Comparative Pharmacology of Ofatumumab Versus Ocrelizumab in Humanised-CD20 Transgenic Mice

P298

Marc Bigaud[1], Daniel Anthony[2], Philipp Lutzenburg[1], Geraldine Zipfel[1], Tatjana Uffelmann[1], Helena Vostiarova[1], Volker Engelhardt[1], Barbara Nuesslein-Hildesheim[1], Bernd Kieseier[1]

[1]Novartis Pharma AG, Basel, Switzerland; [2]Department of Pharmacology and Oncology, University of Oxford, UK

Introduction

- Therapeutic strategies aiming at CD20+ B-cell depletion (anti-CD20 monoclonal antibodies [mAbs]) are effective disease-modifying therapies (DMTs) for treating MS. However, the route of administration, subcutaneous (s.c.) versus intravenous (i.v.), might have a profound impact on dosing, B-cell depletion and treatment outcome[1]
- In humanised-CD20 (huCD20) transgenic mice, direct comparison of ^{111}indium-radiolabelled ofatumumab (OMB, a fully human mAb) and ocrelizumab (OCR, a humanised mAb) showed that s.c. delivery of OMB led to best lymph node (LN) targeting, which may in turn lead to improved efficacy given these are the sites of pathogenic B- and T-cell interactions[1]
- Further pharmacological differentiation of OMB and OCR is expected from a head-to-head comparison

Objective

- To assess the potency of OMB-s.c. and OCR-i.v. at depleting B cells in mice expressing huCD20

Methods

Mice

- Adult huCD20 C57BL/6 (Ms4a1tm2[hCD20]Smoc) mice (Shanghai Model Organisms Center, China) expressing human CD20 exclusively on B cells treated with OMB-s.c. or OCR-i.v. were observed for dose-dependent effects at following doses:
  - OMB-s.c.: 2, 6 (human equivalent dose) and 20 µg/mouse (n=7 each)
  - OCR-i.v.: 6, 20 and 200 (human equivalent dose) µg/mouse (n=7 each)
- The drug exposures achieved by OMB-s.c. and OCR-i.v. treatments were measured by LC-MS/MS
- The levels of CD19+ B cells in blood and lymphoid organs were assessed via flow cytometry and changes versus baseline were used to estimate B-cell depletion
- Flow cytometry was also used to assess marginal zone B cells (MZ; CD19+CD21+CD23-), follicular (FO) B cells (CD19+CD21-CD23+) and plasma cells (CD138+)

Model of T-dependent humoral response

- Adult huCD20 C57BL/6 mice expressing human CD20 exclusively on B cells and treated with DNP-KLH (2,4-Dinitrophenyl hapten conjugated to keyhole limpet hemocyanin) vaccination were used to investigate the potency of OMB-s.c. versus OCR-i.v. at depleting MZ and FO B cells in lymphoid organs and in bone marrow (BM)
  - DNP-KLH vaccination was used to induce germinal center reaction with specific IgM/IgG responses
- Therapeutic antibody levels were monitored using IgG-binding ELISA assays and B-cell counts using flow cytometry in blood and lymphoid organs (spleen and inguinal LN)

Results

Time-dependent exposures of OMB-s.c. versus OCR-i.v. at 6 µg/mouse in serum and lymphoid organs of huCD20 mice

- In serum, OMB-s.c. treatment achieved a much lower (at least 4-fold) early peak drug levels versus OCR-i.v., with a slower washout up to 3 days post-treatment. However, exposures were similar for both treatments from Day 3 onwards (**Figure 1A**)

