

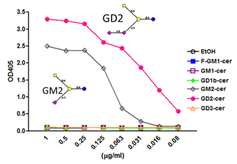
[1]St. Jude Children's Research Hospital, Memphis, TN 38105 and [2]MabVax Therapeutics, San Diego, CA .

Background: The GD2 ganglioside is a glycolipid that is significantly overexpressed in neuroblastoma, sarcoma and other cancers. A panel of fully human anti-GD2 mAbs has been recovered from patients immunized with a GD2-KLH conjugate vaccine. HuMab-1B7 and HuMab-31F9 were selected for further evaluation based on low nM affinity antigen binding and antigen specificity. Complement-(CDC) and antibody dependent cytotoxicity (ADCC) as well as antibody internalization and cytotoxicity of MMAE conjugates were measured to evaluate for clinical utility. Moreover, we used PET imaging to determine targeting specificity and biodistribution in patient derived xenograft models.

Table 1: Specificity of anti-GD2 HuMabs (IgG) Measured by ELISA

HuMab	Lot	PBS	GM2-PAA	GD2-PAA	GD3-PAA	Globo-H	MUC1	Tn-PAA	sTn-PAA	TF-PAA	sLeA-PAA	EtOH	GM2-cer	GD2-cer	GD3-cer	F-GM1-cer	GM3-cer
1B7	1	0.13	**2.24**	**2.97**	0.09	0.09	NT	0.10	0.08	0.09	0.10	0.13	**1.97**	**2.07**	0.08	0.08	0.07
1B7	4	0.11	**2.02**	**2.49**	0.09	0.09	NT	0.09	0.09	0.13	0.11	0.09	**1.84**	**1.96**	0.08	0.08	0.08
1B7	5	0.17	**3.45**	**3.67**	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.09	**0.95**	**2.77**	0.10	0.09	0.10
1B7 w/cast	7	0.10	**1.89**	**2.56**	0.09	0.11	NT	0.09	0.13	0.10	0.13	0.10	**1.26**	**1.85**	0.12	0.08	0.09
31F9	1	0.09	0.09	**0.92**	0.09	0.08	0.09	0.08	0.08	0.08	0.09	0.10	0.10	**0.66**	0.11	0.12	0.13
31F9	3	0.08	0.09	**1.36**	0.08	0.08	0.08	0.08	0.14	0.08	0.19	0.09	0.09	**1.19**	0.09	0.10	0.16
31F9	4	0.10	0.11	**1.61**	0.09	0.10	NT	0.09	0.15	0.10	0.12	0.08	0.13	**1.38**	0.15	0.09	0.33

HuMabs tested at 2 µg/ml (table) or as indicated (graph), OD405 values (duplicates)



anti-GD2	Affinity* (nM)
14.G2a (m)	4.1
Unituxin (ch)	5.5
1B7 (hu)	1.1 ± 0.4
31F9 (hu)	3.2 ± 1.6

* measured by BiaCore on GD2-PAA

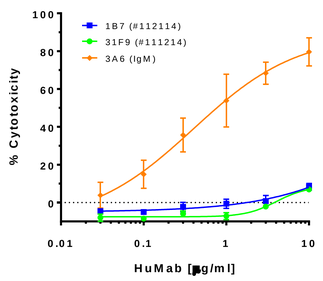
HuMab-1B7 or -31F9 binding (representative lots) is measured by ELISA on plates coated with the respective antigens and detected with HRP-labelled secondary antibodies. Synthetic biotinylated x-PAA antigens are captured on Neutr-Avidin coated plates, and natural x-cer antigens are dissolved in ethanol and directly coated on plates.

