IGM-55.5, a novel monoclonal human recombinant IgM antibody with potent activity against B cell leukemia and lymphoma

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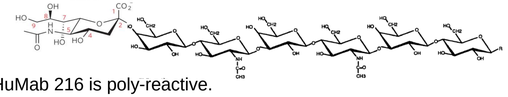
Abstract

IGM-55.5 is an engineered monoclonal human recombinant IgM antibody that was derived from a natural monoclonal antibody 216 isolated at Stanford University from the splenocytes of a patient with Non-Hodgkin's lymphoma. HuMab 216 was previously used in a B-cell acute lymphoblastic leukemia phase I trial and was demonstrated to be well tolerated with significant decrease in peripheral blasts observed (*Liedtke et al, Haematologica, 2012*). IGM-55.5 has been engineered to be mono-reactive and recognizes a carbohydrate determinant as an epitope on normal human B cells as well as B-cell lymphoma and B-progenitor lymphoblasts.

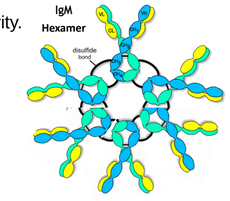
Here, we report new preclinical studies that describe the mechanism of action and potent activity of IGM-55.5. In-vitro analysis of IGM-55.5 was found to have high surface binding on a broad panel of B cell leukemia and lymphoma cells lines by flow cytometry. IGM-55.5 binding leads to the disruption of the plasma membrane and formation of large membrane pores resulting in cell lysis. This non-classical apoptosis occurs in the absence of complement fixation but in vitro cytotoxicity is increased in assays in the presence of human complement. Studies with aggressive disseminated tumor xenograft models (Namalwa, Granta, & Nalm-6) demonstrate that IGM-55.5 significantly prolongs survival and reduces circulating tumor cells. Peripheral blood samples from non-hodgkin's lymphoma patients demonstrated the potent activity of IGM-55 in eliminating B cell lymphoblasts in comparison with Rituxan when treated in-vitro. These data taken together highlight the potent activity of IGM-55.5 as therapeutic for advanced B cell malignancies, especially indicated for Rituxan resistant or refractory patients.

Background: CDIM & IGM-55.5

- HuMab 216 is based on natural human antibody isolated from patient.
- HuMab 216 recognizes a linear lactosamine epitope structurally similar to the "i" neonatal blood group antigen, CDIM:

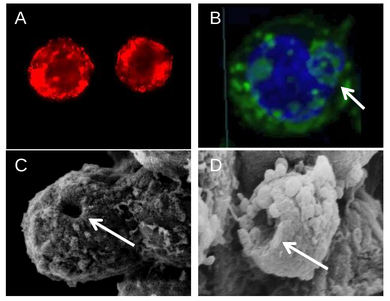


- HuMab 216 is poly-reactive.
- Functionally, HuMab 216 binds and kills patient-derived malignant B cells, normal human B cells, and human and murine B cell lines, and extended overall survival in an acute lymphoblastic leukemia (ALL) Nalm-6 xenograft model.
- HuMab 216 was tested in a Phase I clinical trial with an excellent safety profile and functional capacity to ablate leukemic blasts in ALL.
- IGM-55.5 was engineered to have similar antigen specificity and restricted polyreactivity.
- IGM-55.5 has improved preclinical potency.
- The current study shows mechanisms of action of IGM-55.5 and demonstrates its efficacy on human B cell lines *in vitro*, in xenograft mouse models, and on normal human and patient-derived samples.



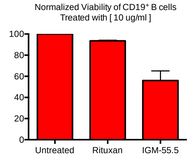
Mab Binding Leads to Membrane Pore Formation

A) HuMab 216 & **B)** IGM-55.5 bind the surface of Nalm-6 cells (Confocal microscopy). **C)** Nalm-6 or **D)** Splenic B cells treated with HuMab 216 for 15 minutes lead to pore formation (SEM). White arrows indicate observable pores.

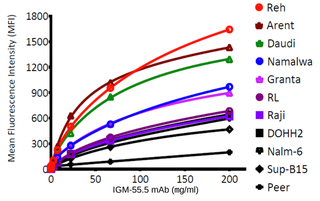


IGM-55.5 Pore Formation Lead to Loss of B cell Viability

CD19⁺ purified human B cells treatment with IGM-55.5 for 24 hours in the absence of complement leads to reduced B cell viability as determined by flow cytometry (propidium iodide staining & normalization; n=4 normal healthy donors shown.)



CDIM Expression on Human Hematologic Cell Lines

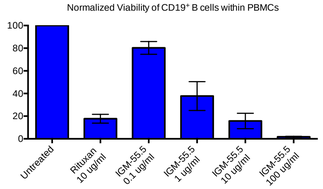


IGM-55.5 Alexa-488 detects CDIM expression on B cell lines as determined by flow cytometry analysis. Dose response binding experiments demonstrated that CDIM expression is high on non-hodgkin's lymphoma cell lines with modest binding to T cell lines.

