

C53 Potent CDC, ADCC and Anti-Tumor Activity of Human Monoclonal Antibodies to Sialyl-Lewis A

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Objective

The long-term objective of our studies is to select a clinical candidate human monoclonal antibody for the treatment of recurring cancers. Monoclonal antibodies recovered from individuals vaccinated with cancer vaccines are characterized to select the most biologically active candidates.

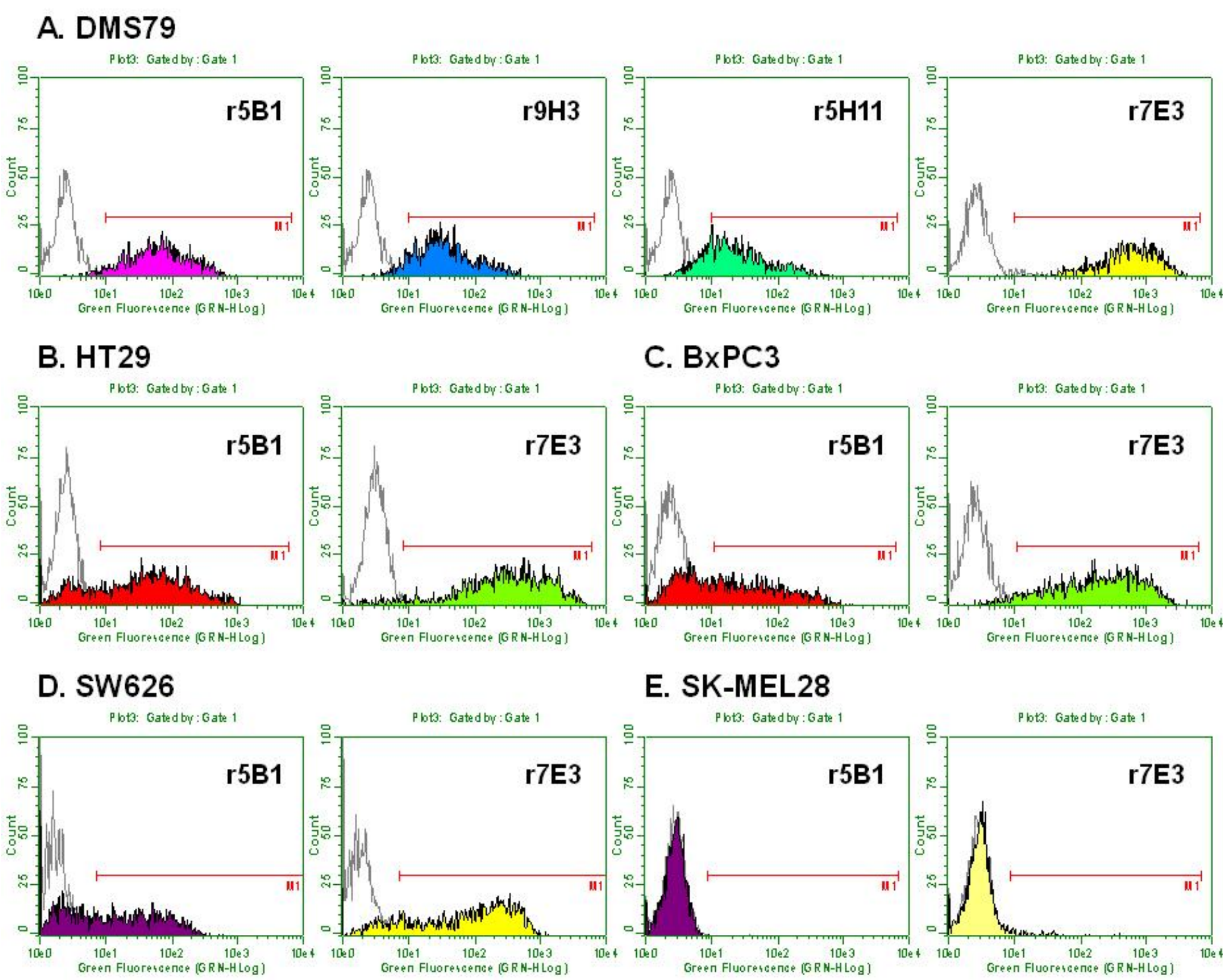
Methods

In this study we evaluated antibodies against the carbohydrate antigen sialyl-Lewis A (sLe^a), also known as CA19.9, which is widely expressed on epithelial tumors of the gastrointestinal tract, on breast cancer cells, and also on small cell lung cancer cells. sLe^a serves as a ligand for epithelial leukocyte adhesion molecules and over-expression of sLe^a appears to be a key event in enabling invasion and metastasis of many tumor cells. Since 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.