Figure 1A. OMB-s.c. versus OCR-i.v. 6 ug/mouse in serum of huCD20 mice

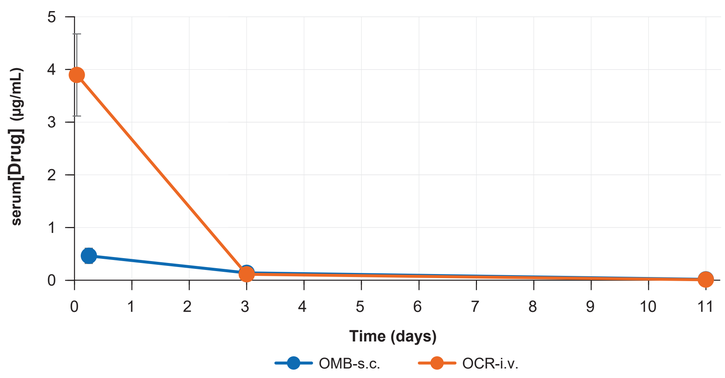


Figure 1B. OMB-s.c. in serum and lymphoid organs of huCD20 mice

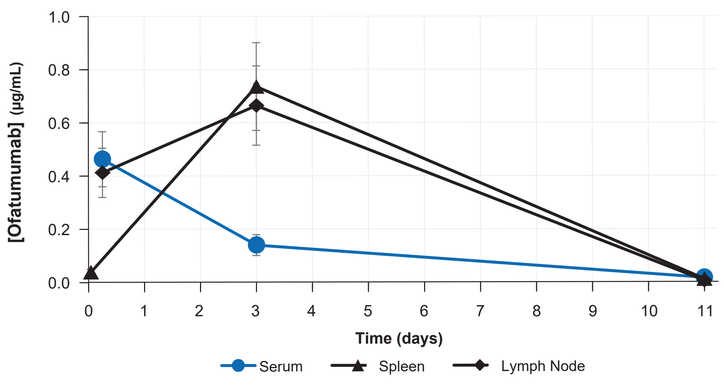
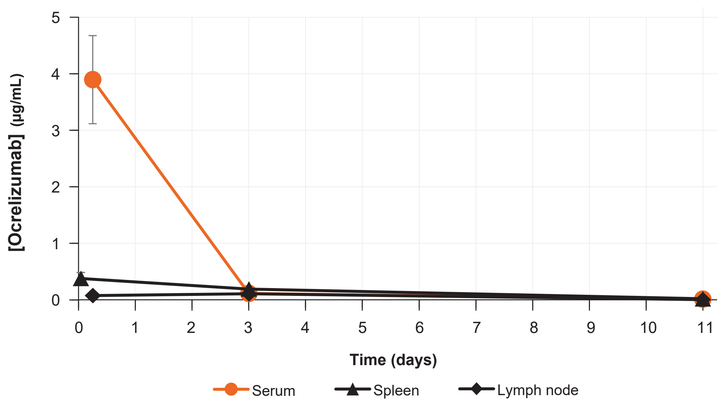


Figure 1C. OCR-i.v. in serum and lymphoid organs of huCD20 mice



- In LN and spleen, at Day 3, OMB-s.c. showed higher drug exposure versus serum, whereas lower or similar drug exposures were observed for OCR-i.v. (**Figure 1B** and **1C**)
- Although both treatments at the same dose (6 µg/mouse) achieved similar serum exposures within 3 days, s.c. route of administration enabled higher OMB exposure in the lymphatic compartment versus serum

Potency of OMB-s.c. versus OCR-i.v. at depleting B cells in blood and lymphoid organs

- In blood, OMB-s.c. appears ~20-fold more potent versus OCR-i.v. for depleting circulating B cells versus baseline levels
  - At 3 days following treatment, serum levels needed for achieving 50%/90% efficacy (EC_{50}/EC_{90}) were ~0.01/0.3 µg/mL for OMB-s.c. versus ~0.2/5.0 µg/mL for OCR-i.v. (**Figure 2**)
- At human-equivalent dose, OMB-s.c. (6 µg/mouse) reached its EC_{50} value for depleting circulating B cells, whereas OCR-i.v. (200 µg/mouse) was supramaximal (~40-fold above its EC_{90}) (**Figure 2**)
- OMB-s.c. showed a ~20-fold higher potency for depleting B cells in blood as compared to lymphoid organs (**Figure 2**)
- OCR-i.v. is equipotent for depleting circulating B cells in blood versus lymphoid organs (**Figure 2**)
- With similar EC_{50}s around 0.2 µg/mL, both OMB-s.c. and OCR-i.v. treatments appeared equipotent at depleting non-circulating B cells (**Figure 2**)
- In spleen and LN, drug level ratios versus serum were 5–20 for OMB-s.c. and ≤1 for OCR-i.v., respectively while OMB-s.c. appeared ~2-fold more efficacious at depleting B cells versus OCR-i.v. (**Figure 3**)

