

# A Humanized Anti-CD30 Monoclonal Antibody, XmAb™2513, with Enhanced *In Vitro* Potency Against CD30-Positive Lymphomas Mediated by High Affinity Fc-Receptor Binding



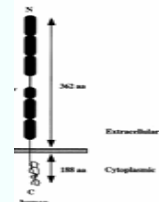
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## ABSTRACT

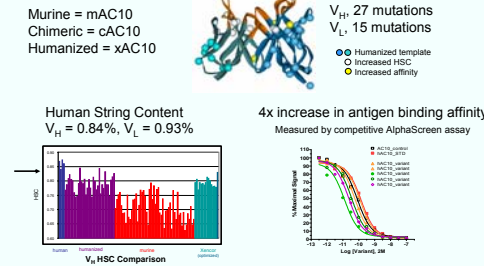
CD30 (also known as Ki-1), a member of the TNF-receptor superfamily, is normally expressed at low levels on activated lymphocytes and has been implicated in cell death and T-cell proliferation. CD30 is highly expressed in Hodgkin's disease, (HD) and in Anaplastic Large Cell Lymphoma (ALCL). Unmodified CD30 antibodies as well as anti-CD30 based bi-specific antibodies, antibody-toxin conjugates, and radioimmunotherapeutics have examined CD30 as a therapeutic target in pre-clinical and clinical studies. Unmodified antibodies have met with limited success and a lack of engagement of immune effector cells may be one of the major short-comings. Although bispecific antibodies proved among the most clinically effective through the recruiting of host effector functions to tumor cells, they pose significant manufacturing challenges. Similarly, toxin- and radio-conjugates require complicated manufacture and handling. Recent advances in antibody engineering have led to the development of "naked" antibodies with greatly enhanced effector function through mutagenesis at the Fc-receptor binding interface. This approach has been applied to a humanized antibody specific for CD30 to produce an antibody with enhanced potency and efficacy coupled with the ease of manufacture and handling of a traditional IgG antibody. The murine human chimeric antibody cAC10 was humanized using the novel method of human string content optimization. The humanized antibody (hAC10) has an affinity for antigen 4-fold higher than that of the corresponding chimeric antibody. The humanized variable domain was then combined with a modified Fc region and exhibited an approximately 20 fold increase in affinity for the FcγRIIIa receptor resulting in the therapeutic lead XmAb2513. Expression levels from a stable cell line were close to 1 g/ml. In preliminary development, the cytotoxic activity of XmAb2513 was measured by Antibody-Dependent Cell-mediated Cytotoxicity (ADCC) assay. ADCC assays used PMBCs isolated from peripheral blood as effector cells and the human Hodgkin's cell line L540 which expresses high levels of CD30 as the target at an effector target ratio of 25:1. cytotoxicity was measured by release of LDH or cell counting. The activity of XmAb2513 was compared to that of cAC10 with no Fc-receptor binding enhancement (IgG1) as well as the antibody SF11 (also human IgG1). Significant improvements were observed in both potency (concentration of antibody required to effect 50% of maximal lysis) and efficacy (maximal percent lysis at saturating antibody concentration). The potency of XmAb2513 is ~3 fold higher than that of cAC10-IgG1 and 10-fold higher than SF11 with an increase in efficacy of 4-fold relative to cAC10-IgG1. XmAb2513 has advantageous properties for a therapeutic compound against CD30-positive lymphomas including high levels of cytotoxicity and ease of manufacture and handling. The promising results reported herein clearly warrant further investigation.

## CD30 is a lymphoma tumor marker

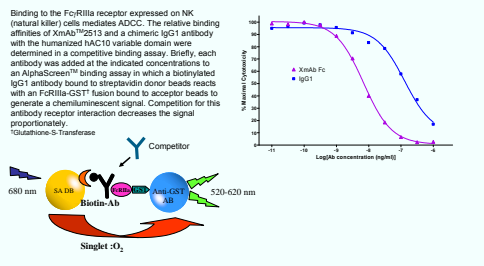
- CD30 (Ki-1):
  - Member of the TNF receptor super family (TNFRSF5)
  - Integral membrane protein (120 kd)
  - Soluble (sCD30, 85 kd) produced by TACE cleavage
  - Lacks a Death Domain
- Function:
  - Positive regulator of apoptosis (cell death)
  - Limits proliferation of autoreactive CD8+ T-cells
- CD30 Expression is activation dependent:
  - Viral infected lymphocytes (EBV, HTLV1)
  - Variable proportion (3-31%) of circulating T cells may be CD30+, primarily CD8 Thymus medullary tissue
  - Paraneoplastic areas of LN
  - Eosinophils
- Neoplasms
  - Hodgkin's and Reed-Sternberg cells of classic Hodgkin's disease
  - Anaplastic Large Cell Lymphoma/Cutaneous T cell
  - Mediastinal B cell tumors
  - Embryonal Cell Carcinoma, mesothelioma



