

XmAb™ 5574: an Fc-Engineered Anti-CD19 Monoclonal Antibody with *In Vitro* and *In Vivo* Efficacy against Lymphoma and Leukemia



Eugene Zhukovsky, Holly Horton, Christopher Lawrence, Sher Karki, John Richards, Erik Pong, Matthew Bernett, Seung Chu, Patrick Joyce, Matthias Peipp[#], Roland Repp[#], John Desjarlais
 Xencor, Inc., Monrovia, CA, USA and [#]Division of Stem Cell Transplantation and Immunotherapy; University of Kiel, Kiel, Germany

A Abstract

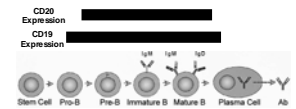
CD19 is a pan-B cell surface receptor expressed from early stages of pre-B cell development through terminal differentiation into plasma cells. It is an attractive target for cancers of lymphoid origin since it is expressed on the vast majority of Non-Hodgkin Lymphomas (NHL) as well as different types of leukemia, including those lacking CD20 expression (e.g. pediatric ALL). The majority of NHL patients will relapse after the current treatment regimen of chemotherapy combined with rituximab (CHOP-R) despite major improvements in response rates and progression free survival. Thus salvage regimens with novel non-cross resistant antibody therapies are warranted. Here we present the characterization of a novel Fc-engineered and humanized anti-CD19 antibody (XmAb™ 5574) that was generated using our XmAb™ antibody engineering technologies. This antibody is highly cytotoxic against a panel of lymphoma and leukemia cell lines as well as primary cancer cells. The main features of this antibody are: i) increased affinity for Fc gamma receptors (FcγR), ii) improved effector function, and iii) significantly increased antitumor potency. Humanization and affinity maturation technologies were applied to this antibody in order to: i) decrease immunogenicity, ii) increase affinity, and iii) increase stability of the engineered antibody. Since internalization is expected to impact a naked antibody's effector functions, we assayed its internalization rate in the Raji cell line using Eu-labeled anti-CD19 antibodies and observed an unexpectedly low rate of internalization. Therefore, we proceeded to investigate several direct and indirect (Fc-mediated) mechanisms of antibody-mediated cytotoxicity. The potency of XmAb5574 in antibody-dependent cell-mediated cytotoxicity (ADCC) increased 10- to 100-fold relative to a native/non-Fc-engineered version (anti-CD19 IgG1) in a screen of nine NHL and leukemia cell lines (chronic lymphocytic leukemia [CLL], B-cell acute lymphoblastic leukemia [B-ALL], hairy cell leukemia [HCL], mantle cell lymphoma [MCL], chronic myelogenous leukemia [CML], and Burkitt's lymphoma [BL]). ADCC potency (EC50) and efficacy (% lysis) of the Fc-engineered anti-CD19 antibody were superior to that of rituximab in CLL - 10- and 1.5-fold higher, in B-ALL - 10- and 100-fold higher, and in HCL - 6- and 1.2-fold higher, respectively. Unlike XmAb5574, native anti-CD19 IgG1 mediated little ADCC. Moreover, XmAb5574 mediated potent ADCC of primary patient-derived ALL and MCL cells that was also substantially increased in potency and efficacy relative to rituximab and anti-CD19 IgG1. Furthermore, XmAb5574 demonstrated enhanced antibody-dependent cellular phagocytosis (ADCP) relative to anti-CD19 IgG1. XmAb5574 also exhibited robust anti-proliferative and apoptotic activity that was significantly more potent than that of rituximab. Xenograft mouse models, Ramos and Raji, demonstrated that XmAb5574 has remarkable protection against tumor growth. Moreover, in the Ramos xenograft model of an established sc tumor, XmAb5574 showed statistically significant benefit for tumor inhibition compared to anti-CD19 IgG1. Finally, XmAb5574 showed rapid and efficient depletion of B cells in cynomolgus monkeys. In summary, our data suggest that our anti-CD19 Fc variant antibody engineered for increased effector function is a promising next-generation immunotherapeutic for a variety of leukemias and lymphomas.