Figure 2. Drug exposure-dependent B-cell depletion in blood and lymphoid organs

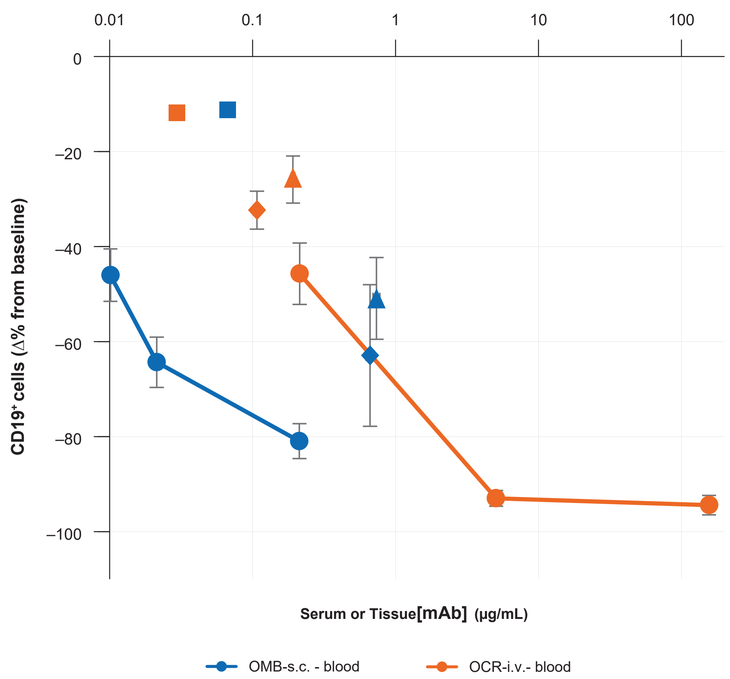
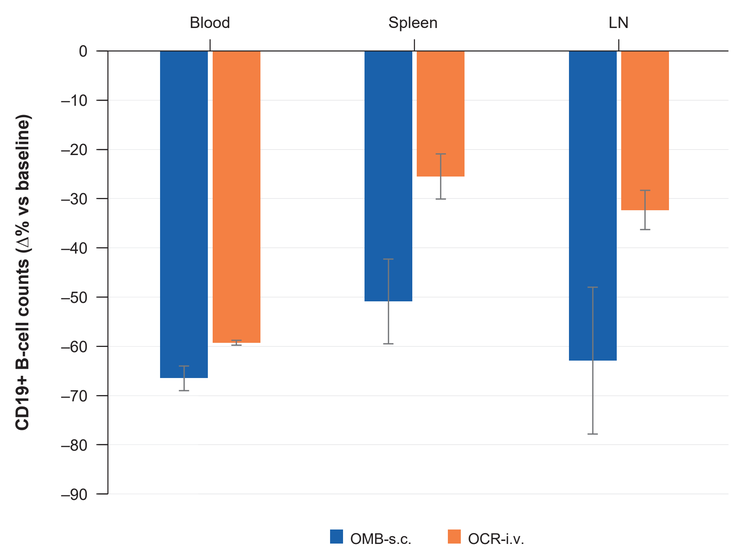


