

# XmAb™ Fc Engineered Anti-CD19 Monoclonal Antibodies With Enhanced *In Vitro* Efficacy Against Multiple Lymphoma Cell Lines



Eugene Zhukovsky, Matthew Bennett, Erik Pong, Seung Chu, Sher Karki, John Richards, Shaotang Ren, Wei Dang, Collin Edler, Nephi Polder, Patrick Joyce, Cheryl Chan, Philip Hammond, Jonathan Jacinto, John Desjarlais  
Xencor, Monrovia, CA, ezhukovsky@xencor.com

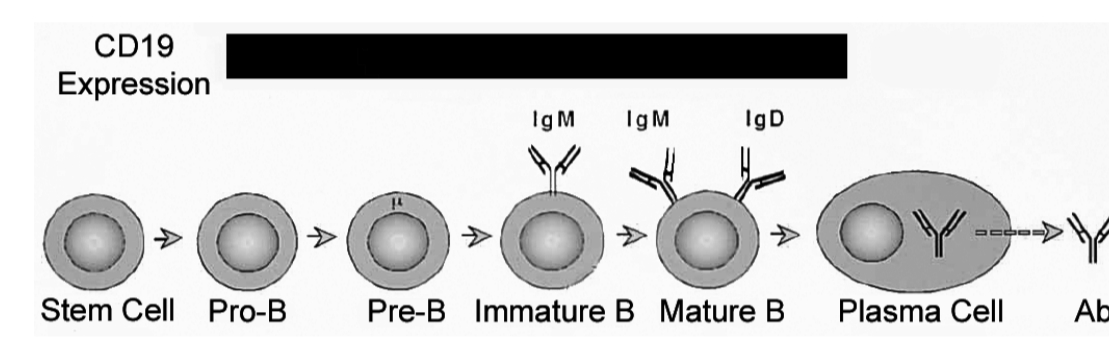
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## ABSTRACT

CD19 is a pan-B cell surface receptor that is expressed from early stages of pre-B cell development through terminal differentiation into plasma cells. It is an attractive immunotherapy target for cancers of lymphoid origin since it is also expressed on the vast majority of Non-Hodgkin's Lymphoma (NHL) cells as well as some leukemias. Despite major improvements in response rates and progression free survival the majority of NHL patients will continue to relapse from current combination chemotherapy with anti-CD20, thus salvage regimens with new non-cross resistant antibody therapies are warranted. Our XmAb™ antibody engineering technology increases the affinity of IgG antibodies for Fc gamma receptors (FcγR), improves the effector function of antibodies, and significantly increases their antitumor potency. This technology was applied to a humanized anti-CD19 antibody to generate a variant with significantly enhanced antibody-dependent cell-mediated cytotoxicity (ADCC) (10- to 100-fold). The resulting XmAb™CD19 variant was assayed for ADCC against multiple cell lines representative of follicular lymphoma (FL), chronic lymphocytic leukemia (CLL), B-cell acute lymphoblastic leukemia (B-ALL), mantle cell lymphoma (MCL), hairy cell leukemia (HCL), chronic myelogenous leukemia (CML), and Burkitt's lymphoma (BL). The ADCC activity of the XmAb™CD19 was in striking contrast to a wild type IgG1 version of the antibody that mediates little ADCC. Moreover, ADCC potency and efficacy of the anti-CD19 Fc variant antibody were superior to that of rituximab: CLL – 10- and 1.5-fold higher, ALL – 10- and 100-fold higher, and HCL – 6- and 1.2-fold higher, respectively. XmAb™CD19 also showed a robust increase in Antibody Dependent Cellular Phagocytosis (ADCP) of more than 50-fold relative to a wild type IgG1 antibody. XmAb™CD19 antibody also displayed potent anti-proliferative activity that was 10-fold more potent than that of rituximab; however, no difference was observed between either IgG1 wild type or XmAb™ antibody. In summary, these data suggest that the anti-CD19 Fc variant antibody engineered for increased effector function could be a promising next-generation NHL immunotherapeutic.

## CD19 is a lymphoma/leukemia marker

- CD19:
  - Member of the immunoglobulin superfamily of receptors
  - Integral membrane protein (95 kDa)
  - Extracellular domain contains two Ig-like domains
  - Cytoplasmic domain (~240 aa) is conserved between species
- CD19 is expressed/over-expressed by a variety of B cell neoplasms
  - Non-Hodgkin's Lymphomas (B-NHL)
  - Pre-B Acute Lymphocytic Leukemia (B-ALL)
  - B cell chronic lymphocytic leukemia (B-CLL)
  - Hairy cell leukemia (HCL)
- Function and expression of CD19:
  - Regulates humoral immune responses by B cells
  - In complex with CD21, CD81 and CD225, forms a multimeric cell surface signal transduction complex involved in co-signaling with BCR:
    - Regulates negative selection of B2 cells
    - Regulates positive selection of B1 cells
  - Ubiquitously expresses on B cell lineage from pre-B cells to terminal differentiation into plasma cells

