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Human monoclonal antibodies to sialyl-Lewis-A with potent CDC and ADCC activity.

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Abstract.

The carbohydrate antigen sialyl-Lewis A (sLe^a) is widely expressed on epithelial tumors of the gastrointestinal tract, on breast cancer cells, and also on small cell lung cancer cells, but is expressed minimally or not at all on normal tissues. sLe^a serves as a ligand for epithelial leukocyte adhesion molecules and higher expression of sLe^a was observed in patients with greater node involvement. Since over-expression of sLe^a appears to be a key event in invasion and metastasis of many tumor cells and tumor cells expressing sLe^a are highly susceptible to antibody mediated lysis mechanisms, sLe^a presents an attractive molecular target for tumor therapy in a minimal disease setting.

Here we report the discovery and initial characterization of fully human antibodies that were generated from blood lymphocytes from individuals immunized with sLe^a vaccine at MSKCC. Antibodies were identified by ELISA using sLe^a-HSA conjugates and binding to the native antigen was verified by FACS analysis on DMS-79 cells. Two antibodies were selected for further studies based on the apparent high affinity, which was estimated by Biacore at 0.14 nM for 5B1 (IgG/λ) and 0.04 nM for 7E3 (IgM/κ). These antibodies did not bind to sialyl-Lewis X, Lewis A and other related carbohydrates. Both antibodies have been expressed as fully functional human recombinant antibodies in CHO cells. Complement dependent cytotoxicity (CDC) against DMS-79 cells was approximately 60% and 70-90% for r5B1 and r7E3, respectively. Moreover, r5B1 antibodies showed approximately 50% ADCC of DMS-79 cells with human NK cells (at 5:1 ratio) and 80-90% ADCC with human peripheral blood mononuclear cells with two different blood donors (at 100:1 ratio). These results are very encouraging and we believe that further studies to scale up and test these antibodies in various tumor challenge models are warranted. Since sLe^a is widely expressed on human cancers, such antibodies could eventually find utility in approximately half of the new cancer cases occurring each year.

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Summary and Conclusion:

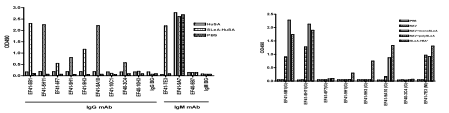
- Human antibodies to sLeA have been recovered from individuals immunized with sLeA vaccine.
- Two antibodies, 7E3 (IgM) and 5B1 (IgG) showed high affinity binding to sLeA–HSA conjugates.
- 7E3 and 5B1 bind well to the native antigen on DMS-79 cells.
- 7E3 and 5B1 show strong complement mediated cytotoxicity (CMC) on DMS-79 and SW626 cells.
- 5B1 shows strong ADCC activity with PBL and NK cells isolated from human blood donors.

These preliminary data are encouraging and further experiments to evaluate the *in vivo* activity of these fully human antibodies in suitable animal models are warranted.

References:

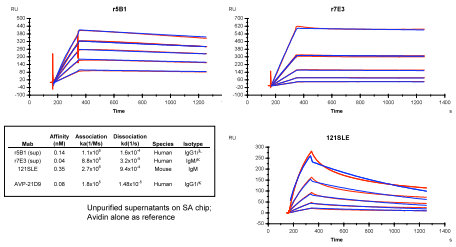
Ragupathi et al., Cancer Immunol Immunother, 58:1397, 2009.

Fig. 1: Detection of human anti- sLeA antibodies by ELISA



ELISA plates were coated with sLeA-APD-HSA (GlycoTech #07-011) and blocked with 0.1% BSA/0.05% Tween 20/ PBS. Hybridoma supernatants were incubated for 1hr. After washing, the plates were incubated with HRP conjugated goat anti-human Fc Ab for 45 min followed by OPD substrate addition and colorimetric detection.

