Novel Fully Human Anti-GD2 Monoclonal Antibodies with Potent Therapeutic Activity against Neuroblastoma, Sarcoma and Melanoma.

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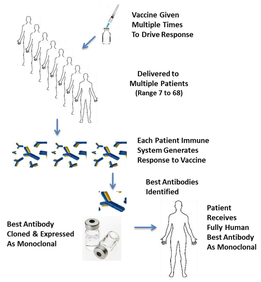
Introduction

Patients vaccinated with ganglioside-KLH conjugates produce antigen-specific antibodies. B cells are rescued from each patient to identify best antigen-specific binders, which are cloned and expressed as monoclonal antibodies and further characterized *in vitro*. Antibodies that meet our *in vitro* selection profile are selected for efficacy testing in relevant animal models. The best in class antibody will be selected for clinical development.



Abstract.

Background: Gangliosides such as GD2, GD3 and GM2 are promising targets for antibody-mediated cancer therapy since they are expressed at high levels on the surface of several cancers, including neuroblastomas, sarcomas and melanomas. Anti-GD2 monoclonal antibodies have shown promising clinical outcomes in neuroblastoma and a chimeric anti-GD2 antibody (Unituxin™) was recently approved by FDA. However, murine-derived antibodies discovered so far show adverse effects that limit their clinical utility. Fully human antibodies derived from immunized patients might be able to overcome these limitations, since epitope specificity has evolved in the human tissue environment. Here we describe the characterization and functional evaluation of two potential development candidates.

Methods: PBMCs from patients immunized with GD2 lactone-keyhole limpet hemocyanin (GD2L-KLH) conjugate vaccine were utilized to isolate fully human monoclonal antibodies (huMabs). HumAbs were selected for 1) specificity against GD2 initially by ELISA and cell surface binding by flow cytometry 2) by glycan array and affinity assays and 3) by effector functions in complement dependent cytotoxicity (CDC) and antibody dependent cellular cytotoxicity (ADCC) assays. The therapeutic potential of lead antibodies was further evaluated in xenograft models in SCID mice using GD2 positive CHLA255 neuroblastoma as well as with TC71 and SaoS2 sarcoma cell lines.

Results: Antibodies that bind specific to purified GD2 by ELISA were expressed as recombinant antibodies in CHO cells. Many recovered antibodies showed cross-reactivity with several gangliosides in ELISA assays. Two antibodies with high specificity for GD2, 1B7 and 31F9V2 were selected for further studies. Binding to native antigen expressed on the cell surface of different cell lines was confirmed by FACS analysis. 31F9V2 was monospecific for GD2 (affinity by Surface Plasmon Resonance was ~4 nM) while 1B7 showed dual specificity for GD2 and GM2 with affinities of ~1 nM for GD2 and ~ 370 nM for GM2, respectively. Complement dependent cytotoxicity against the 4 cell-lines ranged between 35% and 80% with both huMabs, and 1B7 antibodies showed ADCC activity between 40% and 65% on all 4 cell lines in assays with human PBMCs. Survival following IV or SC challenge with SaoS2 and TC71 cells (respectively) was at least doubled following six IP treatments with 200 mcg of either 1B7 or 31F9V2 twice weekly.

Fig. 1: Binding of human anti-GD2 Antibodies to Various Carbohydrates (ELISA)

Clone ID	PBS	GM2-PAA	GD2-PAA	GD3-PAA	Globo-H	MUC1	Tn-PAA	sTn-PAA	TF-PAA	sLeA-PAA
1B7	0.08	**2.54**	**3.21**	0.08	0.08	0.08	0.08	0.08	0.08	0.08
2H12	0.08	**2.37**	**2.98**	0.08	0.08	0.08	0.08	0.08	0.08	0.08
1G2	0.09	0.09	**2.77**	0.10	0.09	0.09	0.09	0.09	0.09	0.09
2F7	0.10	**1.03**	**2.89**	0.13	0.12	0.11	0.10	0.10	0.10	0.10
2E12*	0.12	**0.38**	**2.94**	0.09	0.08	0.09	0.09	0.09	0.09	0.39
31F9	0.08	0.09	**1.36**	0.08	0.08	0.08	0.08	0.14	0.08	0.19
32E2	0.09	0.11	**2.80**	*0.25*	0.10	0.11	0.14	0.21	0.11	0.11

Clone ID	EtOH	GM2-cer	GD2-cer	GD3-cer	F-GM1-cer	GM3-cer
1B7	0.08	**0.82**	**2.14**	0.08	0.08	0.07
2H12	0.08	*0.23*	**1.86**	0.08	0.09	0.08
1G2	0.09	0.10	**1.45**	0.08	0.08	0.08
2F7	0.09	0.12	**2.27**	0.09	0.08	0.08
2E12*	0.08	0.17	**2.06**	0.10	0.08	0.08
31F9	0.09	0.09	**1.19**	0.09	0.10	0.16
32E2	0.09	0.09	**0.62**	0.11	0.09	0.09

*IgM

ELISA plates were coated with Ganglioside ceramides or biotinylated polyacrylamide-carbohydrate constructs captured on avidin and blocked with 2.5% HSA in PBS. Hybridoma supernatants were incubated for 1hr. After washing, the plates were incubated with AP-conjugated goat anti-human Fc Ab for 45 min followed by OPD substrate addition and colorimetric detection.

