Poster #4120

Depletion of tissue-resident B cells by a CD20xCD3 IgM bispecific T cell engager in cynomolgus monkeys demonstrates effective tissue penetration and potent target cell killing

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Background

- Imvotamab (IGM-2323) is an engineered high-affinity, high-avidity bispecific anti-CD20 IgM antibody T cell engager (TCE) that is being studied as a combination therapy in a clinical trial for relapsed/refractory non-Hodgkin's lymphoma (NHL).
- Imvotamab offers a novel treatment strategy in NHL by depleting CD20-expressing tumor cells through multiple mechanisms, including the recruitment of T cells to kill tumor cells through T cell dependent cellular cytotoxicity (TDCC), complement-dependent cytotoxicity (CDC), and enhanced immune modulation via IFNγ-dominant cytokine stimulation.
- We evaluated the activity of a surrogate cynomolgus monkey (cyno) cross-reactive CD20xCD3 IgM bispecific TCE, IGM-2324, in depleting CD20-expressing B cells in peripheral blood and lymphoid tissues of cynomolgus monkeys in vivo.
- We hypothesized that the high affinity and valency of IGM-2324 would enable potent B cell killing in blood and tissues even when B cells express low levels of CD20.
- B cell depletion was assessed by the immunohistochemical (IHC) signal of CD20, a B cell surface antigen expressed on pro-B cells through mature B cells in the B cell lineage. B cell depletion was also assessed by the IHC signal of CD19, a comparative and confirmatory alternative marker of B cells since CD19 is expressed on a wider range of the B cell lineage from pre-B cell to early plasmablast, and it shares co-expression with CD20 on pro-B cells to mature B cells.

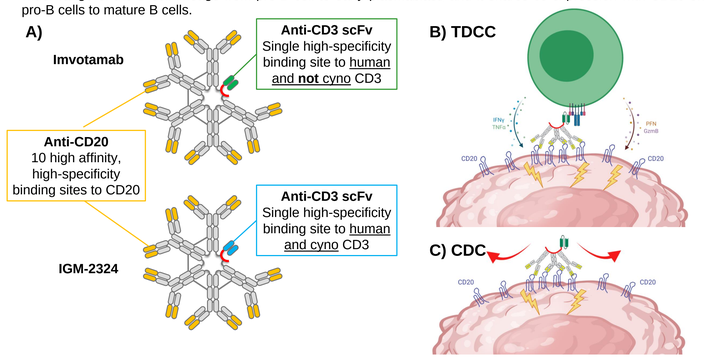

Figure 1. Molecular structure of imvotamab and surrogate IGM-2324. Ten anti-CD20 binding domains of imvotamab and IGM-2324 provide high affinity and high avidity binding to CD20 **(A)**. Functional comparability was confirmed across *in vitro* and *ex vivo* studies evaluating the mechanism of action of the two IgM antibodies. Schematic mode of action (MoA) of TDCC **(B)** and CDC **(C)** for imvotamab in humans and IGM-2324 in humans and cynomolgus monkeys.

Experimental design of GLP toxicology study in cynomolgus monkeys, IHC methods, and image analysis by HALO®

Group No.	Test Material	Dose Level (mg/kg)	No. of Animals			
			Main Study		Recovery	
			Male	Female	Male	Female
1	Vehicle	0	3	3	2	2
2	IGM-2324	5	3	3	0	0
3	IGM-2324	25	3	3	2	2

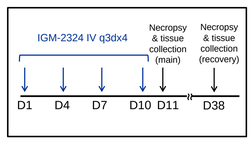

Table 1. Cynomolgus monkeys were administered vehicle or IGM-2324 at 5 mg/kg or 25 mg/kg through intravenous (IV) infusion twice weekly for a total of four doses on days 1, 4, 7, and 10. Depletion of tissue-resident B cells was evaluated in the spleen, mesenteric lymph node (MLN) and bone marrow (BM) of monkeys at 24 hours post the last dose of vehicle or IGM-2324 on day 11, and at 28 days post the last dose of vehicle or IGM-2324 on day 38 in recovery animals.