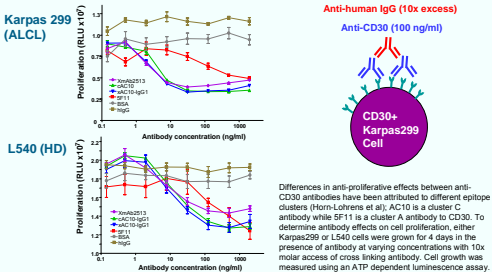
## Humanized with an increase in affinity



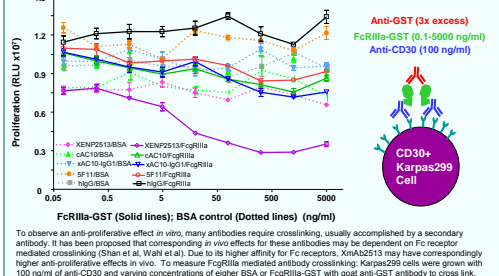
## Increased FcγRIIIa binding affinity



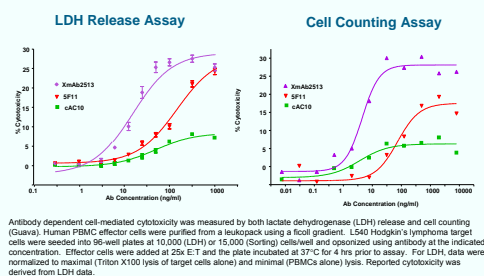
## XmAb™2513 is anti-proliferative



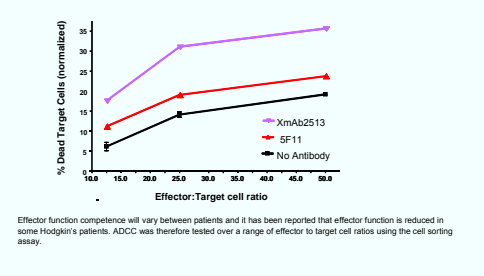
## Increased FcR-mediated antiproliferation



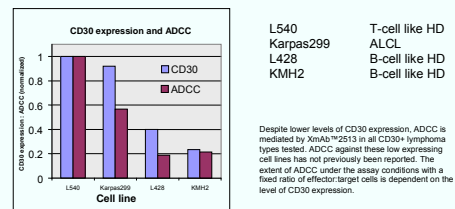
## XmAb™2513 mediates enhanced ADCC



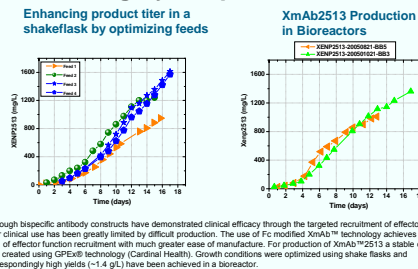
## ADCC is enhanced at all E:T ratios



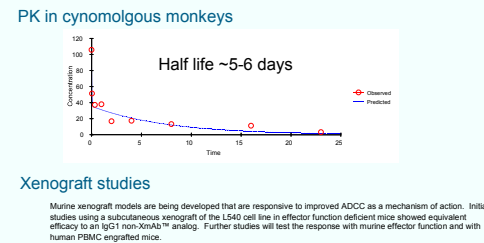
## XmAb™2513 mediates ADCC on low CD30 expressing cell lines



## High yield production



## Preclinical Pharmacology



## Summary/Conclusions

The XmAb™2513 engineered antibody has been shown to have enhanced potency and efficacy as compared to IgG1 antibodies. This was observed both in ADCC assays as well as in antiproliferation assays where the antibody crosslinking required for an antiproliferative effect was mediated by Fc receptor binding. Published results from clinical trials with bispecific antibodies directed to CD30 provide clinical evidence in Hodgkin's Lymphoma that enhanced recruitment of effector function is a successful means of generating a cytotoxic antibody. However, manufacturing limitations prevent specifics from being practical in widespread use. Preclinical results with XmAb™2513 support further testing in the clinic to validate the role of enhanced Fc effector function.

## References

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## Acknowledgements

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