Fig. 2: Binding to Sarcoma and Neuroblastoma Cell Lines (Flow Cytometry)

	Sarcoma								Neuroblastoma	
	ST88 (5ug/ml)		LS141 (5ug/ml)		TC71 (5ug/ml)		SaoS2 (5ug/ml)		Lan1-Luc (5ug/ml)	
Clone ID	MFI	% Positive	MFI	% Positive	MFI	% Positive	MFI	% Positive	MFI	% Positive
1B7	89.7	**87.5**	31.7	**69.7**	**1869.1**	**99.5**	**1159.6**	**99.7**	**782.5**	**100.0**
2H12	69.3	**91.8**	37.3	**90.8**	**820.6**	**100.0**	**979.8**	**99.9**	**1776.7**	**100.0**
1G2	4.1	2.3	3.7	0.8	82.1	**95.4**	86.3	**96.0**	188.0	**94.8**
2F7	6.4	5.7	4.3	2.2	**377.3**	**99.4**	**317.1**	**99.6**	**612.5**	**99.7**
2E12*	9.9	5.2	3.7	1.7	209.9	**85.6**	**664.3**	**99.0**	**307.1**	**94.7**
31F9	**402.3**	**98.1**	93.6	**94.9**	**298.2**	**95.0**	**1270.1**	**100.0**	**751.5**	**99.6**
32E2	7.1	**11.2**	6.0	**11.1**	68.1	**94.9**	163.0	**99.6**	105.1	**86.2**

Fig. 3: Kinetic Parameters of Binding to GD2-PAA-biotin captured on Avidin Chip (BiaCore Analysis)

HuMab	KA (1/M)	KD (M)	ka (1/Ms)	kd (1/s)	Species	Isotype
1B7	$1.4x10^{9}$	$7.0x10^{-10}$	$1.5x10^{6}$	$1.0x10^{-3}$	Human	IgG1/k
2H12	$3.7x10^{8}$	$2.7x10^{-9}$	$6.8x10^{5}$	$1.8x10^{-3}$	Human	IgG1/k
31F9	$2.0x10^{8}$	$5.0x10^{-9}$	$1.6x10^{5}$	$7.7x10^{-4}$	Human	IgG1/k
31F9V2	$3.5x10^{8}$	$2.9x10^{-9}$	$4.0x10^{5}$	$1.1x10^{-3}$	Human	IgG1/k
32E2	$1.1x10^{8}$	$9.3x10^{-9}$	$5.0x10^{4}$	$4.7x10^{-4}$	Human	IgG1/k
1G2	$4.0x10^{8}$	$2.5x10^{-9}$	$4.5x10^{5}$	$1.1x10^{-3}$	Human	IgG1/k
2F7	$7.0x10^{8}$	$1.4x10^{-9}$	$1.1x10^{6}$	$1.5x10^{-3}$	Human	IgG1/k
2E12	$9.0x10^{9}$	$1.1x10^{-10}$	$8.9x10^{5}$	$9.9x10^{-5}$	Human	IgM/k

Fig. 4: Binding Specificity in Glycan Array 4.1 (CFG analysis).

