Comparative Pharmacology of Ofatumumab vs Ocrelizumab in Humanized CD20 Transgenic Mice

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Disclosures

- **Marc Bigaud**, **Geraldine Zipfel**, **Tatjana Uffelmann**, **Helena Vostiarova**, **Volker Engelhardt**, **Barbara Nuesslein-Hildesheim**, and **Bernd Kieseier** are employees of Novartis
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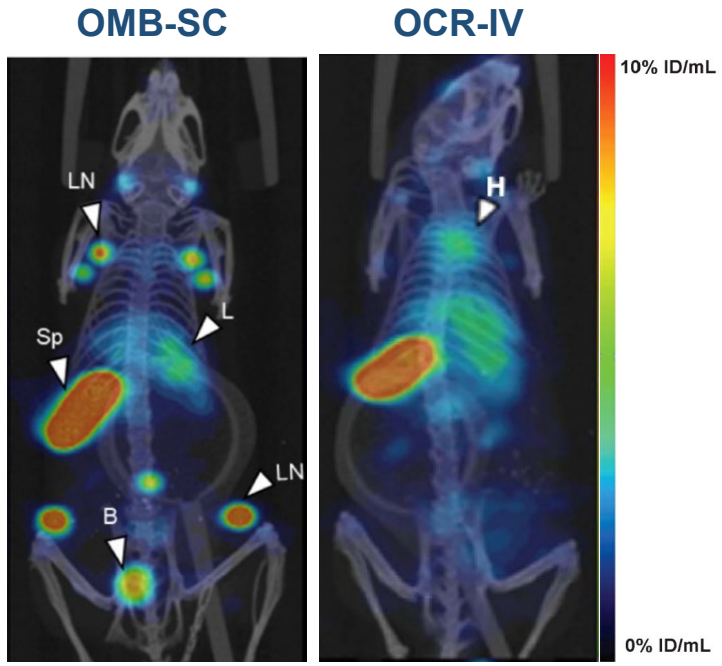
Background and Objectives

- Therapeutic strategies aiming at B-cell depletion via anti-CD20 mAbs are effective disease-modifying therapies for treating MS
- Because ~85% of B cells are within the lymphatic system and only ~2% are circulating in the blood,[1] the route of administration (SC or IV) might have a profound impact on dosing, B-cell depletion, and treatment outcomes
- Pilot work has suggested improved LN targeting for OMB-SC vs OCR-IV (huCD20 mice; SPECT images 72 hours post treatment with ^{111}In-OMB or ^{111}In-OCR [5 µg, 5 MBq])



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% ID/mL, percentage of injected dose per milliliter; B, bladder; H, heart; L, liver; LN, lymph node; Sp, spleen.

➔ **Studies were undertaken to benchmark the potency of OMB-SC and of OCR-IV at depleting CD20$^+$ B cells in humanized CD20 transgenic mice**

