Imvotamab, a CD20-Targeted Bispecific IgM T Cell Engager, Effectively Depletes Low-Expressing CD20+ B cells in Preclinical Models of Autoimmune Disease

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Background

- B cell depletion therapy (BCDT) with conventional IgG antibodies (e.g. rituximab) has been used to treat autoimmune (AI) disease for several decades
- However, many patients do not achieve long term disease control or remission
- Failure of these therapies to fully deplete tissue-resident B cells, including low-expressing CD20+ B cell subsets that are precursors to autoantibody-producing plasmablasts and plasma cells, may result in persistent reservoirs of pathogenic clones that contribute to the ongoing generation of autoantibodies and disease activity
- Bispecific IgM antibody T cell engagers (TCEs) are exciting drug candidates with the potential to deplete tissue-resident target cells more effectively through T cell-dependent cellular cytotoxicity (TDCC) and complement-dependent cytotoxicity (CDC) as compared to conventional BCDT mechanisms of action, which rely predominantly upon ADCC
- Imvotamab (IGM-2323) is an engineered high-affinity, high avidity bispecific anti-CD20 IgM antibody TCE
- Imvotamab has been evaluated in ~100 patients with non-Hodgkin's lymphoma (NHL)[1]
- Given the preliminary clinical profile of imvotamab in NHL, which shows durable response rates and a favorable safety profile, we evaluated its potential to deplete peripheral and tissue-resident B cells in preclinical models of AI disease

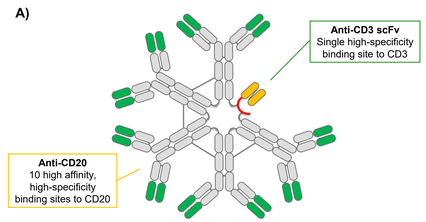


Figure 1. (A) Structure of imvotamab. Imvotamab is a fully human pentameric anti-CD20 IgM antibody with ten CD20 binding domains, and with a J-chain fused to a single chain variable fragment (scFv) targeting CD3ε. **(B)** Imvotamab has two potential mechanisms of action of killing B cells: TDCC and CDC.

CD20 receptor expression on B cells throughout B cell development

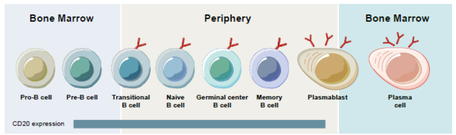
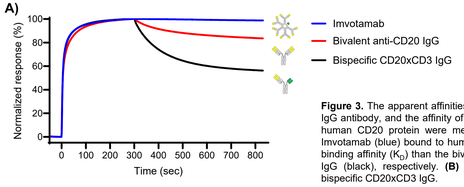


Figure 2. CD20 receptor expression on B cell subsets throughout B cell development in the bone marrow and periphery. Shown in the schematic are early B cell populations, including pro- and pre-B cells, and plasma cells in the bone marrow as well as immature and mature B cells in the periphery. Peripheral B cells include transitional, naïve, germinal center, memory B cells, and plasmablasts. Schematic is modified from Crickx et al. *Kidney Int* 2020.

Imvotamab binds to CD20 with high apparent affinity



B)

Antibody	$K_D$app (nM)
Imvotamab	0.008
Bivalent anti-CD20 IgG	0.320
Bispecific CD20xCD3 IgG	2.133

Figure 3. The apparent affinities of imvotamab, its corresponding bivalent anti-CD20 IgG antibody, and the affinity of a bispecific CD20xCD3 IgG antibody to recombinant human CD20 protein were measured by surface plasmon resonance (SPR). **(A)** Imvotamab (blue) bound to human CD20 with an apparent 40- and ~300-fold higher binding affinity (K_D) than the bivalent anti-CD20 IgG (red), and bispecific CD20xCD3 IgG (black), respectively. **(B)** K_D values of imvotamab, bivalent anti-CD20 IgG, bispecific CD20xCD3 IgG.

Imvotamab is more effective at killing a low CD20-expressing B cell line compared to obinutuzumab and rituximab

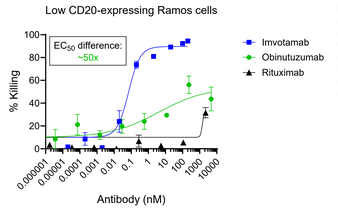


Figure 4. An *in vitro* cellular cytotoxicity assay shows imvotamab is more effective at killing low CD20-expressing cells compared to obinutuzumab and rituximab in the absence of complement. Percentage of killing of Oregon-green 488-labeled low CD20-expressing Ramos cells by imvotamab (blue squares), obinutuzumab (green circles), and rituximab (black triangles) in PBMCs from healthy donor PBMCs (n=3 healthy donor PBMCs, each donor was run in duplicate). Black box describes fold difference in EC_{50} values between imvotamab and obinutuzumab. Data points are represented as mean ± SEM.