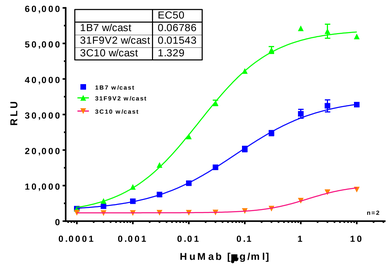
Table 2: Survey of Cell Surface Staining on Various Tumor Cell Lines

		HuMab 31F9		HuMab 1B7	
Cell Line	Tumor	% Positive	MFI	% Positive	MFI
ST88	sarcoma	89.5	122	87.5	90
LS141		88.2	57	69.7	32
TC-71		91.8	**416**	99.5	**1869**
$SaOS_2$		99.7	**1485**	99.7	**1160**
SK-MEL28	melanoma	91.0	18	2.0	4
SK-MEL19		99.2	36	8.5	5
BxPC3	pancreas	81.7	58	5.1	8
Capan-2		20.4	19	33.0	19
DMS79	SCLC	98.9	**1229**	43.6	23
H524		99.4	**2631**	97.3	**2837**
Lan1-Luc	NB	99.6	**677**	99.6	**1483**
Jurkat	lymphoma	76.9	103	74.6	125
MCF 7	breast	8.7	7	4.8	5
MDA-MB-231		4.2	16	83.8	98
Hs578T		99.6	**495**	99.1	**332**
MDA-MB-231-1833		0.3	12	0.8	17
MDA-MB-231-1834		0.6	24	0.4	29
MDA-MB-231- Brain		5.0	6	98.0	84
MDA-MB231-Br1		0.2	12	0.5	13
MDA-MB-231-Br2		11.8	33	99.2	**184**
HT29	colon	8.9	37	2.9	7

Cell surface staining with HuMab at 5 µg/ml plus anti-huIgG-AF488, analyzed on a Guava PCA-96 system.

Fig. 1: CDC and ADCC Activity of HuMabs Against SaOS2 Cells

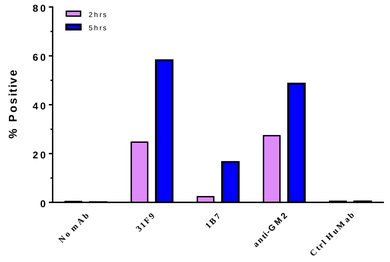


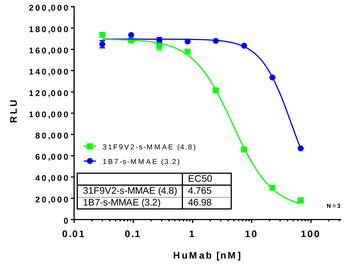


CDC activity: HuMab 1B7 or HuMab 31F9 antibodies at the indicated concentration were incubated with Calcein-AM loaded SaOS2 cells plus human complement for 2 hrs. HuMab anti-GM2 (3A6, IgM) was used as assay positive control. Fluorescence is measured in samples (Exp), presence of C' (Spon) and NP-40 lysed (Max) wells. % Cytotoxicity = (Exp-Spon)/(Max-Spon)*100.

ADCC Reporter Assay: HuMab 1B7 or -31F9 produced in presence of Castanospermine were incubated with 12.5k SaOS2 cells in presence of 7.5k Jurkat reporter cells (Promega) and incubated for 18 h. Induction of Relative Light Units (RLU) corresponding to ADCC activity is measured by chemiluminescence (CellTiter Glow). HuMab anti-GM2 (3C10, IgG) is an assay control.

Fig. 2: HuMab-1B7 and HuMab-31F9 Internalization and ADC Activity

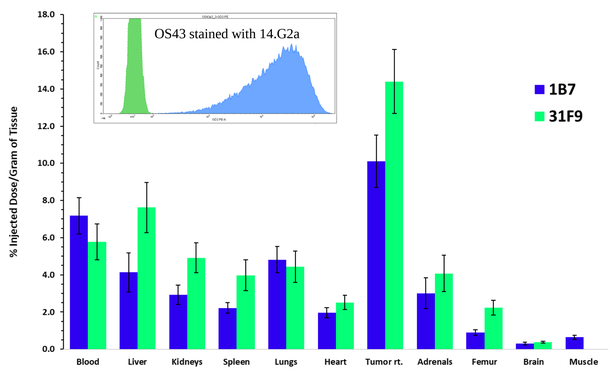




Internalization: HuMab 1B7 or HuMab 31F9 antibodies were precomplexed with pHAb-labelled secondary anti-HuIgG-$F(ab)_2$ antibodies [2]. pHAb is a pH sensitive dye that is fluorescent at low pH (when internalized) and non-fluorescent at neutral pH. Fluorescence was measured after 2 and 5 hours of incubation at 37ºC on a Guava PCA-96 system.