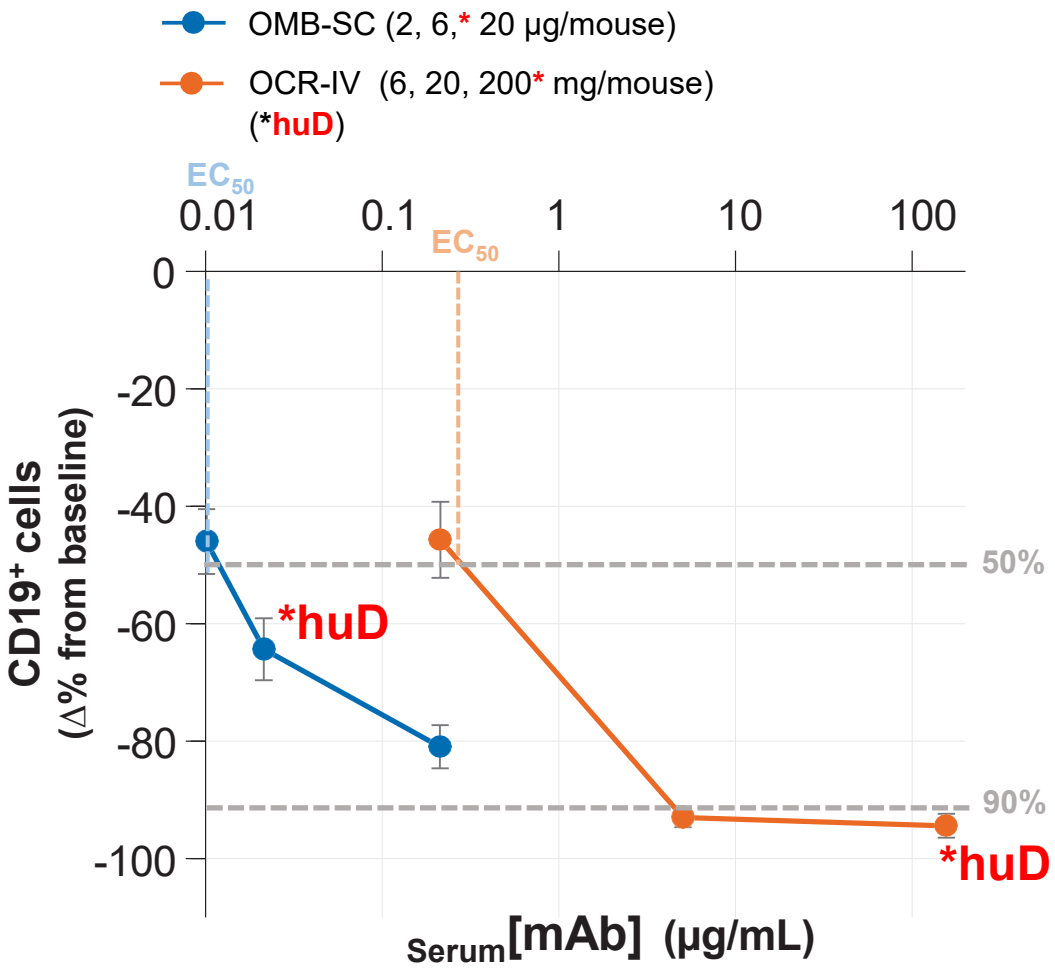
1. Blum KS, Pabst R. *Immunol Lett*. 2007;108(1):45-51. 2. Torres JB et al. *Front Immunol*. 2022;3:814064.
^{111}In, radiolabeled with indium-111; huCD20 mice, transgenic mice expressing human CD20; IV, intravenous; LN, lymph node; mAb, monoclonal antibody; MBq, megabecquerel; MS, multiple sclerosis; OCR, ocrelizumab; OMB, ofatumumab; SC, subcutaneous; SPECT, single-photon emission computed tomography.

Methods

- Adult huCD20 C57BL/6 (Ms4a1tm2[hCD20]Smoc) mice purchased from Shanghai Model Organisms Center (China)
- PK/PD studies: Naïve mice
  Mice vaccinated with DNP-KLH to develop a T-dependent germinal center reaction
- Treatments: OMB-SC, single dose: 2, 6 (huD), or 20 µg/mouse (n=7 each)
  OCR-IV, single dose: 6, 20, or 200 (huD) µg/mouse (n=7 each)
- PK readouts: Drug levels in blood and tissues (spleen, LN, BM) via IgG-binding ELISA assays
- PD readouts: B-cell ($CD19^+CD20^+$) depletion (vs baseline) in blood and tissues via flow cytometry
  MZ B cells ($CD19^+CD21^+CD23^-$), FO B cells ($CD19^+CD21^-CD23^+$), and antibody-producing cells ($CD138^+$) in spleen, LN, and BM

BM, bone marrow; DNP-KLH, 2,4-dinitrophenyl hapten conjugated to keyhole limpet hemocyanin; ELISA, enzyme-linked immunosorbent assay; FO, follicular; huCD20 mice, transgenic mice expressing human CD20; huD, human-equivalent dose; IgG, immunoglobulin G; IV, intravenous; LN, lymph node; MZ, marginal zone; OCR, ocrelizumab; OMB, ofatumumab; PD, pharmacodynamic; PK, pharmacokinetic; SC, subcutaneous.