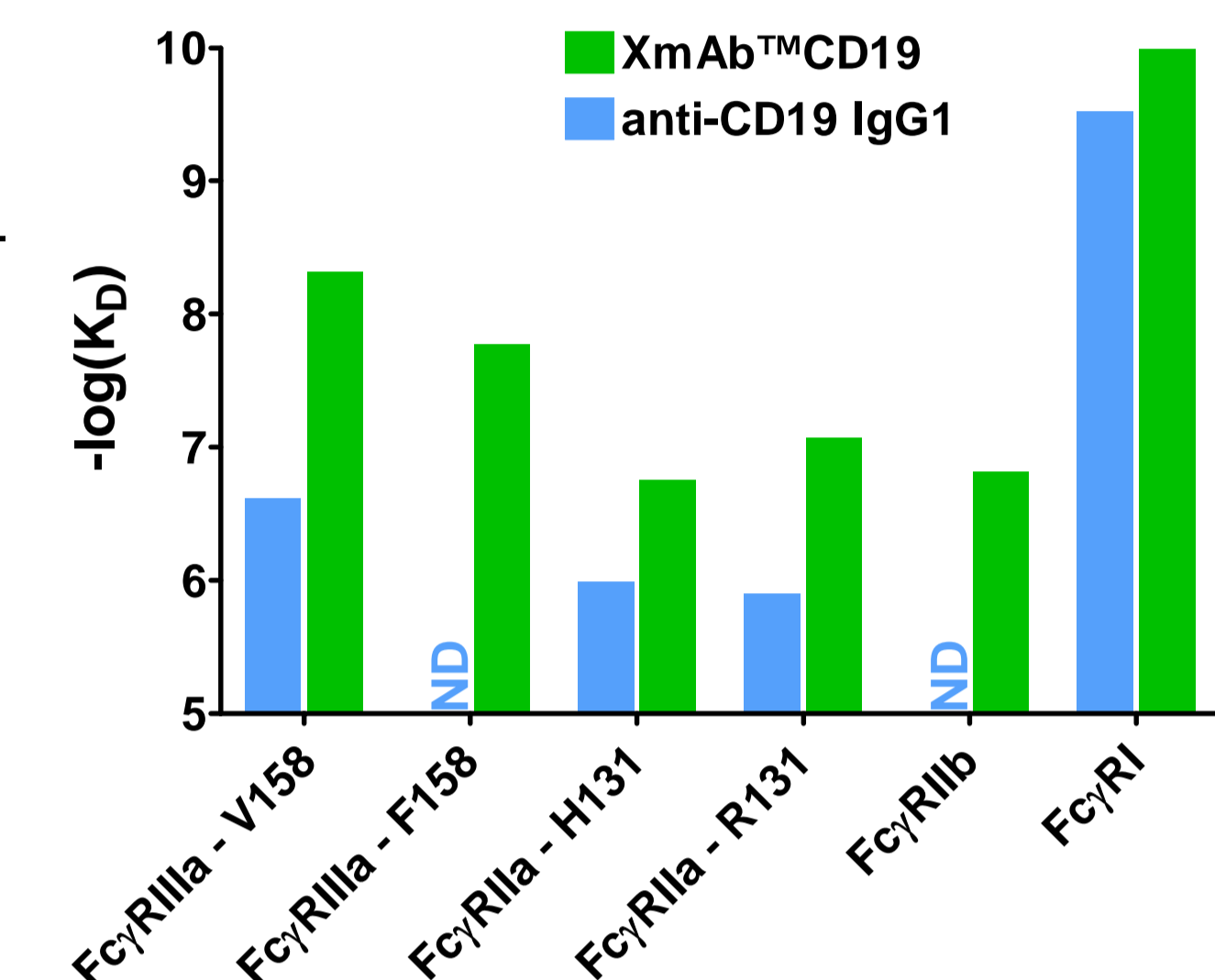


## Development of XmAb™CD19

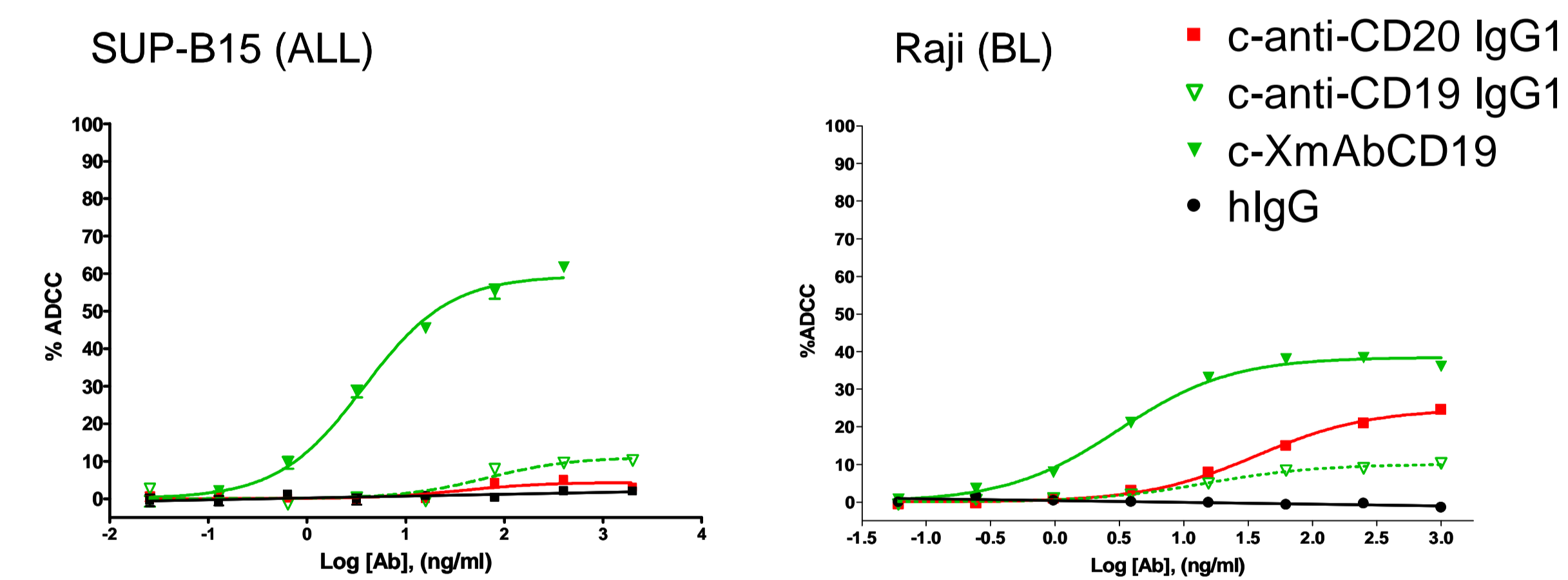
- Selection
  - Starting antibody is a murine anti-CD19
  - Humanized library contained 16 members
  - Affinity optimization yielded a humanized XmAb™CD19 with better binding relative to that of the parent antibody
    - Screening Fab libraries of CDR variants
    - Selecting the leads by screening IgG libraries of the Fab library hits
  - Selection of the lead XmAb™ was based on potency, stability, and other development-related characteristics
- Characterization
  - All variants are characterized by PAGE (reducing and non-reducing conditions), and gel filtration chromatography
  - Direct binding measured by Biacore or FACS
  - Competition binding measured by FACS
  - Antibody Dependent Cell-mediated Cytotoxicity assay (ADCC)
  - Antibody Dependent Cellular Phagocytosis (ADCP)
  - Growth inhibition/viability assay
  - Accelerated stability assay at 60°C

## Increased FcγRIIIa binding affinity

Binding to the FcγRIIIa receptor expressed on NK (natural killer) cells mediates ADCC. Using Xencor's XmAb™ Fc engineering technology we selected a two point variant (I332E/S239D) that greatly enhanced binding of Fc to FcγRIIIa. The relative binding affinities of XmAb™CD19 and a chimeric IgG1 antibody were calculated by determining binding parameters on Biacore. Briefly, protein A/G was coupled to a flow cell of a CM5 chip. IgG was first diluted in Biacore running buffer (HBS-EP, pH 7.4) to 25 nM and immobilized to protein A/G channel to ~ 1000 RUs. FcγR-His was serially diluted in the same buffer and injected at 30 μL/min for 2 min followed by dissociation for 3 min. To determine K<sub>D</sub> the resulting sensorgrams are "group-fitted" using the 1:1 interaction model available in BIAevaluation software.

