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

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Abstract

Background: 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 Lymphoma (NHL) and leukemia cells. The majority of NHL patients relapse after the current treatment regimen of chemotherapy combined with rituximab (CHOP-R), thus salvage regimens with non-cross resistant antibody therapies are warranted. We present the characterization of a humanized Fc-engineered anti-CD19 antibody (XmAb5574).

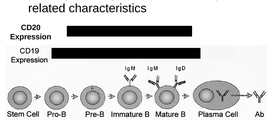
Methods: We applied XmAb technology to a chimeric CD19 antibody to humanize it, increase its affinity for Fc gamma receptors, improve its effector function, and thus significantly increase its antitumor potency. This approach yielded a pre-clinical candidate (XmAb5574) that was tested in a series of in vitro cytotoxicity assays and in mouse xenograft models of NHL. We also tested the depletion of B cells in peripheral blood of cynomolgus monkeys and evaluated the half life.

Results: XmAb5574 is an anti-CD19 antibody that is highly cytotoxic against a panel of lymphoma and leukemia cell lines. This antibody does not appreciably internalize, and it mediates ADCC (EC50 ~ 1 – 0.03 ng/ml) and phagocytosis (EC50 ~ 3 – 0.1ng/ml) superior to that of native anti-CD19 IgG1, rituximab, and alemtuzumab. XmAb5574-mediated ADCC is also significantly increased relative to the native IgG1 analog and rituximab in patient-derived ALL and MCL cells. Furthermore, XmAb5574 demonstrates enhanced antibody-dependent cellular phagocytosis (ADCP) relative to anti-CD19 IgG1. XmAb5574 exhibits apoptotic activity that is significantly more potent than that of both the native IgG1 analog and rituximab. Subcutaneous xenograft mouse models with Ramos and Raji cells show that XmAb5574 has robust protection in both protocols. Moreover, in the Ramos xenograft model of an established sc tumor XmAb5574 showed statistically significant benefit for tumor inhibition compared to anti-CD19 IgG1. Notably, we observed sustained depletion of peripheral B cells in cynomolgus monkeys dosed with XmAb5574. Finally, the PK of XmAb5574 was similar to that of the native IgG1 antibody.

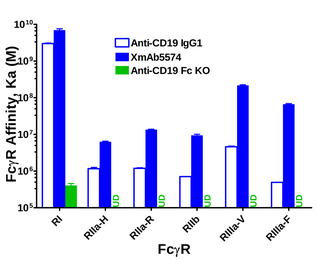
Conclusions: The main features of XmAb5574 are: i) improved cytotoxicity in tumor cells, ii) robust antitumor activity in mouse xenograft models, and iii) B cell depletion in cynomolgus monkeys. Thus XmAb5574, our anti-CD19 Fc variant antibody engineered for increased effector function has potent direct and indirect effector properties and warrants clinical evaluation as an immunotherapeutic for CD19+ hematological malignancies.

Introduction

- 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
- 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)
- Selection of XmAb5574
  - Starting antibody is a murine anti-CD19
  - XmAb mutations, I332E/S239D, in the Fc region increased binding to FcγR 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



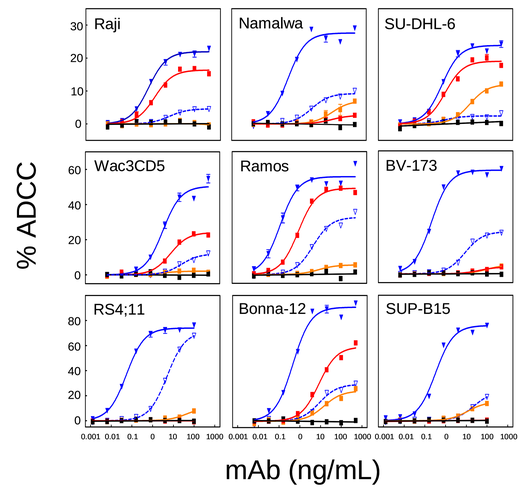
Fc engineering increases FcγRIIIa binding



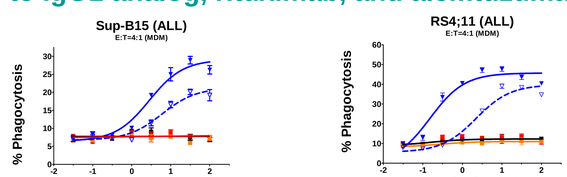
FcγR	Fold increased affinity (XmAb5574 vs anti-CD19 IgG1
IIIa-V	47
IIIa-F	136
IIa-H	6
IIa-R	11
IIb	13
I	2

in vitro

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

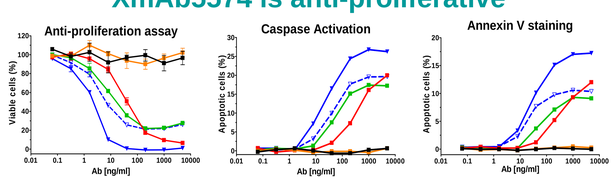


XmAb5574 has enhanced phagocytosis relative to IgG1 analog, rituximab, and alemtuzumab