OMB vs OCR Exposure-Dependent B-Cell Depletion in Blood
(Naïve Mice; 3 Days Post Treatment)



OMB-SC: [MW: 146k]
- EC_{50} ~0.01 µg/mL (~0.07 nM)
- EC_{90} ≥0.3 µg/mL

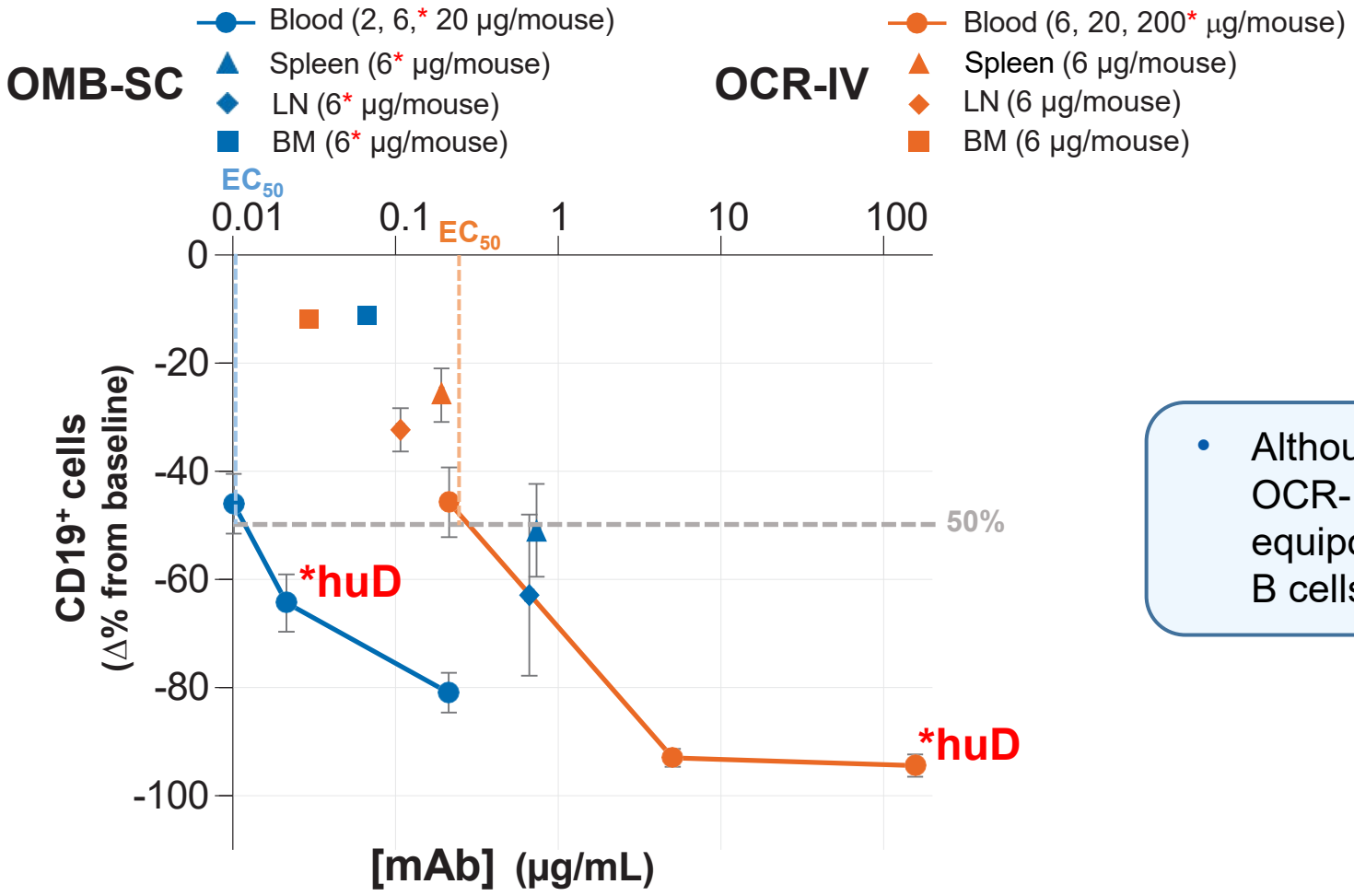
OCR-IV: [MW: 148k]
- EC_{50} ~0.2 µg/mL (~1.4 nM)
- EC_{90} ~5 µg/mL