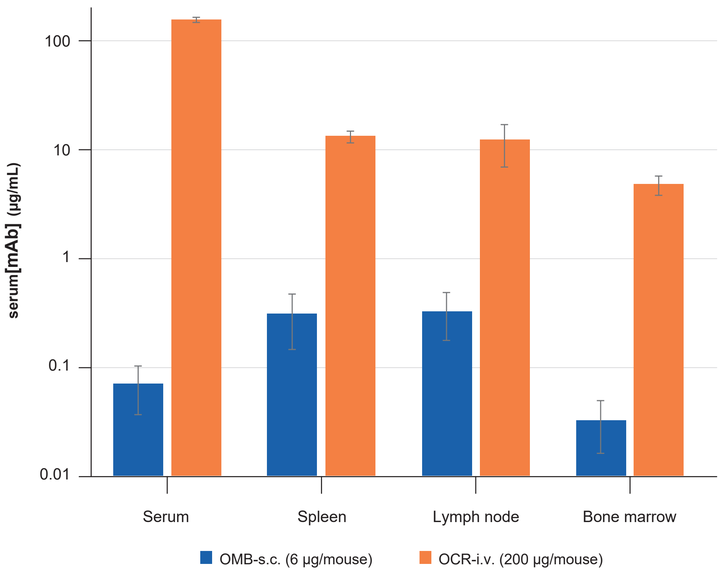
Figure 3. OMB-s.c. versus OCR-i.v. at 6 µg/huCD20 mice



Dose-dependent exposures of OMB-s.c. versus OCR-i.v. in lymphoid organs of huCD20 mice

- Both OMB-s.c. and OCR-i.v. achieved dose-proportional drug levels in all compartments
- At human equivalent doses, OCR-i.v. (200 µg/mouse) achieved markedly higher (30 to 1000-fold) drug exposures versus OMB-s.c. (6 µg/mouse; **Figure 4**)

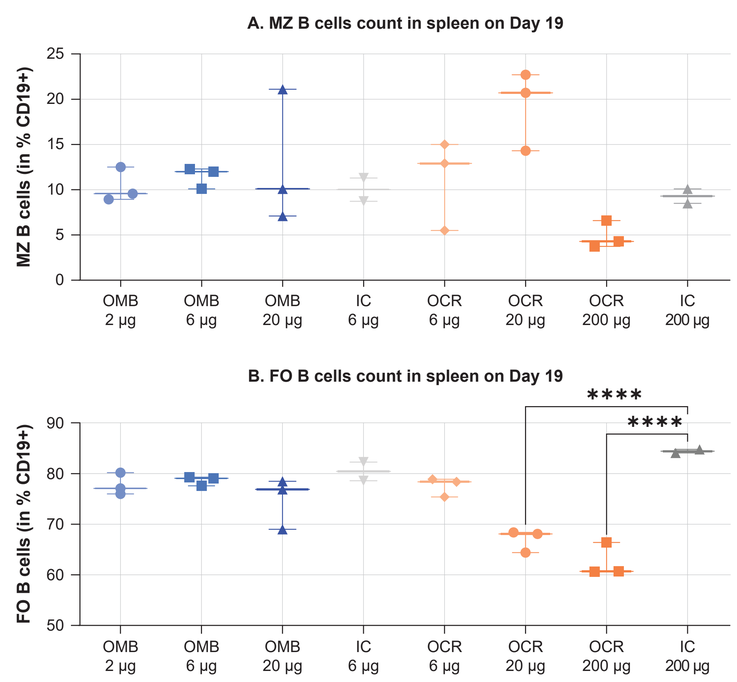
Figure 4. Exposure of OMB-s.c. versus OCR-i.v. in huCD20 mice at human equivalent doses



Potency of OMB-s.c. versus OCR-i.v. at depleting MZ and FO MZ B cells in lymphoid organs

- At human equivalent doses, MZ and FO B cells in secondary lymphoid organs were markedly depleted by OCR-i.v. but spared by OMB-s.c. (**Figure 5**)