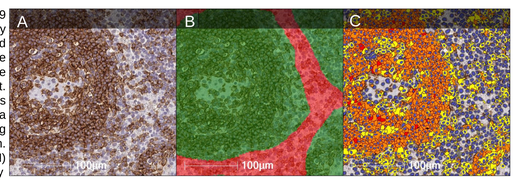

Figure 2. The CD20 and CD19 immunoreactivities were visualized by DAB chromogen and counterstained with hematoxylin **(A)**. Both of the antibodies were raised against the intracellular domain of each target. Quantitative Digital Image Analysis was performed by the Pharma Services Group at Indica Labs, using the HALO® image analysis platform. A Convoluted Neural Network (CNN) based classifier was trained to identify the white pulp/germinal centers (green) and the red pulp/cortex (red) **(B)**. The Multiplex IHC Module was used to quantitate the percentage of biomarker positive cells in these regions. An AI based nuclear segmentation was used to detect the nuclei, and optical density thresholds were set for low (yellow), medium (orange) and high (red) staining for the cytoplasmic and membrane compartments **(C)**. The percentage of CD20 and CD19-expressing B cells in spleen, MLN & BM was calculated for days 11 and 38.

Peripheral blood CD19+ B cell depletion by IGM-2324 in cynomolgus monkeys

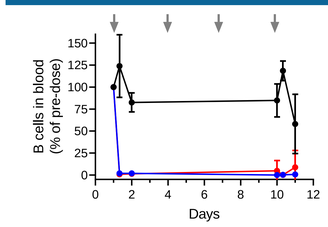

Figure 3. Groups of male and female monkeys received 4 twice-weekly infusions of 0 (vehicle), 5 or 25 mg/kg IGM-2324. Blood samples were collected at various time points and analyzed for CD19+ B cells by flow cytometry. Percentages are relative to individual baseline CD19+ B cell numbers. Repeated IV administration of surrogate IGM-2324 at 5 and 25 mg/kg/dose to cynomolgus monkeys resulted in rapid and pronounced depletion of circulating CD19+ B cells in all treated animals that persisted at least through Day 11, 24 hours after the last dose.

CD20+ B cell depletion by IGM-2324 in the spleen of cynomolgus monkeys

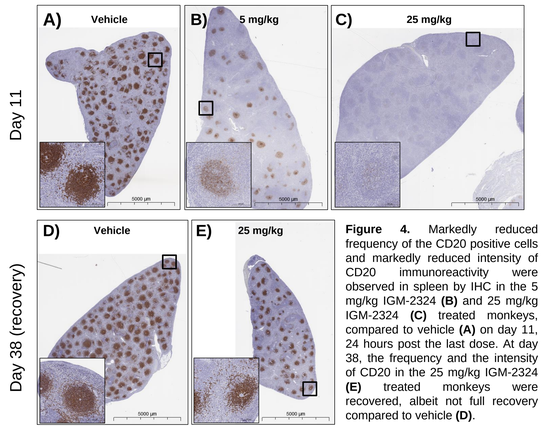

Figure 4. Markedly reduced frequency of the CD20 positive cells and markedly reduced intensity of CD20 immunoreactivity were observed in spleen by IHC in the 5 mg/kg IGM-2324 **(B)** and 25 mg/kg IGM-2324 **(C)** treated monkeys, compared to vehicle **(A)** on day 11, 24 hours post the last dose. At day 38, the frequency and the intensity of CD20 in the 25 mg/kg IGM-2324 **(E)** treated monkeys were recovered, albeit not full recovery compared to vehicle **(D)**.

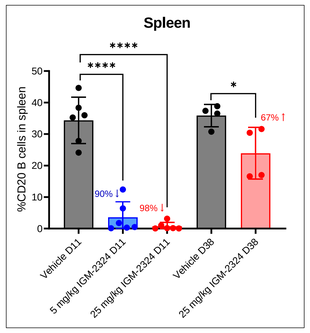

Figure 5. Quantitative analysis revealed significant reduction in the frequency of CD20 expressing B cells in the spleen by IGM-2324 treatment. A substantial portion of CD20 expressing B cells recovered at day 38. Error bars represent mean ± SD. Statistical analyses (unpaired t-test) represent comparisons to vehicle control groups (ns (not significant): p > 0.05, *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001).