ADC cytotoxicity: Chemoenzymatic site-specific MMAE-labeled HuMab 1B7 or -31F9 ADCs were prepared by SiteClick Chemistry as described [1]. Cytotoxicity against SaOS2 cells was measured after 3 days incubation and detection of viable cells by chemiluminescence (CellTiter Glow, Promega)

Fig. 3: Biodistribution of ^{64}Cu-NOTA-1B7 and ^{64}Cu-NOTA-31F9 Antibodies in OS43 Osteosarcoma Xenograft Bearing Nude Mice



Biodistribution.[4] Female nude mice were implanted s.c. on the right rear flank with human OS43 patient-derived xenografts (PDX) (99% GD2+ by flow cytometry using commercially available 14G2a-PE anti-GD2 antibody) and tumors were allowed to grow to ~5-7 mm. Mice were then injected r.o. with 90 µCi of ^{64}Cu-NOTA-1B7 or ^{64}Cu-NOTA-31F9 and sacrificed at 48 hours post-injection. Tissues were dissected, weighed and counted for radioactivity. Radioactivity concentration is expressed as % of injected dose per gram tissue (wet weight; Mean ± STD, n = 5 or 8 {1B7 or 31F9}) .

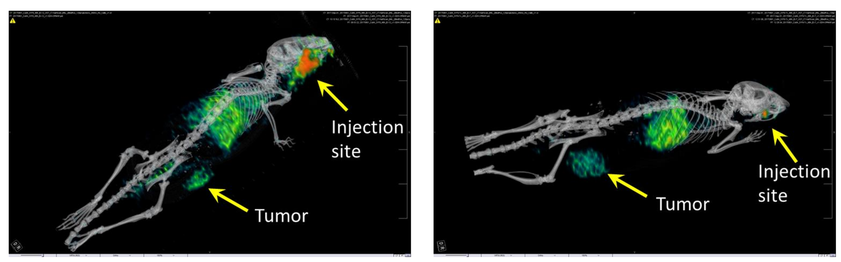
Fig. 4: PET Imaging of OS43 Osteosarcoma Xenografts with ^{64}Cu-NOTA-HuMabs

^{64}Cu-NOTA-1B7 ^{64}Cu-NOTA-31F9

PET-CT: Female nude (nu/nu) mice bearing s.c. OS43 osteosarcoma PDX were injected r.o. with 90 µCi of ^{64}Cu-NOTA-1B7 or ^{64}Cu-NOTA-31F9. At 48 hours post-injection, mice were anesthetized (isoflurane) and static 30 min PET-CT scans performed.

Fig. 5: PET Imaging of 143B Osteosarcoma Xenografts with ^{64}Cu-NOTA-31F9 - Effect of cold blocking dose [3].

Hot only Cold Pre-injected + Hot



Static 30 min PET-CT scan of a moderately GD2-expressing 143B osteosarcoma tumor (34% GD2+ by flow cytometry using 14G2a-PE anti-GD2 antibody) in a nude (*nu/nu*) mouse, imaged 48 hours post-injection of 90 µCi of ^{64}Cu-NOTA-31F9.

Static 30 min PET-CT scan of a 143B osteosarcoma tumor-bearing nude mouse, pre-treated with 100 µg unlabeled 31F9, 4 hours pre-injection of radiotracer and imaged 48 hours post-injection of 90 µCi of ^{64}Cu-NOTA-31F9.

Conclusions:

- Both anti-GD2-HuMabs show very little complement mediated cytotoxicity while retaining significant ADCC activity.
- Both HuMabs are internalized and MMAE-ADCs show *in vitro* cell cytotoxicity in the low nM range.
- ^{64}Cu-NOTA-1B7 and ^{64}Cu-NOTA-31F9 PET imaging shows accumulation in GD2+ OS43 and 143B osteosarcoma xenografts.
- ^{64}Cu-NOTA-31F9 shows better specificity and supports continued evaluation of the HuMab-31F9 antibody as a valuable candidate for the development of an imaging probe for Sarcoma.

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References:

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