Hybridomas were generated with human B cells isolated from individuals vaccinated with sLe^a-KLH conjugate cancer vaccine at MSKCC. Antibody producing cells were identified by ELISA using sLe^a-HSA and synthetic sLe^a – PAA-biotin conjugates. Binding to the native antigen expressed on the cell surface was tested by FACS analysis on sLe^a positive DMS-79 cells and others, and the biological activity was measured in CDC and ADCC assays. The binding affinity was determined by Surface Plasmon Resonance (BiaCore) and the specificity of the carbohydrate binding was evaluated by ELISA and by glycan array analysis (Consortium for Functional Glycomics, Array 4.1). Recombinant antibodies were generated in CHO cells and purified on Protein A (IgG) or hydroxyapatite (IgM), respectively.

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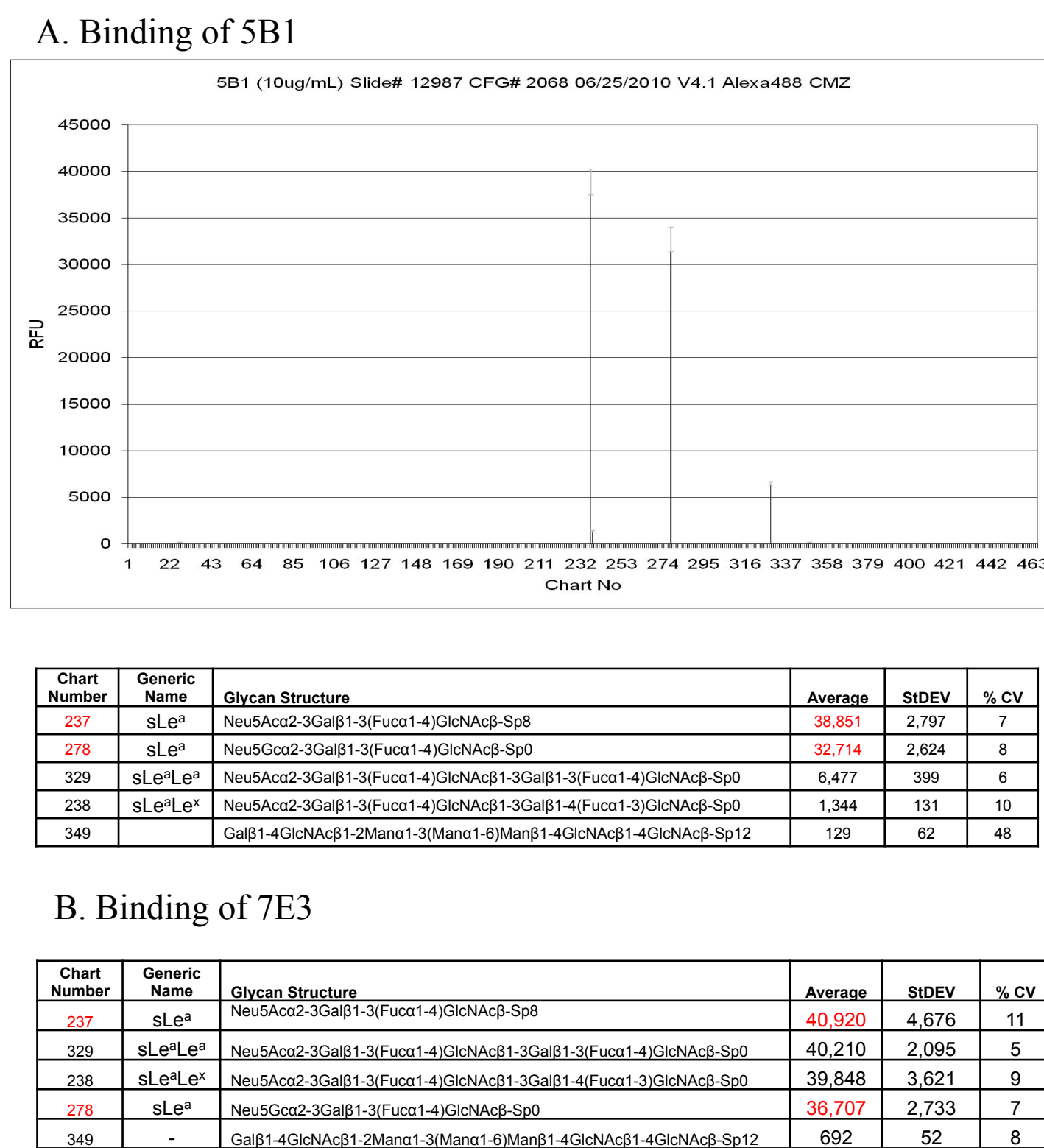
Fig. 1: Selection of antibodies by cell surface staining and high affinity Binding to sLeA-PAA-biotin.