CD19+ B cell depletion by IGM-2324 in the spleen of cynomolgus monkeys

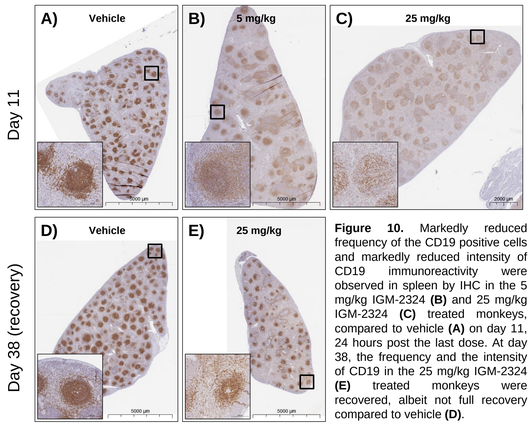

Figure 10. Markedly reduced frequency of the CD19 positive cells and markedly reduced intensity of CD19 immunoreactivity were observed in spleen by IHC in the 5 mg/kg IGM-2324 **(B)** and 25 mg/kg IGM-2324 **(C)** treated monkeys, compared to vehicle **(A)** on day 11, 24 hours post the last dose. At day 38, the frequency and the intensity of CD19 in the 25 mg/kg IGM-2324 **(E)** treated monkeys were recovered, albeit not full recovery compared to vehicle **(D)**.

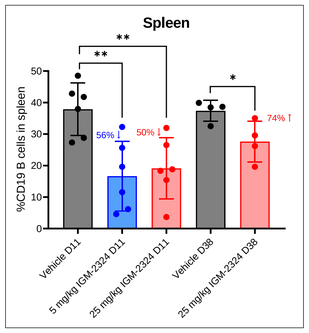

Figure 11. Quantitative analysis revealed significant reduction in the frequency of CD19 expressing B cells in the spleen by IGM-2324 treatment. A substantial portion of CD19 expressing B cells recovered at day 38. Error bars represent mean ± SD. Statistical analyses (Unpaired t-test) represent comparisons to vehicle control groups (ns: p > 0.05, *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001).

CD20+ B cell depletion by IGM-2324 in the mesenteric lymph node (MLN) of cynomolgus monkeys

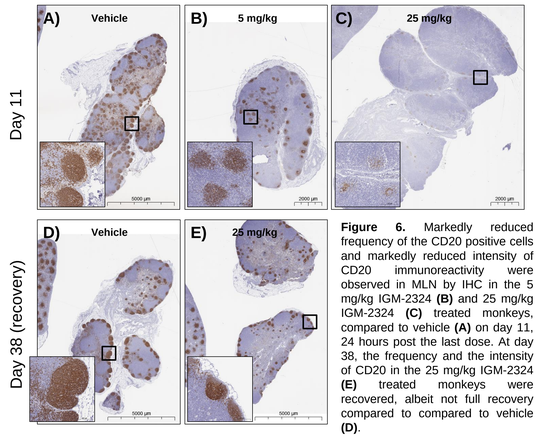

Figure 6. Markedly reduced frequency of the CD20 positive cells and markedly reduced intensity of CD20 immunoreactivity were observed in MLN by IHC in the 5 mg/kg IGM-2324 **(B)** and 25 mg/kg IGM-2324 **(C)** treated monkeys, compared to vehicle **(A)** on day 11, 24 hours post the last dose. At day 38, the frequency and the intensity of CD20 in the 25 mg/kg IGM-2324 **(E)** treated monkeys were recovered, albeit not full recovery compared to compared to vehicle **(D)**.

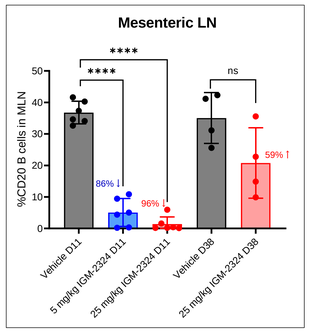

Figure 7. Quantitative analysis revealed significant reduction of the frequency of CD20 expressing B cells by IGM-2324 treatment in the MLN. A substantial portion of CD20 expressing B cells recovered at day 38. Error bars represent mean ± SD. Statistical analyses (unpaired t-test) represent comparisons to vehicle control groups (ns: p > 0.05, *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001).