➔ OMB-SC is ~20-fold more potent vs OCR-IV for depleting circulating B cells

➔ At huD, OMB-SC is near its EC_{50}, whereas OCR-IV is ~40-fold higher than its EC_{90}

EC_{50}, half-maximal effective concentration; EC_{90}, 90% effective dose; huD, human-equivalent dose; IV, intravenous; OCR, ocrelizumab; OMB, ofatumumab; mAb, monoclonal antibody; MW, molecular weight; SC, subcutaneous.

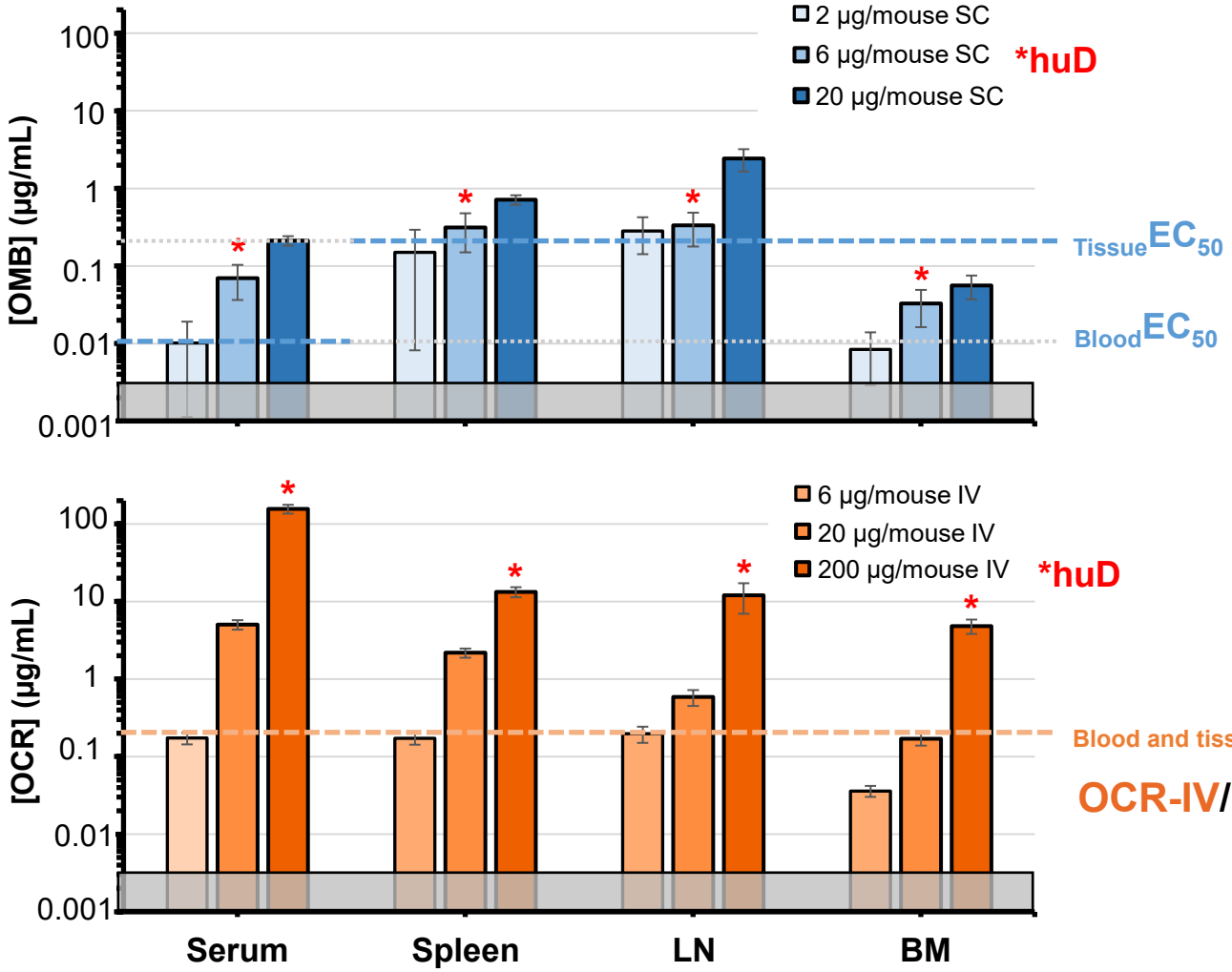
OMB vs OCR Exposure-Dependent B-Cell Depletion in Serum vs Tissues
(Naïve Mice; 3 Days Post Treatment)