Mab	Affinity (nM)	Association ka(1/Ms)	Dissociation kd(1/s)	Species	Isotype
r5B1 (sup)	0.14	1.1x10 ⁶	1.6x10 ⁻⁴	Human	IgG1 ^λ
r7E3 (sup)	0.04	8.8x10 ⁵	3.2x10 ⁻⁵	Human	IgM ^κ
121SLE	0.35	2.7x10 ⁶	9.4x10 ⁻⁴	Mouse	IgM

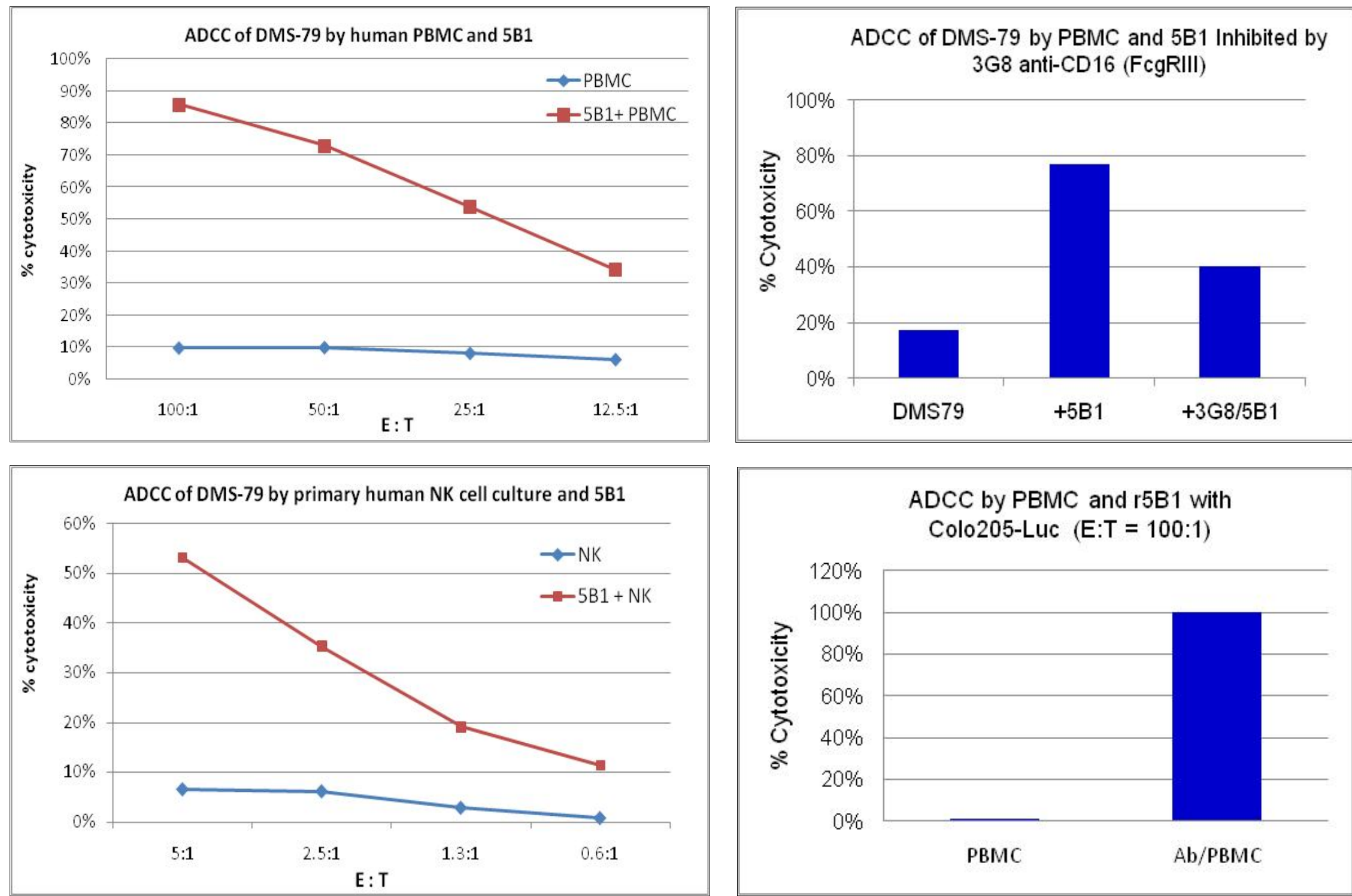
Affinity was measured using sLeA-PAA-biotin (GlycoTech #01-044) captured on a Biacore avidin chip.. and Avidin alone was used as reference.