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
- 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:
 - Amplifies BCR activation
 - 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 early plasma cells

CD19 is expressed/over-expressed by a variety of B cell neoplasms

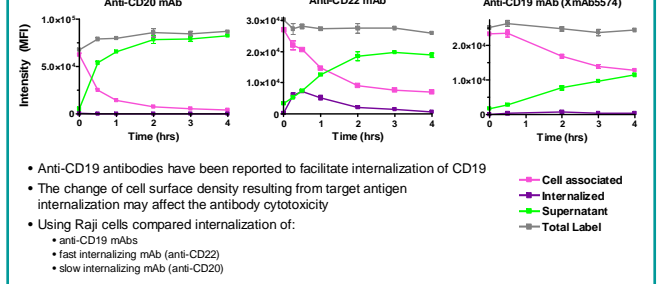
- Non-Hodgkin's Lymphomas (B-NHL)
- Pre-B Acute Lymphocytic Leukemia (B-ALL)
- B cell chronic lymphocytic leukemia (B-CLL)
- Hairy cell leukemia (HCL)



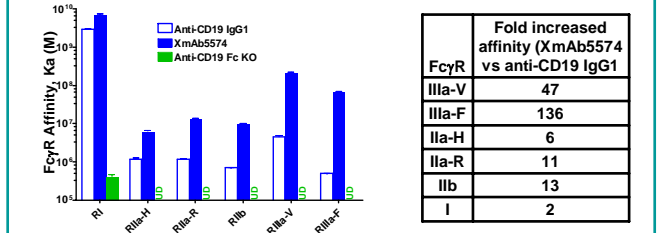
Development of XmAb5574

- Selection
 - Starting antibody is a murine anti-CD19
 - XmAb mutations, I332E/S239D, in the Fc region increased binding to Fcγ Receptors
 - Affinity optimization yielded XmAb5574, a humanized antibody with increased binding to CD19 relative to that of the parent antibody
 - Selection of the lead XmAb was based on potency, stability, and other development-related characteristics
- Characterization
 - All variants are characterized by PAGE 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/apoptosis assays

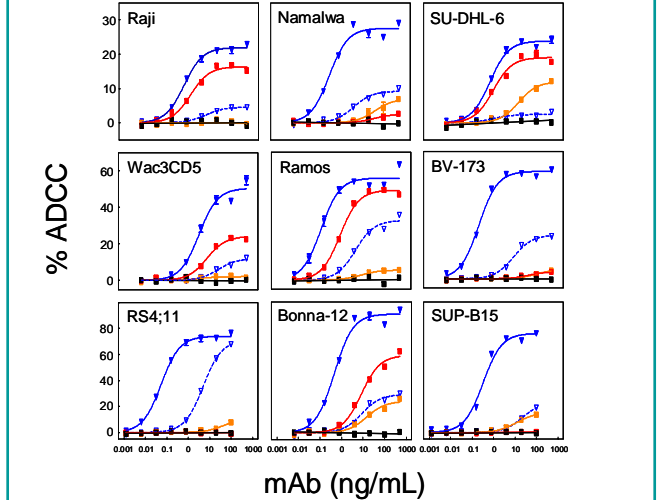
B CD19 internalization is slow



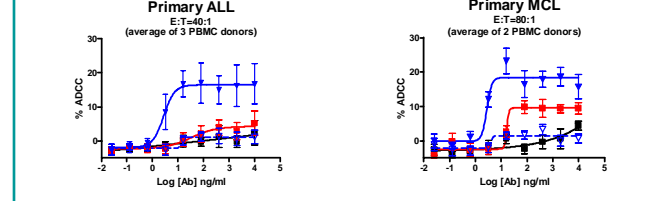
- Anti-CD19 antibodies have been reported to facilitate internalization of CD19
- The change of cell surface density resulting from target antigen internalization may affect the antibody cytotoxicity
- Using Raji cells compared internalization of:
 - anti-CD19 mAbs
 - fast internalizing mAb (anti-CD22)
 - slow internalizing mAb (anti-CD20)



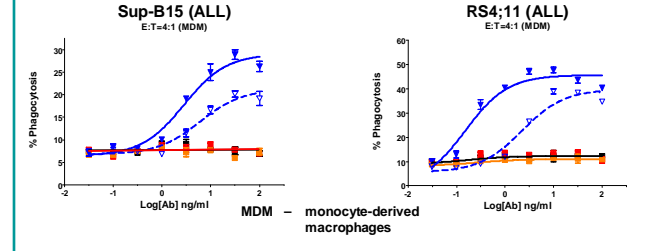
XmAb5574 has enhanced ADCC relative to its IgG1 analog, rituximab and alemtuzumab