CD19+ B cell depletion by IGM-2324 in the MLN of cynomolgus monkeys

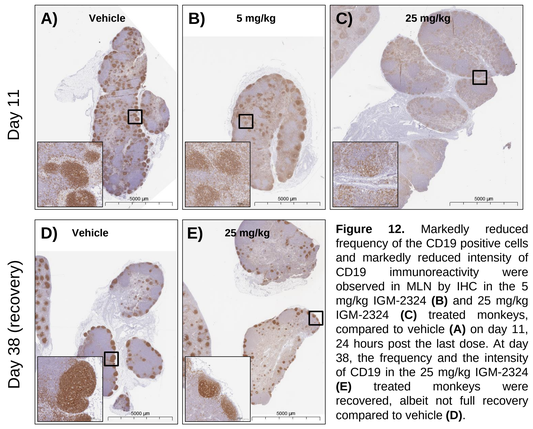

Figure 12. Markedly reduced frequency of the CD19 positive cells and markedly reduced intensity of CD19 immunoreactivity were observed in MLN by IHC in the 5 mg/kg IGM-2324 **(B)** and 25 mg/kg IGM-2324 **(C)** treated monkeys, compared to vehicle **(A)** on day 11, 24 hours post the last dose. At day 38, the frequency and the intensity of CD19 in the 25 mg/kg IGM-2324 **(E)** treated monkeys were recovered, albeit not full recovery compared to vehicle **(D)**.

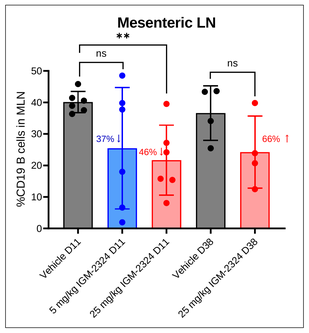

Figure 13. Quantitative analysis revealed significant reduction of the frequency of CD19 expressing B cells by IGM-2324 treatment in the MLN. A substantial portion of CD19 expressing B cells recovered at day 38. Error bars represent mean ± SD. Statistical analyses (Unpaired t-test) represent comparisons to vehicle control groups (ns: p > 0.05, *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001).

IGM-2324 kills low CD20+ expressing B cells in the bone marrow (BM) of cynomolgus monkeys

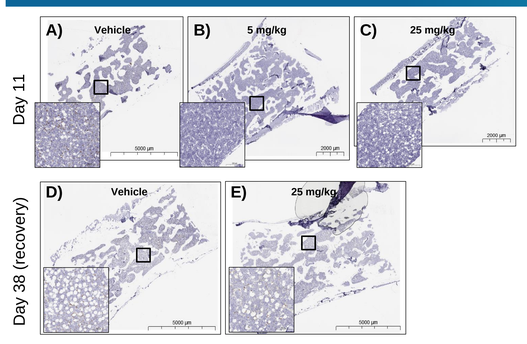

Figure 8. Markedly reduced frequency of the CD20 positive cells in BM was observed by IHC in the 5 mg/kg IGM-2324 **(B)** and 25 mg/kg IGM-2324 **(C)** treated monkeys, compared to vehicle **(A)** on day 11, 24 hours post the last dose. At day 38, the frequency of CD20 in the 25 mg/kg IGM-2324 **(E)** treated monkeys was recovered albeit not full recovery compared to vehicle **(D)**.

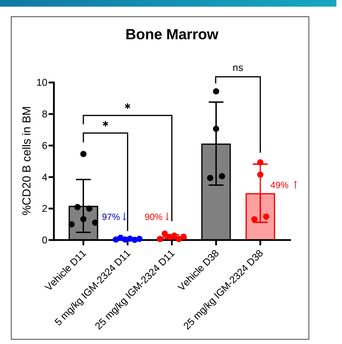

Figure 9. Quantitative analysis revealed significant reduction of the frequency of CD20 expressing B cells in the BM by IGM-2324 treatment. A substantial portion of CD20 expressing B cells recovered at day 38. Error bars represent mean ± SD. Statistical analyses (Unpaired t-test) represent comparisons to vehicle control groups (ns: p > 0.05, *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001).

Summary

- In the GLP toxicology study with cynomolgus monkeys, CD20 expression was measured as it is the direct target of imvotamab and surrogate IGM-2324. CD19 was also measured as a comparative and confirmatory alternative marker of B cells.
- Significant reductions in both CD20 and CD19 expressing B cells were observed in the lymphoid tissues following treatment with 5 and 25 mg/kg of IGM-2324, compared to vehicle treated animals.
- In spleen, MLN and BM, CD20 expressing B cells were reduced by 90-98%, 86-96%, and 90-97% on day 11, respectively, following IGM-2324 treatment.
- In spleen and MLN, CD19 expressing B cells were reduced by 50-56% and 37-46% on day 11, respectively, following IGM-2324 administration.
- By day 38, 49-67% and 66-74% recovery of CD20 and CD19 expressing B cells, respectively, was observed in the lymphoid tissues.
- Together, these data indicate that 4 doses of a CD20xCD3 IgM bispecific TCE leads to deep tissue B cell depletion during the dosing period, but ultimately results in B cell recovery post dosing.

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