Imvotamab kills low CD20-expressing B cell subsets in an *ex vivo* cellular cytotoxicity assay

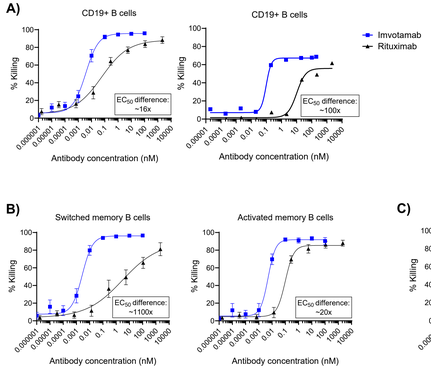


Figure 5. *Ex vivo* cellular cytotoxicity assays show imvotamab has more favorable EC_{50} values than rituximab in the absence of complement. **(A-C)** Percentage of killing of **(A)** total CD19+ B cells by imvotamab (blue squares) and rituximab (black triangles) in PBMCs from healthy donor PBMCs from two separate assay runs (each assay includes n=3 healthy donor PBMCs, each donor was run in duplicate). **(B-C)** Imvotamab (blue squares) depletes low CD20-expressing CD19+ B cell subsets (switched memory and activated memory) with higher potency compared to rituximab (black triangles) in two separate assay runs (each assay includes n=3 healthy donor PBMCs, each donor was run in duplicate). Black box describes fold difference in EC_{50} values between imvotamab and rituximab. Data points are represented as mean ± SEM.

Imvotamab depletes peripheral and tissue B cells in CD34+ human engrafted NSG-SGM3 mice

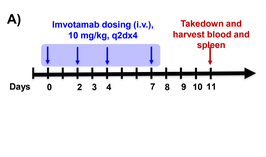


Figure 6. Preliminary *in vivo* data show Imvotamab depletes total CD19+ B cells in the blood and spleen of humanized mice. CD34+ human engrafted NSG-SGM3 mice received repeat doses of imvotamab as shown in **(A)**. **(B)** Total CD19+ B cells were measured in the blood and spleen 4 days after the last dose of imvotamab (n=4 mice/group). Data points are represented as mean ± SEM.

Imvotamab depletes peripheral and splenic B cells expressing low CD20, including activated memory B cells, *in vivo*

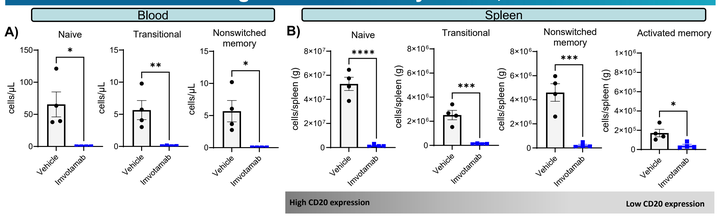


Figure 7. CD19+ B cell subsets are depleted in the blood and spleens of CD34+ human engrafted NSG-SGM3 mice following repeat doses of imvotamab **(A-B)**. **(A)** Naïve, transitional and nonswitched memory B cells were measured in the blood of vehicle (black circles) and imvotamab-treated (blue squares) NSG-SGM3 mice. **(B)** Naïve, transitional, nonswitched memory and activated memory B cells were measured in the spleens of vehicle (black circles) and imvotamab-treated (blue squares) NSG-SGM3 mice (n=4 mice/group). Data points are represented as mean ± SEM.

Imvotamab predominantly activates CD8+ T cells in the spleens of NSG-SGM3 mice

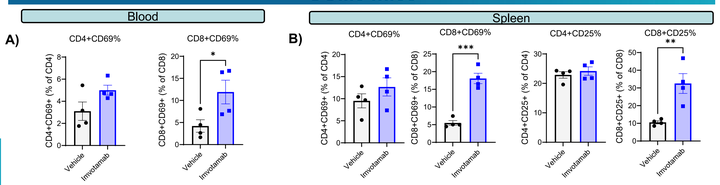


Figure 8. Flow cytometric analyses demonstrate CD8+ T cells are significantly activated by repeat dosing of imvotamab in the blood and spleens of CD34+ human engrafted NSG-SGM3 mice. **(A-B)** Frequencies of activated (CD69% or CD25%) CD4+ and CD8+ T cells in the **(A)** blood and **(B)** spleens of vehicle and imvotamab-treated mice. Data points are represented as mean ± SEM. Student's *t* test; *p<0.05, **p<0.01, ***p<0.001.

Summary

- Imvotamab binds with high affinity to recombinant human CD20 with an apparent 300-fold higher binding affinity than the bivalent anti-CD20 IgG
- Imvotamab depletes low CD20-expressing B cell subsets both *in vitro/ex vivo* using low CD20-expressing Ramos cells or healthy donor PBMCs and *in vivo* using CD34+ human engrafted NSG-SGM3 mice
- *Ex vivo* cytotoxicity assays with healthy donor human PBMCs show imvotamab trends toward more potent killing of low CD20-expressing B cells compared to traditional IgGs (e.g. rituximab)
- Preliminary data show imvotamab penetrates splenic tissue of CD34+ human engrafted NSG-SGM3 mice to deplete both high and low CD20-expressing B cells, including activated memory B cells
- Both *ex vivo* and *in vivo* data show imvotamab depletes the key B cell subsets implicated in driving autoimmune disease pathology

[1]Budde et al. *American Society of Hematology Annual Congress* 2021

[2]Hernandez et al. *American Society of Hematology Annual Congress* 2022

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