- Although OMB-SC is ~20-fold more potent vs OCR-IV for depleting circulating B cells, it is equipotent to OCR-IV for depleting tissue-resident B cells (with an EC_{50} ~0.2 µg/mL [~1.4 nM])

BM, bone marrow; EC_{50}, half-maximal effective concentration; huD, human-equivalent dose; IV, intravenous; mAb, monoclonal antibody; LN, lymph node; OCR, ocrelizumab; OMB, ofatumumab; SC, subcutaneous.

OMB vs OCR Dose-Dependent Levels in Serum vs Tissues
(Naïve Mice; 3 Days Post Treatment)



Drug level ratio vs serum

	Serum	Spleen	LN	BM
OMB-SC:	1	3-15	5-28	≤1
OCR-IV:	1	≤1	≤1	≤1

➔ **OMB-SC:** Confirmed primary access to lymphatic compartment

➔ **OCR-IV:** Confirmed access to lymphatic compartment via blood

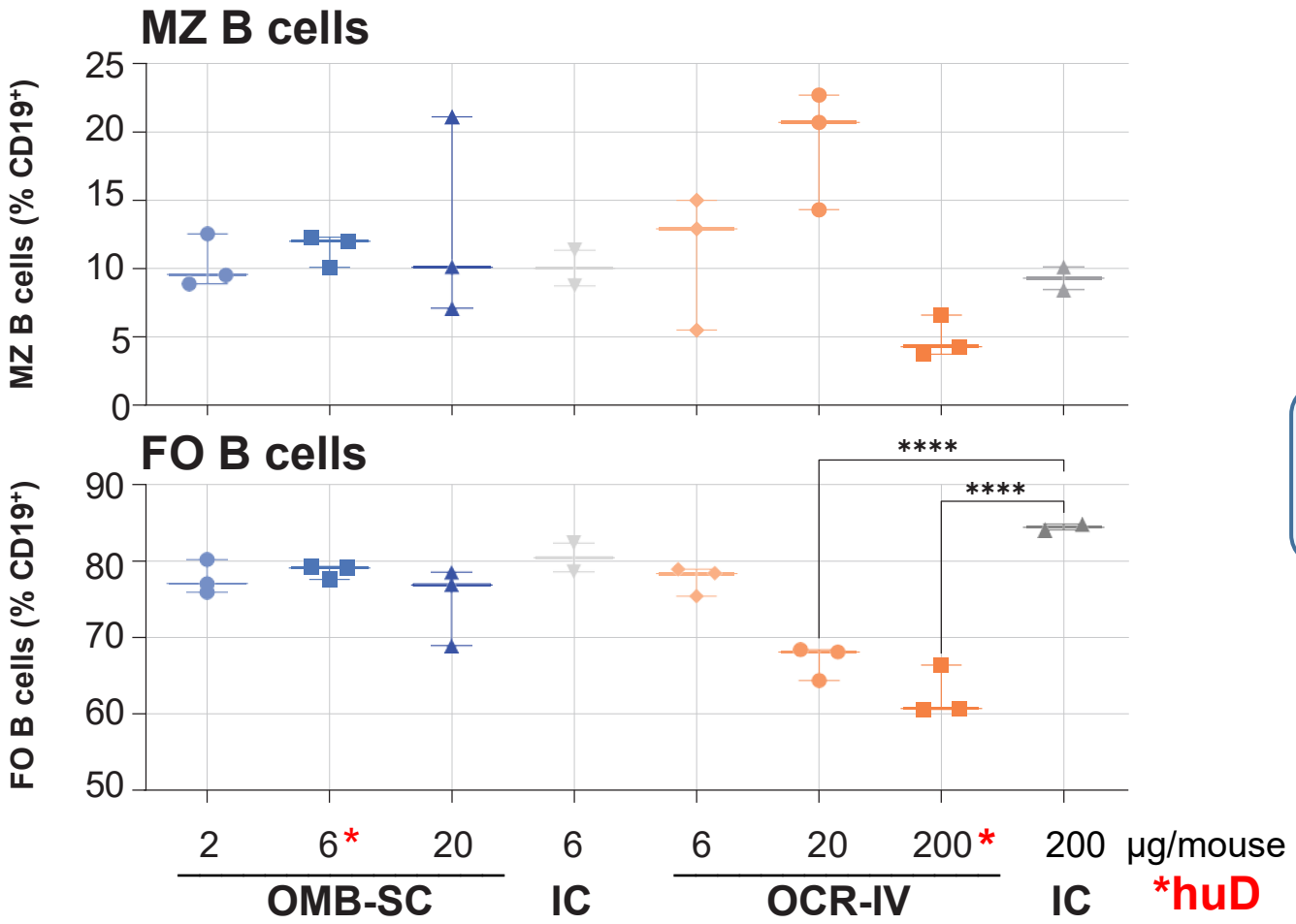
Drug level ratio at huD

	Serum	Spleen	LN	BM
OCR-IV/OMB-SC:	>2000	~40	~40	~150

➔ At huD, markedly higher OCR vs OMB levels in all compartments

BM, bone marrow; EC_{50}, half-maximal effective concentration; huD, human-equivalent dose; IV, intravenous; LN, lymph node; OCR, ocrelizumab; OMB, ofatumumab; SC, subcutaneous.