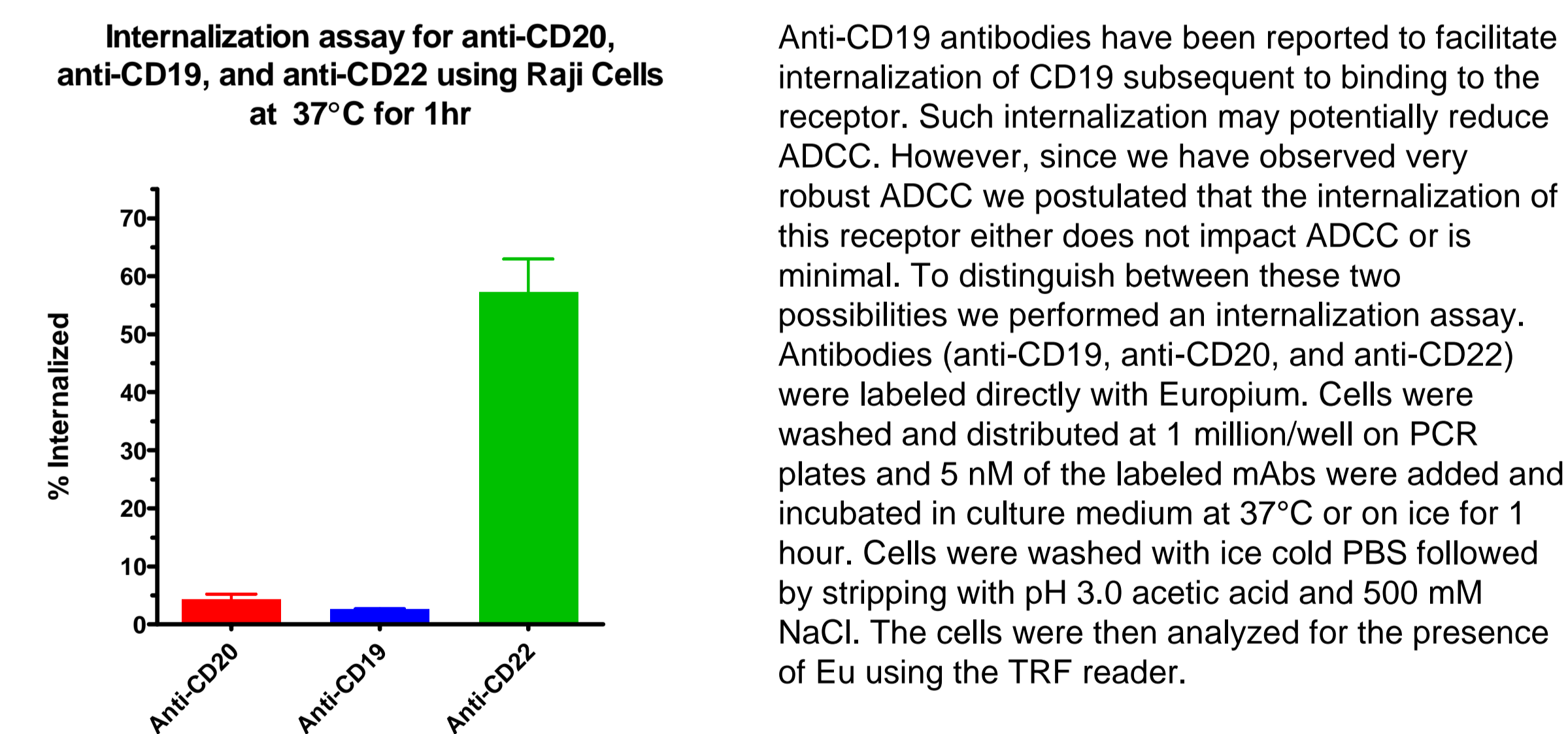


## XmAb™CD19 mediates enhanced ADCC



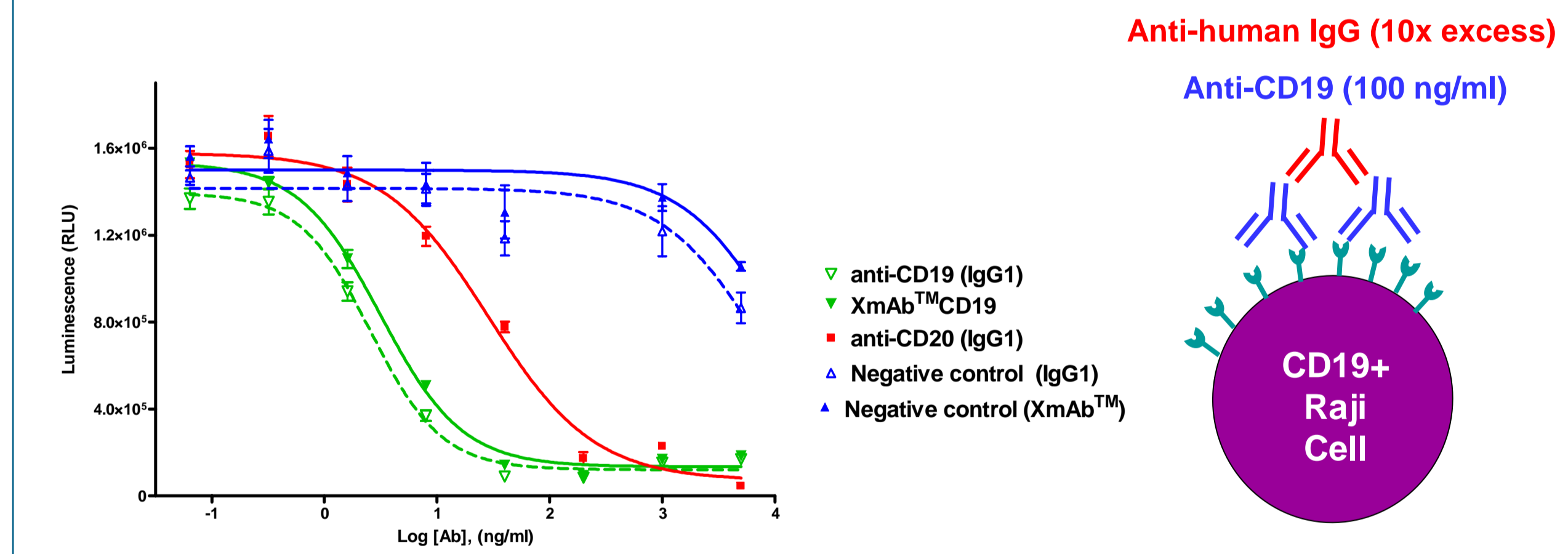
Antibody dependent cell-mediated cytotoxicity was measured by lactate dehydrogenase (LDH) release. Human PBMC effector cells were purified from a leukopak using a Ficoll gradient. SUP-B15 (Acute Lymphocytic Leukemia) and Raji (Burkitt's Lymphoma) target cells were seeded into 96-well plates at 10,000 (Raji) and 20,000 (SUP-B15) cells per well and opsonized using antibody at the indicated concentration. Effector cells were added at 25x E:T and the plate incubated at 37°C for 4 hrs for assay. Data were normalized to maximal (Triton X100 lysis of target cells alone) and minimal (PBMCs alone) lysis.

## XmAb™CD19 internalization is similar to rituximab (anti-CD20)



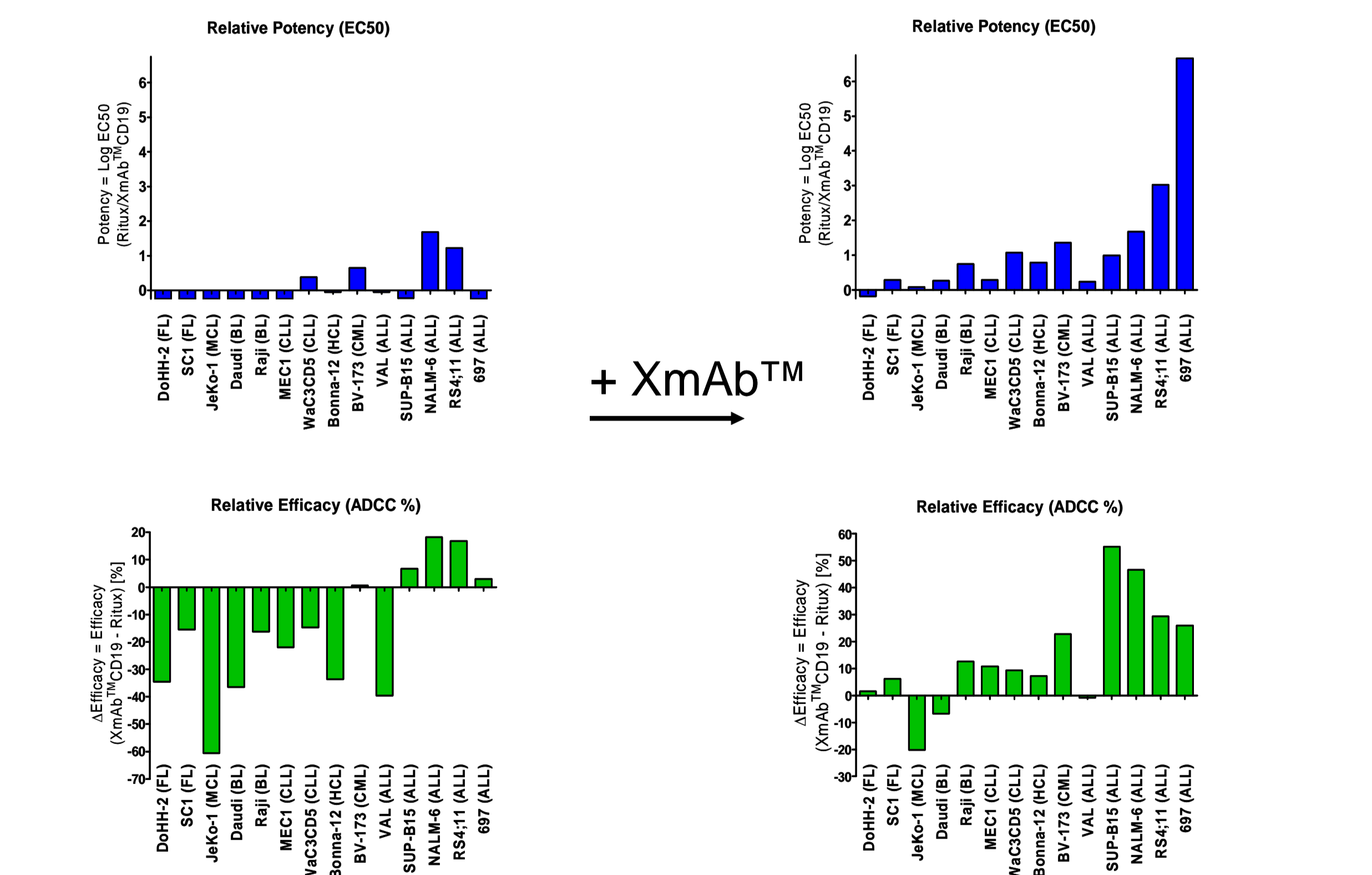
Anti-CD19 antibodies have been reported to facilitate internalization of CD19 subsequent to binding to the receptor. Such internalization may potentially reduce ADCC. However, since we have observed very robust ADCC we postulated that the internalization of this receptor either does not impact ADCC or is minimal. To distinguish between these two possibilities we performed an internalization assay. Antibodies (anti-CD19, anti-CD20, and anti-CD22) were labeled directly with Europium. Cells were washed and distributed at 1 million/well on PCR plates and 5 nM of the labeled mAbs were added and incubated in culture medium at 37°C or on ice for 1 hour. Cells were washed with ice cold PBS followed by stripping with pH 3.0 acetic acid and 500 mM NaCl. The cells were then analyzed for the presence of Eu using the TRF reader.