MDM – monocyte-derived macrophages

XmAb5574 is anti-proliferative



Legend:

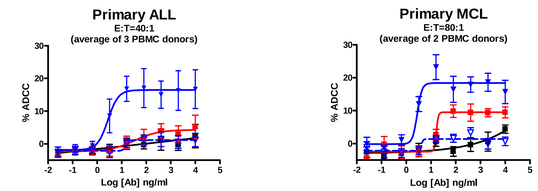
- ▼ XmAb5574
- ▽ Anti-CD19 IgG1
- ■ Anti-CD19 Fc KO*
- ■ alemtuzumab
- ■ rituximab
- ■ XmAb (-) control

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

Cell Line: SU-DHL-6 (DLBCL); crosslinker: hFcγRIIIa-GST fusion; time point: 48hrs

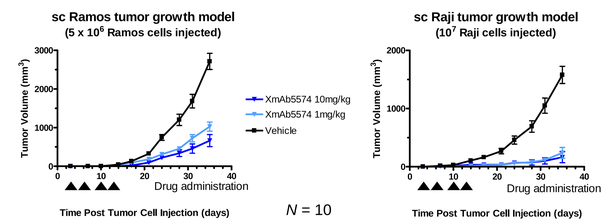
ex vivo and *in vivo*

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



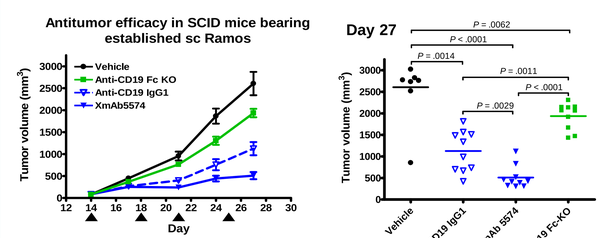
XmAb5574 suppresses tumor growth in SCID mouse models

I. XmAb5574 inhibits tumor growth



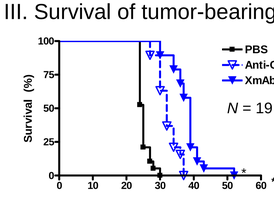
N = 10

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



Tumors between 30-120 mm³; *N* = 10
Dosing at 10mg/kg, BIW x 2

III. Survival of tumor-bearing mice is enhanced by Fc engineering



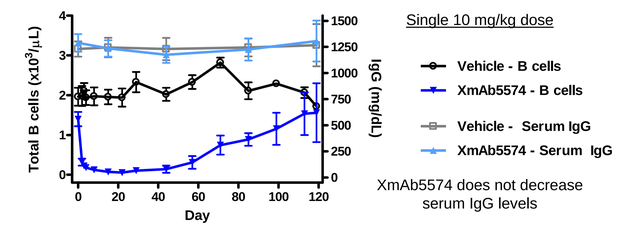
N = 19

- Tumors between 50-130 mm³
- Dosing at 10 mg/kg, BIW x 3
- Criteria for euthanasia:
  - tumor exceeds 2 cm in dimension,
  - tumor ulcerates,
  - body weight loss > 20%,
  - moribund

*XmAb5574 vs Anti-CD19 IgG1, p=0.00018
XmAb5574 vs PBS, p=0.000001

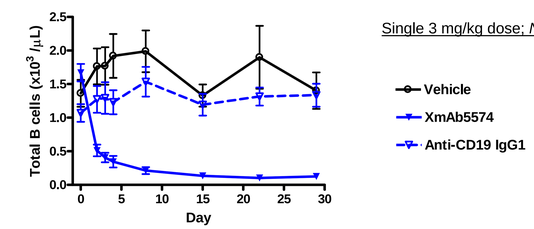
cynomolgus monkey

XmAb5574 efficiently depletes B cells in cynomolgus monkeys

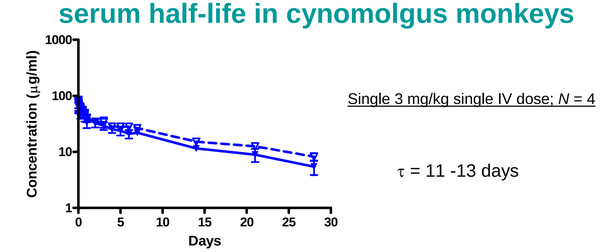


XmAb5574 does not decrease serum IgG levels

Fc engineering is essential for B cell depletion in cynomolgus monkeys



XmAb5574 and IgG1 analog have similar serum half-life in cynomolgus monkeys



τ = 11 -13 days

Summary

XmAb5574 is a promising new immunotherapeutic development candidate for treatment of lymphoma and leukemia. It possesses the following properties:

- Enhanced effector functions: NK cell-mediated ADCC and macrophage-mediated phagocytosis are increased relative to anti-CD19 IgG1 and anti-CD20 IgG1
- Robust anti-proliferative apoptotic activity
- Efficient B cell depletion capacity in cynomolgus monkeys
- Enhanced efficacy: tumor inhibition in SCID mouse xenograft models of human B cell lymphomas and B cell depletion in cynomolgus monkeys are increased relative to anti-CD19 IgG1

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