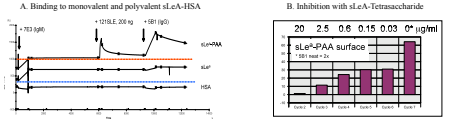
Fig. 2: High Affinity Binding to sLeA-PAA-biotin captured on Avidin Chip



Mab	Affinity (nM)	Association ka(1/Ms)	Dissociation kd(1/s)	Species	Isotype
r5B1 (sup)	0.14	$1.1x10^5$	$1.6x10^{-5}$	Human	IgG1/λ
r7E3 (sup)	0.04	$8.8x10^4$	$3.2x10^{-6}$	Human	IgM/κ
121SLE	0.35	$2.7x10^5$	$9.4x10^{-4}$	Mouse	IgM
AVP-21D9	0.08	$1.8x10^5$	$1.46x10^{-5}$	Human	IgG1/λ

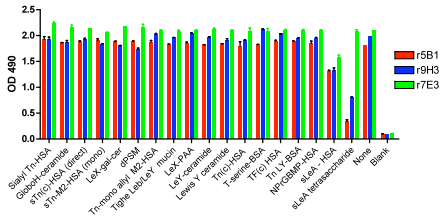
Unpurified supernatants on SA chip; Avidin alone as reference

Fig. 3: Evaluation of Binding Specificity.



(A) Binding of 7E3, 5B1 and 121SLE to sLeA-PAA-biotin (GlycoTech #01-044) or sLeA-sp-biotin (GlycoTech #02-044) captured on a Biacore avidin chip. HSA-biotin served as control and Avidin alone was used as reference. (B) Binding of 5B1 antibody to captured sLeA-PAA-biotin in presence of decreasing amounts of sLeA tetrasaccharide (Sigma #S2279).

C. Binding to sLeA-PAA-HSA in Presence of 20 µg/ml of Various Related Glycoconjugates.



(C) Binding of 7E3, 5B1 and 9H3 to sLeA-HSA coated ELISA plates in presence of 20 µg/ml of the indicated glycoconjugates. sLeA-tetrasaccharide inhibits binding of 5B1 and 9H3 (IgG) but not 7E3 (IgM).

Fig. 4: Binding to DMS-79 Cells

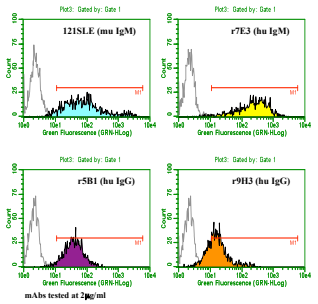


Fig. 5: Complement Mediated Cytotoxicity (CMC) Against DMS-79 Cells.

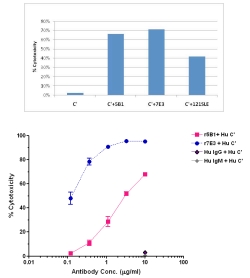
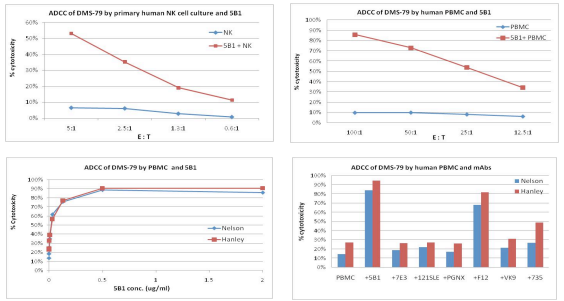


Fig. 6: Cytotoxic Activity in Antibody Dependent Cell-mediated Cytotoxicity (ADCC).



Lead candidate human antibodies tested for activity in antibody dependent cell mediated cytotoxicity (ADCC). ADCC activity against DMS-79 cells at different effector:target ratios with human NK cells (top left) and PBMC (top right). Dose dependent ADCC with PBMC from two independent donors (bottom left). Comparison of ADCC activity against other antibodies (bottom right).

Table 1: cDNA Analysis of Recombinant Antibodies.

Antibody		VH					VL			
Clone ID	Specificity	VH	Mutations from germline	DH (RF)	CDR length	JH	VL	Mutations from germline	CDR length	JL
5B1	SLeA	3-9*01	16	6-25*01 (1)	8, 8, 16	4*02	L1-47*01	4	8, 3, 12	JL1*01
9H3	SLeA	3-9*01	5	2-8*01 (2)	8, 8, 16	4*02	L1-47*01	2	8, 3, 11	JL2*01
7E3	SLeA	3-30*03	6	2-15*01 (2)	8, 8, 19	4*02	K3-15*01	3	6, 3, 10	JK2*01
5H11	SLeA	3-9*01	3	6-25*01(1)	8, 8, 16	4*02	L1-47*01	1	8, 3, 12	JL3*01

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