## XmAb™CD19 is anti-proliferative



To observe an anti-proliferative effect *in vitro*, many antibodies require cross-linking, usually accomplished by a secondary antibody. It has been proposed that corresponding *in vivo* effects for these antibodies may be dependent on cross-linking mediated by Fc receptors expressed on the surface of effector cells. In this experiment Raji cells were grown for 3 days in the presence of XmAb™CD19 and control antibodies at varying concentrations with 10x molar excess of cross-linking antibody. Cell growth was measured using an ATP-dependent luminescence assay.

## XmAb™CD19 has enhanced cytotoxicity over a broad range of cell lines



In order to evaluate cytotoxic properties of XmAb™CD19 we have performed ADCC assays on a panel of 14 cell lines representing various lymphomas and leukemias. Both parameters, potency (EC50) and efficacy (% ADCC) were normalized to that of rituximab (anti-CD20). This screen has demonstrated the cytotoxic superiority *in vitro* of XmAb™CD19 over a broad range of cell lines, especially representing the lympho-proliferative disease that originates in early stages of B cell development.

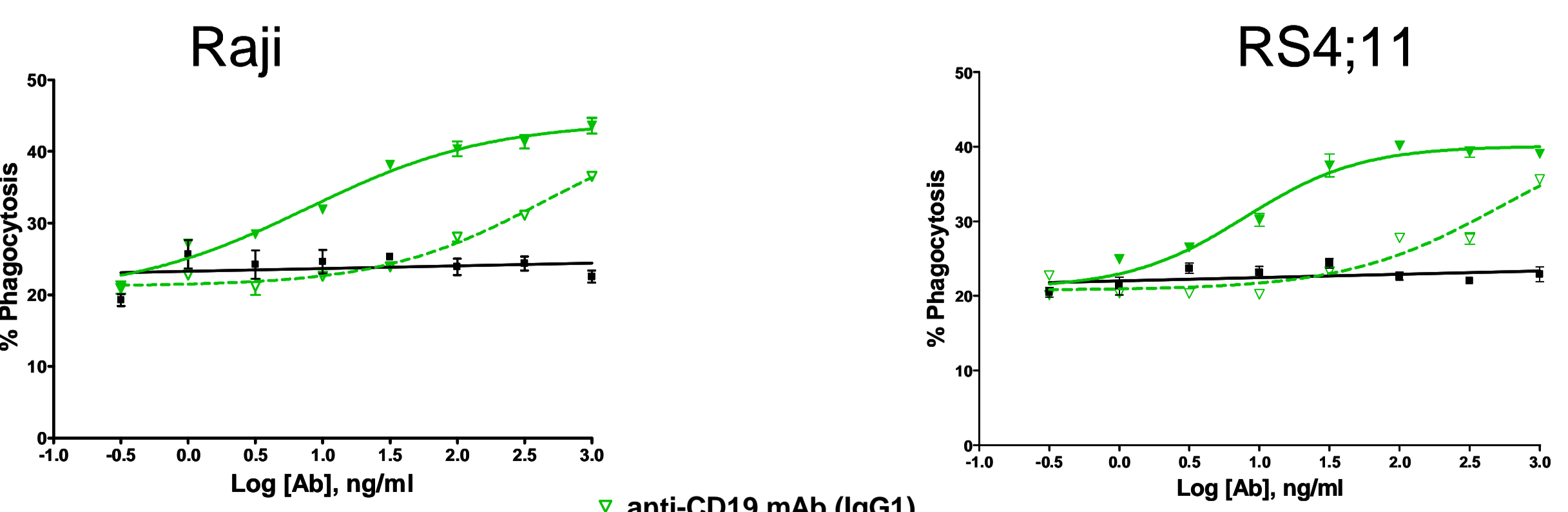
## Summary

The XmAb™CD19 engineered antibody has been shown to have enhanced *in vitro* potency and efficacy as compared to IgG1 antibodies; this was accomplished by optimizing both binding of the Fc domain to FcγR and of the Fv domain to the CD19 antigen. The enhanced cytotoxicity was observed both in ADCC and in ADCP assays; we also observed that this antibody had robust anti-proliferative activity, however this activity was similar to that of IgG1 antibody. The humanized XmAb™CD19 was selected to have an optimized stability profile and represents a new promising immunotherapeutic development candidate for treatment of lymphoma and leukemia.

## Acknowledgements

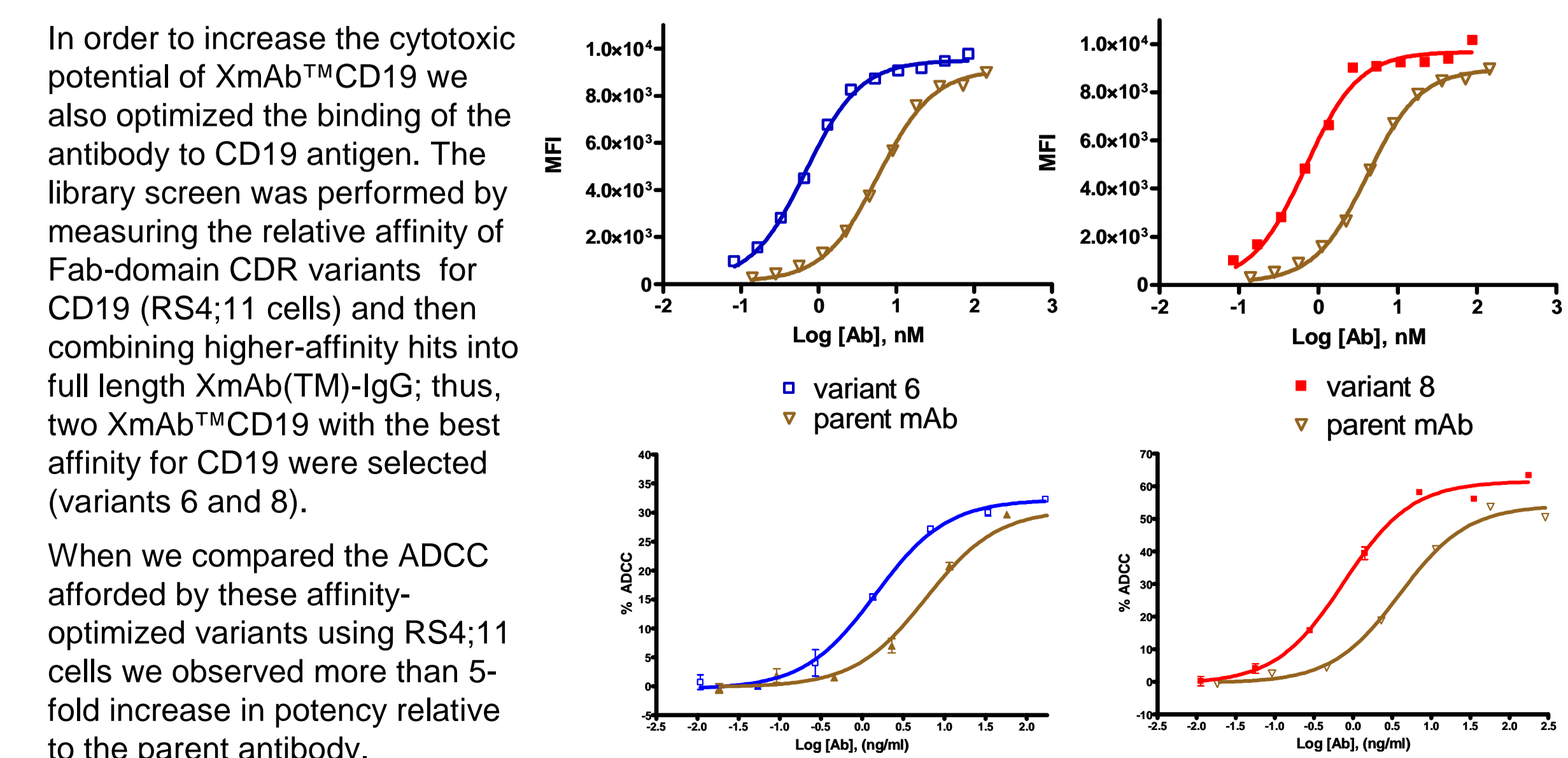
We would like to thank Araz Eivazi, Ethan Li, and Greg Moore for their contribution to this work.