Figure 5. OMB-s.c. versus. OCR-i.v. in depleting A) MZ B cells and B) FO B cells

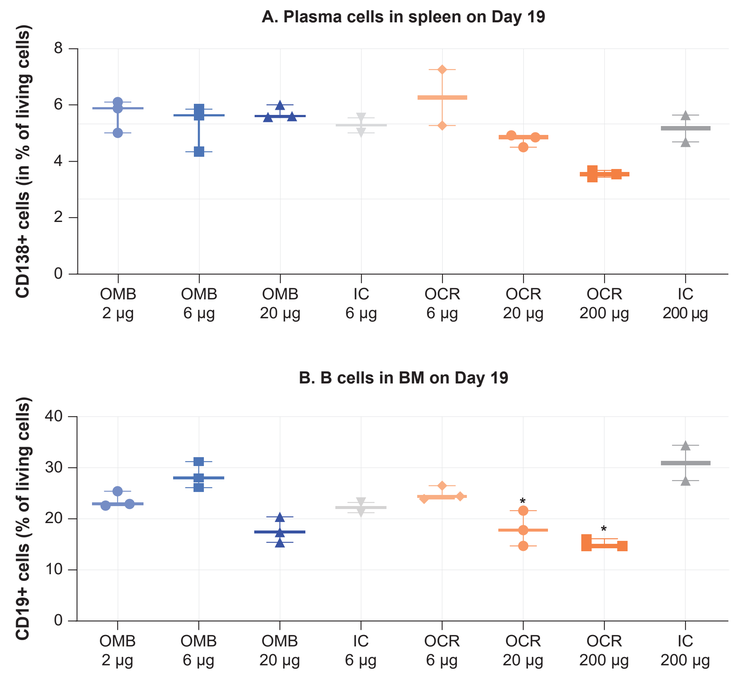


****p<0.0001; IC, isotype control

Impacts of OMB-s.c. versus OCR-i.v. at depleting B cells in BM

As shown in **Figure 4**, at human equivalent dose, OMB-s.c. achieved in BM drug levels within the range of EC_{50} in blood, whereas OCR-i.v. reached its EC_{90} in blood, suggesting a lower impact for OMB-s.c. on BM-resident CD20 expressing cells. This is confirmed in **Figure 6**, with CD138+ plasma cells population (comprising both CD138+CD20- plasma-cells and cells that transiently express CD20 (CD138+CD20+) in spleen is not affected by OMB-s.c. up to 20 µg/mL (>3-fold human equivalent dose), but significantly reduced by OCR-i.v. at 200 µg/mL (human equivalent dose) and by direct effect on CD19+ cells in BM.

Figure 6. OMB-s.c. versus OCR-i.v. - Impacts on plasma cells in A) spleen and B) B cell on BM



*p<0.05; IC, isotype control

Conclusions

- OMB-s.c. demonstrated better efficiency versus OCR-i.v. at targeting lymphoid organs and showed a 20-fold higher depleting potency on circulating B cells and equipotency on non-circulating B cells
- A sparing effect on MZ and FO B cells, key for the development of germinal center reactions and immune surveillance, as well as on BM, important for B-cell repletion and preservation of immune responses, was observed with OMB-s.c.
- The current study explored dose/route of administration dependent differences in an approach to B-cell depletion and supports the intentional design and development of a s.c. and low dose treatment option (OMB) versus high dose i.v. (OCR). The MZ/FO B-cell sparing effect of OMB (versus OCR) observed in this study may be relevant to understanding some differences in safety profiles observed between OMB and OCR in patients with MS

Reference

1. Torres JB, et al. *Front. Immunol.* 10.3389/fimmu.2022.814064

Acknowledgements

The authors acknowledge the following Novartis employees: **Bhavesh Kshirsagar** and **Sreelatha Komatireddy** for medical writing assistance and coordinating author reviews, and **Bal Reddy Telekala** for creative design assistance. The final responsibility for the content lies with the authors.

Disclosures

This study was sponsored by Novartis Pharma AG, Switzerland. **Marc Bigaud, Philipp Lutzenburg, Geraldine Zipfel, Tatjana Uffelmann, Helena Vostiarova, Volker Engelhardt, Barbara Nuesslein-Hildesheim, Bernd Kieseier** are employees of Novartis. **Daniel Anthony** is an employee of Oxford University and Somerville College.

Poster presented at the 38th congress of the European Committee for Treatment and Research in Multiple Sclerosis, RAI Amsterdam, Amsterdam, Netherlands, 26th–28th October 2022

Visit the web at: https://bit.ly/ectrims2022

Copies of this poster obtained through QR (Quick Response) code are for personal use only and may not be reproduced without written permission of the authors

Presenter email address: marc.bigaud@novartis.com

Scan this QR code to download a copy Poster