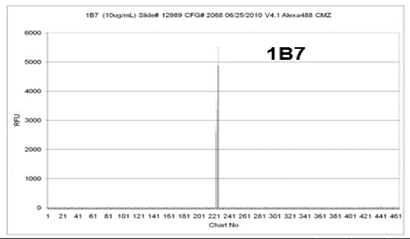


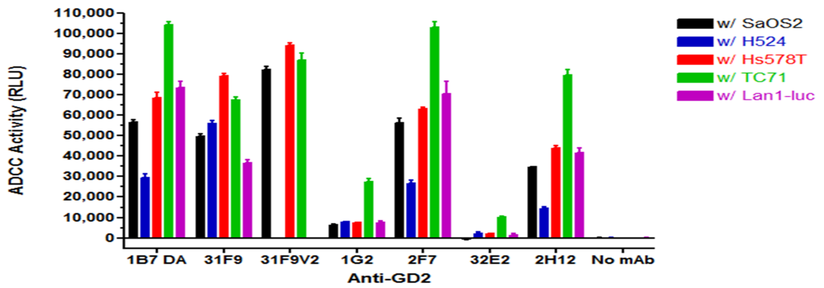
Chart Number	Glycan Structure	Average	StDEV	SEM	% CV
226	Neu5Aca2-8Neu5Aca2-3GalNAcβ1-4Galβ1-4Glcβ-Sp0	5188	601	300	12
224	Neu5Aca2-8Neu5Aca2-8Neu5Aca2-3GalNAcβ1-4Galβ1-4Glcβ-Sp0	3523	328	164	9
223	Neu5Aca2-8Neu5Aca2-8Neu5Aca2-8Neu5Aca2-3GalNAcβ1-4Galβ1-4Glcβ-Sp0	2667	189	95	7
441	Neu5Aca2-3Galβ1-4GlcNAcβ1-3Galβ-Sp8	23	14	7	61
340	GlcNAcα1-4Galβ1-4GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ-Sp0	14	12	6	88

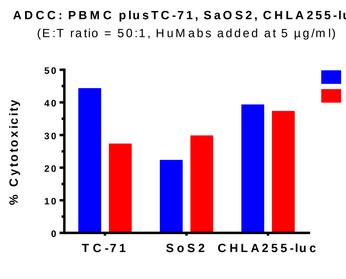
www.functionalglycomics.org/glycomics/HServlet?operation=view&sideMenu=no&psId=primscreen_3423

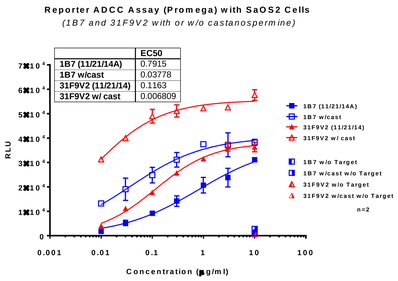
Fig. 5: Activity in Complement Dependent Cytotoxicity (CDC) Assays.

	Cytotoxicity (%)			
Clone ID	H524	Lan1-luc	Jurkat	TC-71
1B7	**98.09**	**106.23**	**69.77**	**94.54**
2H12	**54.73**	**103.76**	**38.38**	NT
1G2	-21.44	-4.97	NT	6.23
2F7	-1.07	**79.63**	**63.56**	**20.57**
2E12*	**125.04**	**103.28**	NT	**91.29**
31F9	10.47	-2.09	-10.28	2.91
31F9V2	**72.89**	**28.9**	-3.51	**45.33**
32E2	-18.43	-8.1	**22.48**	-25.7

Fig. 6: Cytotoxic Activity in Antibody Dependent Cell-mediated Cytotoxicity (ADCC) Assays.

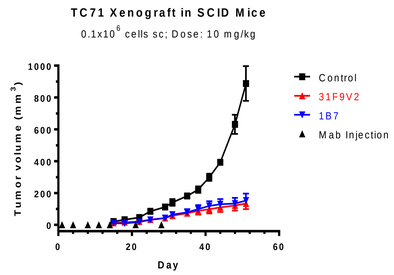


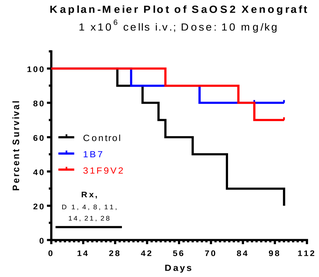




	EC50
1B7 (11/21/14A)	0.7915
1B7 w/cast	0.03778
31F9V2 (11/21/14)	0.1163
31F9V2 w/ cast	0.006809

Fig. 7: Activity of 1B7 and 31F9V2 in Sarcoma Xenograft Models.





Activity of 1B7 and 31F9V2 antibodies was tested in xenograft models. Antibodies (10 mg/kg) were administered twice a week starting on 1 day post inoculation.
Left: TC-71 (Ewing's sarcoma) tumor cells in Matrigel were injected Sub-Q in the hind flank of SCID mice on day 1 (0.1 million cells per mouse, 5 mice per group). Tumor growth was monitored by caliper and the tumor volume is calculated as LxWxW x0.5.
Right: SaOs2 (osteosarcoma) cells were injected intravenously and survival was monitored. Kaplan-Meier Analysis shows the percent survival.

Conclusions:

- Fully human anti-GD2 antibodies were recovered from vaccine trial participants
- 1B7 and 31F9V2 show excellent cytotoxic activity *in vitro* (CDC and ADCC)
- Both antibodies show significant activity in two sarcoma xenograft models
- Studies are ongoing to select an antibody for clinical development

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