## Humanized XmAb™CD19 mediates enhanced phagocytosis



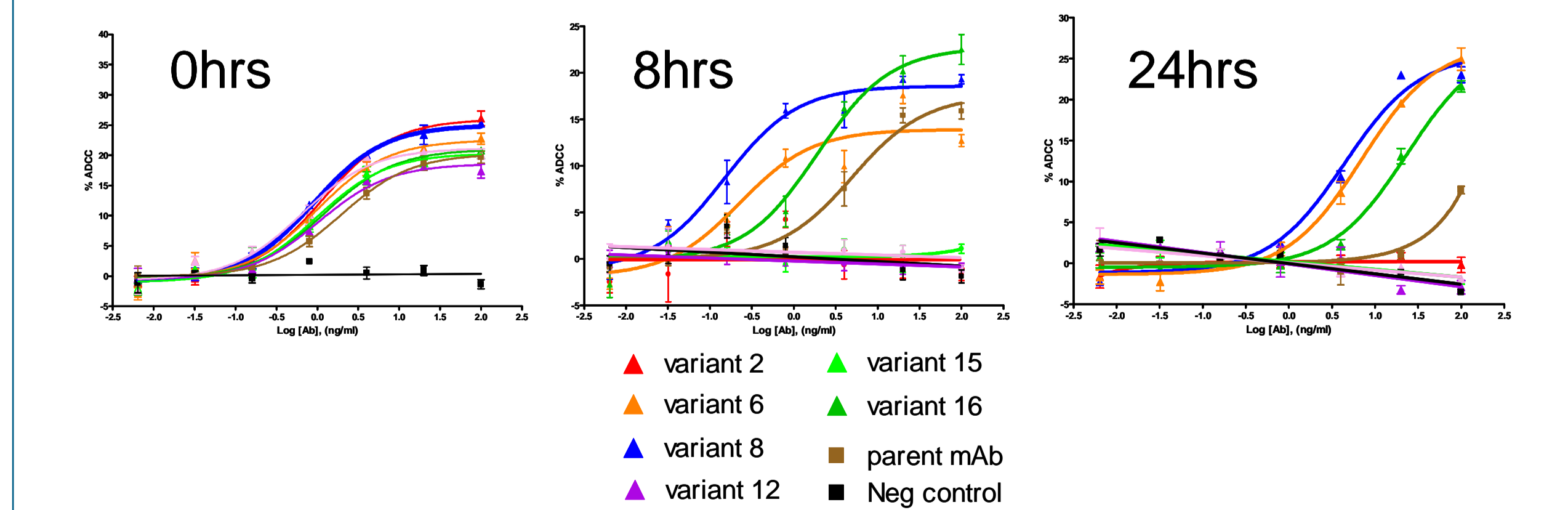
To determine the effect of Fc optimization on phagocytosis we performed ADCP assays. Briefly, tumors were opsonized with anti-CD19 specific antibodies and cultured with monocyte derived macrophages at an E:T ratio 3:1. After 6 hours of incubation cells were harvested, stained, and evaluated by flow cytometry to determine the percentage of phagocytosis.

## Affinity optimization of XmAb™CD19 further increases cytotoxicity



In order to increase the cytotoxic potential of XmAb™CD19 we also optimized the binding of the antibody to CD19 antigen. The library screen was performed by measuring the relative affinity of Fab-domain CDR variants for CD19 (RS4;11 cells) and then combining higher-affinity hits into full length XmAb(TM)-IgG; thus, two XmAb™CD19 with the best affinity for CD19 were selected (variants 6 and 8). When we compared the ADCC afforded by these affinity-optimized variants using RS4;11 cells we observed more than 5-fold increase in potency relative to the parent antibody.

## Affinity optimized XmAb™CD19 is stabilized relative to the parent



Relative stability of XmAb™CD19 antibodies was assessed in accelerated stability studies. Briefly, antibodies were incubated at 60°C for various lengths of time and their cytotoxicity was subsequently tested in ADCC using RS4;11 cells. Some variants lost their cytotoxic potency after 8 hrs of incubation at 60°C. Both variants 6 and 8, selected from the binding and ADCC assays, were active even after 24 hrs incubation and showed higher stability relative to that of the parent antibody.