Fig. 2: Evaluation of Binding Specificity by Glycan Array Analysis



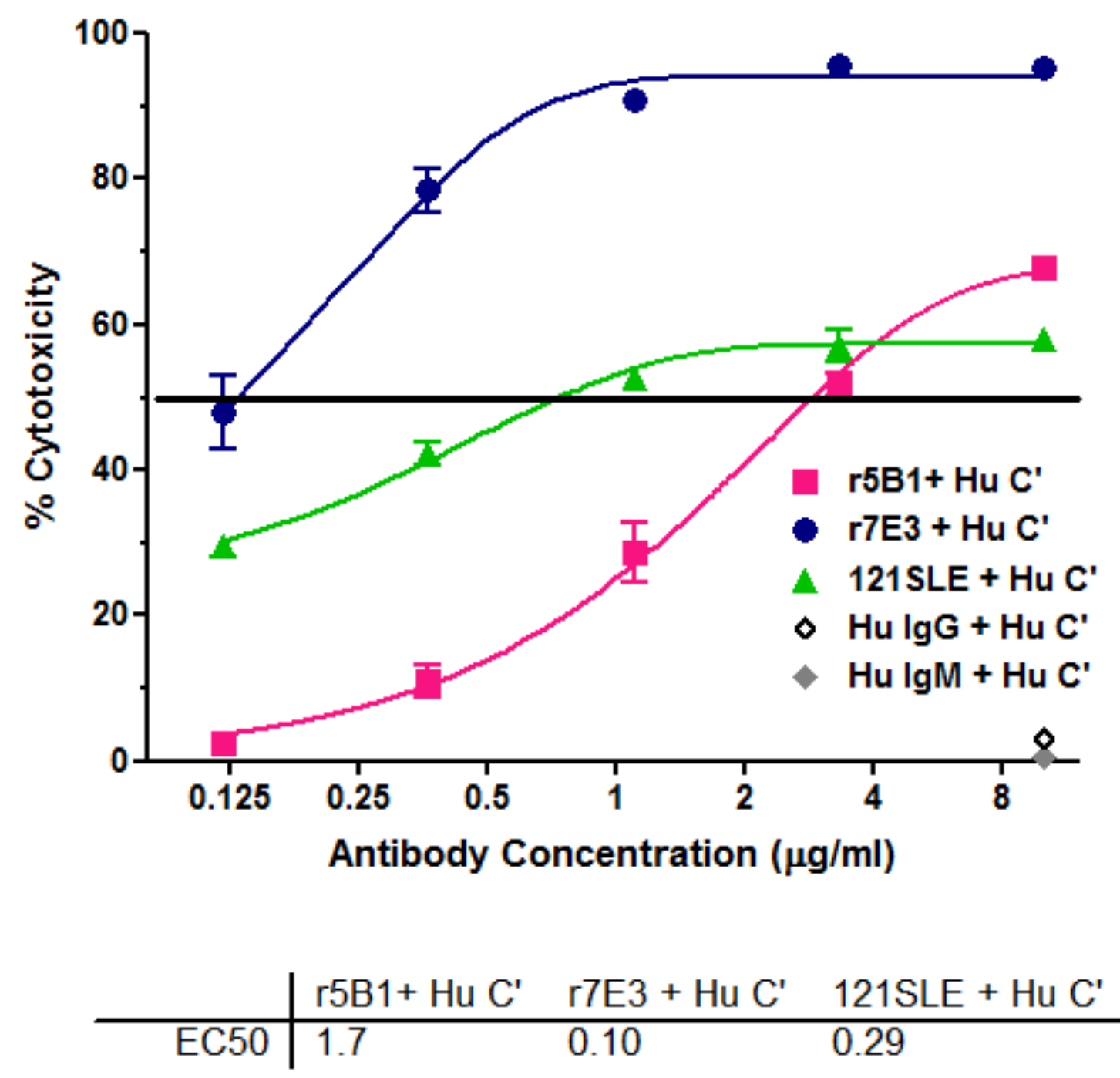
Probing 5B1 (A) and 7E3 (B) binding specificity by glycan array analysis on glycan array 4.1 from the Consortium of Functional Glycomics. Results available at www.functionalglycomics.org. IgD=primscreen_3421 (5B1) and IgD=primscreen_3420 (7E3)

Fig. 4: Cytotoxic Activity in Antibody Dependent Cell-mediated Cytotoxicity (ADCC).