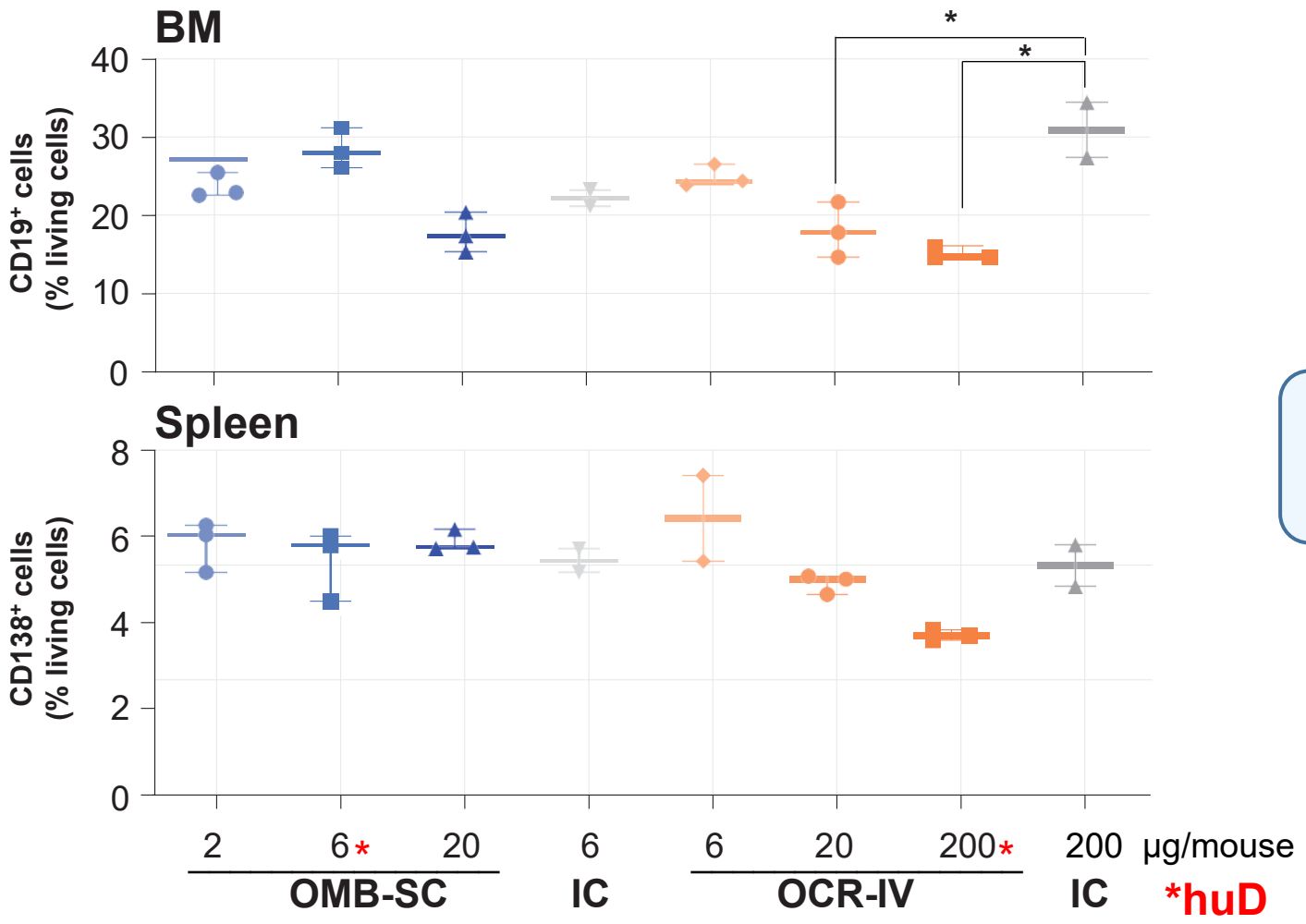
OMB-SC Spares MZ and FO B Cells in Spleen
(DNP-KLH–Immunized Mice; 19 Days Post Immunization)



- At huD, OMB-SC showed no impact on MZ and FO B cells in secondary lymphoid organs, in contrast to OCR-IV

DNP-KLH, 2,4-dinitrophenyl hapten conjugated to keyhole limpet hemocyanin; FO, follicular; huD, human-equivalent dose; IC, isotype control; IV, intravenous; MZ, marginal zone; OCR, ocrelizumab; OMB, ofatumumab; SC, subcutaneous.

OMB-SC Spares B Cells in BM and Plasma Cells in Spleen
(DNP-KLH–Immunized Mice; 19 Days Post Immunization)



- At huD, OMB-SC showed no impact on B cells in BM and plasma cells in spleen, whereas OCR-IV showed signs of depletion

BM, bone marrow; DNP-KLH, 2,4-dinitrophenyl hapten conjugated to keyhole limpet hemocyanin; huD, human-equivalent dose; IC, isotype control; IV, intravenous; OCR, ocrelizumab; OMB, ofatumumab; SC, subcutaneous.

Conclusions

- These results suggest the following major differences for OMB-SC vs OCR-IV:
  - A better efficiency for OMB-SC to target B cells in the lymphatic compartment and, consequently, a higher apparent potency for depleting circulating B cells
  - At huD, a sparing of MZ and FO B cells by OMB-SC, suggesting a sparing of germinal center reactions and development of antibody-producing cells
  - At huD, a sparing of BM-resident B cells

- If there is a good translation to the clinic, OMB-SC may offer high efficacy and lower long-term safety risks with convenient dosing vs OCR-IV in humans

BM, bone marrow; FO, follicular; huD, human-equivalent dose; IV, intravenous; MZ, marginal zone; OCR, ocrelizumab; OMB, ofatumumab; SC, subcutaneous.