Cell Line	Tumor Type	Binding
Reh	ALL	+++
Arent	NHL	+++
Daudi	NHL	+++
Namalwa	NHL	+++
Granta	NHL	+++
Raji	NHL	+++
RL	NHL	++
Ramos	NHL	++
RS411	ALL	++
Nalm-6	ALL	++
K562	CML	+
DOHH2	NHL	+
Sup-B15	ALL	+
RPMI	MM	+/-
Jurkat	T cell	+/-
Peer	T cell	+/-

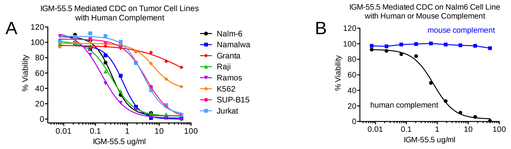
Rapid B Cell Depletion in the Absence of Complement

Isolated human PBMCs from 4 healthy donors were obtained and treated with IGM-55.5 at different concentrations for 24 hours at 37ºC in the absence of human complement. Flow cytometry analysis demonstrated a dose-dependent depletion of CD19⁺ B cells from treated PBMCs.



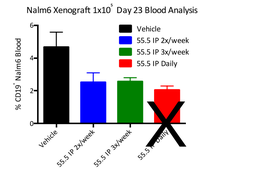
IGM-55.5 has Potent Human CDC on Tumor Cell Lines

Cells were incubated with a dose titration of IGM-55.5 and **A)**10% normal human serum complement or **B)** 10% mouse complement for 1 hour at 37ºC. Cell viability was measured after an additional 3 hour incubation with CCK-SK cell counting reagent.



IGM-55.5 In-vivo Activity in Xenografts

CD19⁺ purified human B cells treatment with IGM-55.5 for 24 hours in the absence of complement leads to reduced B cell viability as determined by flow cytometry (propidium iodide staining & normalization; n=4 normal healthy donors shown.)



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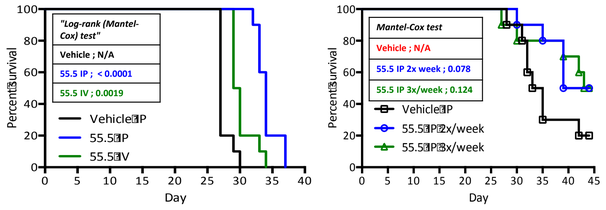
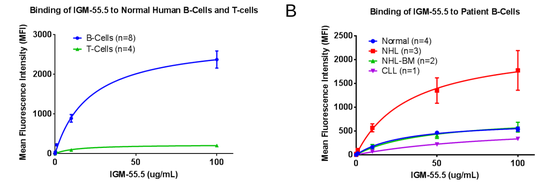


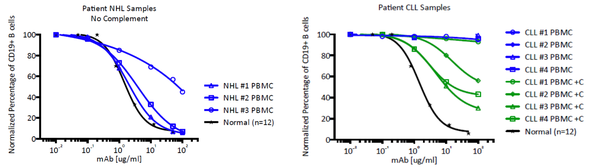
IGM-55.5 Binding to Normal Human and Patient B Cells

IGM-55.5 labeled with Alexa-488 detects CDIM expression on normal CD19⁺ B cells **A)** compared to normal CD3⁺? T cells and **B)** within patient derived samples, by flow cytometry. Binding was highest on NHL patient derived samples and lowest on CLL samples.



IGM-55.5 Causes Cytotoxicity in Normal and Patient B Cells

Ficol isolated human PBMCs from NHL or CLL patients or healthy donors were treated with IGM-55.5 for 24 hours at 37ºC in the absence of human complement. Flow cytometry analysis demonstrated a dose-dependent depletion of CD19⁺ B cells from treated PBMCs.



Summary

- IGM-55.5 kills cells by at least two mechanisms, pore formation and complement dependent cytotoxicity.
- IGM-55.5 binds and kills B cell lines *in vitro* and in mouse models.
- IGM-55.5 binds and kills B cells from normal and patient donors.

References

1. Liedtke M et al. (2012) Phase I trial of a novel human monoclonal antibody mAb216 in patients with relapsed or refractory B-cell acute lymphoblastic leukemia. *Haematologica* 97(1):30-37.

2. Bhat NM et al. (1997) Rapid cytotoxicity of human B lymphocytes induced by VH4-34 (VH4.21) gene-encoded monoclonal antibodies, II. *Clin Exp Immunol* 108:151-159.

3. Bhat NM et al. (1996) Rapid cytotoxicity of human B lymphocytes induced by VH4-34 (VH4.21) gene-encoded monoclonal antibodies. *Clin Exp Immunol* 105:183-190.

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