Effector function of r5B1 antibodies in antibody dependent cell mediated cytotoxicity (ADCC) was measured using DMS-79 cells, a human small cell lung cancer cell line and Colo205-luc colon tumor cells which both expresses sLe^a. Cytotoxicity was evaluated in a 4 hr Calcein-AM (acetoxyethyl) cytotoxicity assay. r5B1 at 2 µg/ml and different effector:target ratios with human PBMC (top left) NK cells (bottom left). Inhibition of 5B1 mediated ADCC by blocking Fc receptors with 3G8 (top right). ADCC activity of 5B1 against Colo-205 cells using PBMC at E:T=100:1 (bottom right).

Fig. 3: Complement Dependent Cytotoxicity (CDC) Against DMS-79 Cells.



Complement dependent cytotoxicity of 5B1 and 7E3 measured against DMS-79 cells. Top: Comparison to murine 121SLE (IgM), all mAbs at 1 µg/ml. Bottom: Dose response for 5B1 (IgG), 7E3 (IgM) and 121SLE (mIgM) and calculated EC50 (µg/ml). Human isotype control antibodies showed <4% cytotoxicity.

Fig. 5: Anti-Tumor Activity in Therapeutic S.C. Xenograft Model with DMS-79 Cells

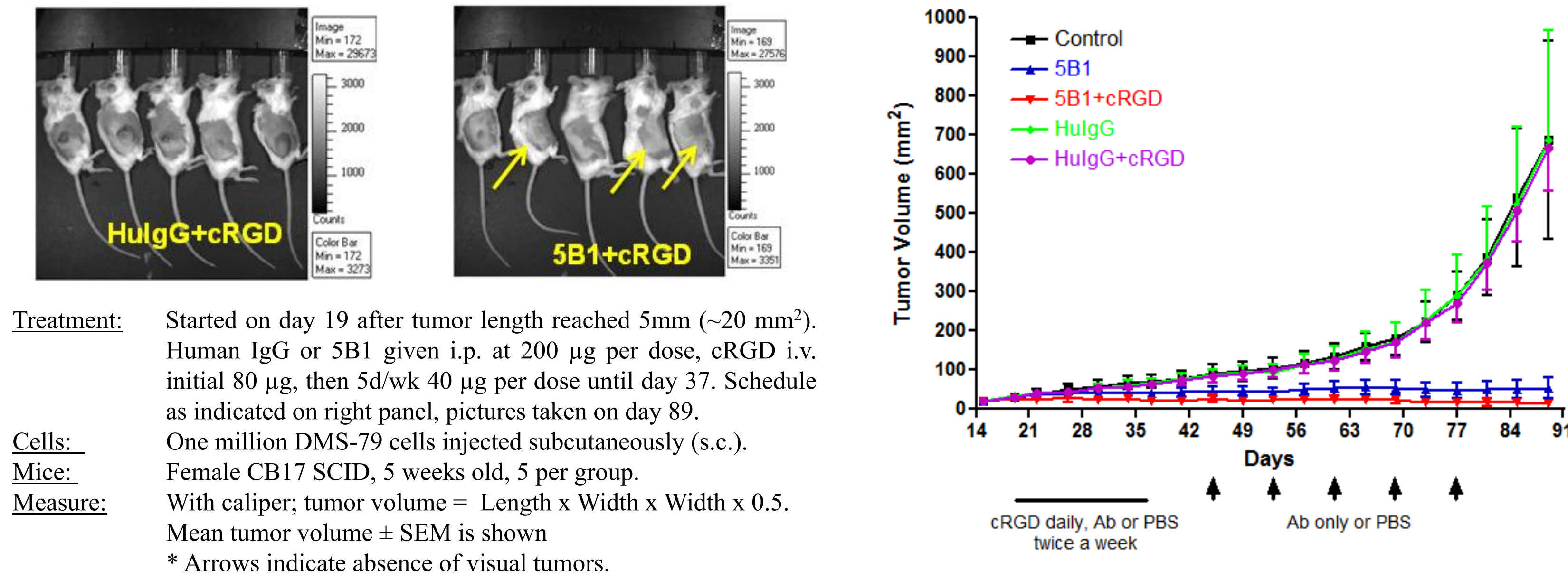
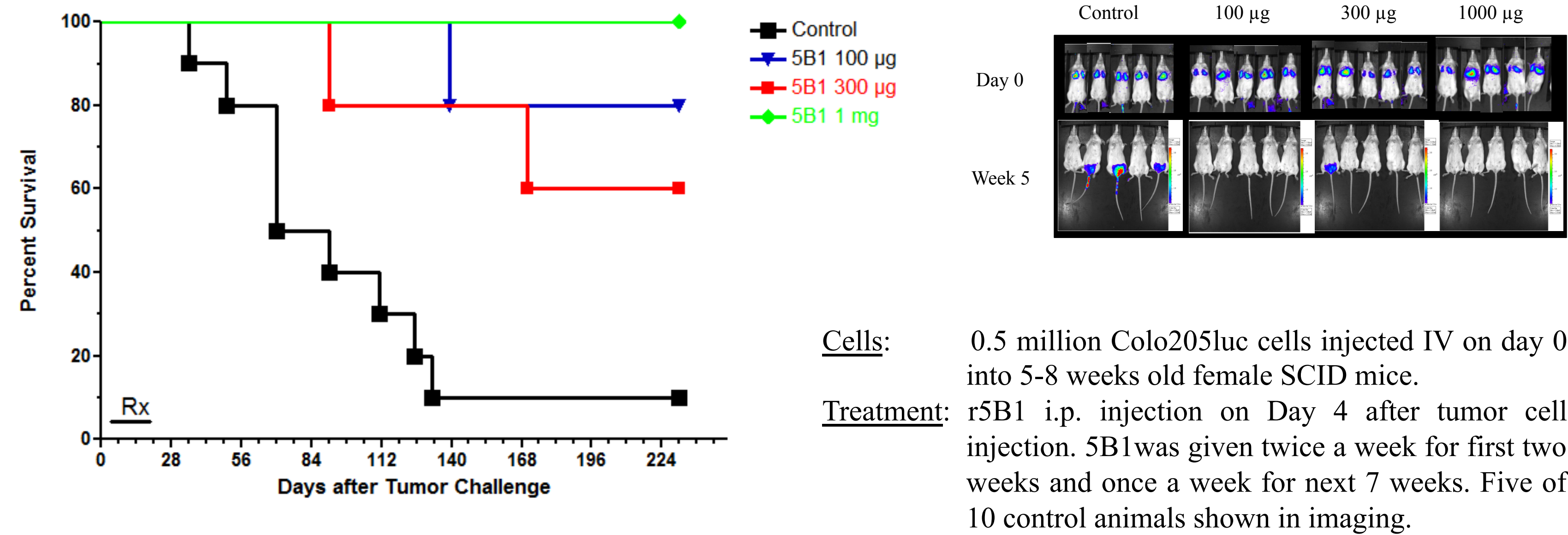


Fig. 6: Effect of r5B1 on Colo205Luc Tumors in SCID Mice: Therapeutic I.V. Model



Results

We report the discovery and initial characterization of fully human antibodies that were generated from blood lymphocytes from individuals immunized with sLe^a –KLH vaccine. Two antibodies (5B1 and 7E3) 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). Both antibodies were highly specific for Neu5Ac2-3Galβ1-3(Fuca1-4)GlcNAcβ and 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 E/T ratio). These antibodies were tested in two xenograft models: 1) Treatment of animals with 5B1 on the day of engraftment with DMS-79 cells in a subcutaneous model completely prevented tumor growth (data not shown). Growth of established DMS-79 tumors was suppressed or regressed in animals treated with 5B1 or a combination of 5B1 plus cRGD. 2) Delayed treatment with various doses of 5B1 showed dose dependent protection up to complete cure in SCID mice engrafted (IV) with Colo205 cells. 7E3 antibodies did not show higher protection despite increased apparent affinity (data not shown).

Conclusion

The specificity and potency of 5B1 antibodies in CDC and ADCC assays translates into good activity in xenograft models. Further studies are warranted to explore the activity against additional cancer specific cell lines in animal models and to pursue preclinical development of 5B1 antibodies.

References

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