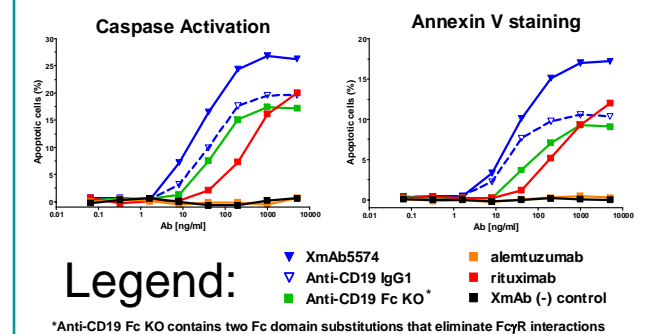
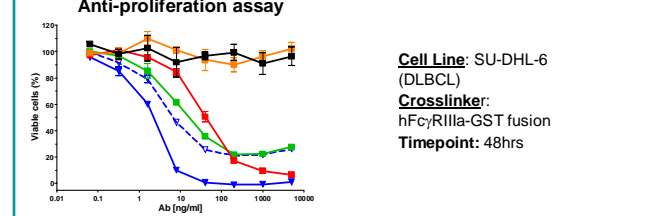
C XmAb5574 is cytotoxic ex vivo against primary leukemia and lymphoma cells



XmAb5574 has enhanced phagocytosis relative to IgG1 analog, rituximab and alemtuzumab



XmAb5574 is anti-proliferative



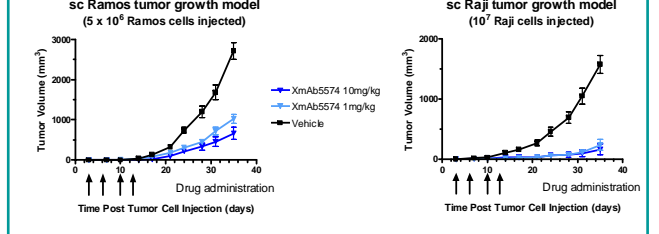
Legend:

- XmAb5574
- Anti-CD19 IgG1
- Anti-CD19 Fc KO*
- alemtuzumab
- rituximab
- XmAb (-) control

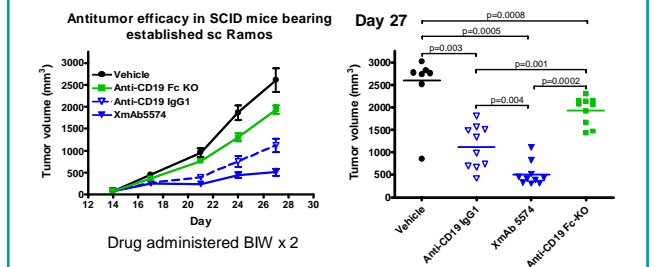
 *Anti-CD19 Fc KO contains two Fc domain substitutions that eliminate FcγR interactions

D XmAb5574 is efficacious in xenograft SCID mouse models

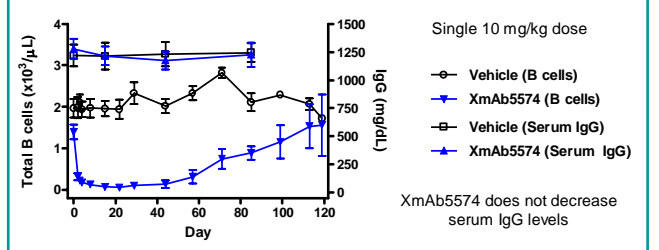
I. XmAb5574 inhibits tumor growth



II. Efficacy against established tumors is FcγR-dependent and enhanced by Fc engineering.



XmAb5574 efficiently depletes B cells in cynomolgus monkeys



Summary

- XmAb5574 represents a promising new immunotherapeutic development candidate for treatment of lymphoma and leukemia with:
- enhanced effector functions: NK cell-mediated ADCC and macrophage-mediated phagocytosis are increased relative to anti-CD19 and anti-CD20 IgG1
 - robust anti-proliferative and apoptotic activity
 - enhanced efficacy in SCID mouse xenograft models of human B cell lymphomas relative to anti-CD19 IgG1
 - efficient B cell depletion capacity in cynomolgus monkeys