

SHORT REPORT

Open Access



# Ibrutinib inhibits antibody dependent cellular cytotoxicity induced by rituximab or obinutuzumab in MCL cell lines, not overcome by addition of lenalidomide

Alexandra Albertsson-Lindblad<sup>1\*</sup> , Catja Freiburghaus<sup>2</sup>, Mats Jerkeman<sup>1</sup> and Sara Ek<sup>2</sup>

## Abstract

**Background:** The Bruton's Tyrosine Kinase (BTK)-inhibitor ibrutinib is highly active in mantle cell lymphoma (MCL) but may inhibit response to anti-CD20 antibody as previously shown in CLL models. We investigated how antibody-dependent cellular cytotoxicity (ADCC) induced by type I/II anti-CD20 antibodies was affected by treatment with ibrutinib in MCL. Furthermore, we investigated if lenalidomide, a potential sensitizer to anti-CD20 treatment, could prevent an inhibitory effect of ibrutinib.

**Methods:** Anti-CD20 (rituximab/obinutuzumab) opsonized MCL cell lines were co-cultured with ibrutinib ( $\pm$  lenalidomide)—exposed effector cells, and analyzed for evaluation of cell death.

**Results:** Cell death induced by rituximab was reduced with 75% at 0.5  $\mu$ M ibrutinib and with 52% at 0.1  $\mu$ M ibrutinib when induced by obinutuzumab, even by addition of lenalidomide. Moreover, obinutuzumab was associated with higher rate of cell death compared to rituximab.

**Conclusion:** Ibrutinib negatively affects anti-CD20 induced cell death in MCL, not reversed by lenalidomide. Explorations of sequential administration and selective BTK-inhibitors may reveal the optimal combination of novel agents in MCL.

**Keywords:** Mantle cell lymphoma, Antibody-dependent cell death, Ibrutinib, Lenalidomide, CD20 antibody

## Background

The addition of anti-CD20 monoclonal antibody (mAb) to chemotherapy has improved disease control and survival rates in mantle cell lymphoma (MCL) and constitutes a backbone in primary treatment [1]. Still, MCL is regarded an incurable disease and exploration of new combinatory regimens is warranted. Ibrutinib, an inhibitor of Bruton's Tyrosine kinase is highly active in MCL and currently combined with anti-CD20 mAb within clinical trials. However, preclinical models in chronic lymphocytic leukemia (CLL) have demonstrated that

ibrutinib has a negative impact on the response to anti-CD20 mAb by inhibiting NK cell activation and thereby reducing antibody-dependent cellular cytotoxicity (ADCC) [2, 3].

Consequently, we hypothesized that ibrutinib inhibits immune mediated cell death induced by a type I/II anti-CD20mAb in MCL in vitro (i). Furthermore, we aimed to investigate whether the reduced response to anti-CD20 mAb treatment by ibrutinib could be prevented by pre-treatment of effector cells with the immune modulator lenalidomide (ii). Third, we compared the potency of inducing immune mediated cell death between type I and II anti-CD20 mAb bodies (iii).

\*Correspondence: alexandra.albertsson\_lindblad@med.lu.se

<sup>1</sup> Department of Oncology, Skåne University HOSPITAL, Lund University, Lasarettsgatan 23, 221 85 Lund, Sweden

Full list of author information is available at the end of the article



**Methods and results**

To investigate whether ibrutinib affects the immune-mediated response to anti-CD20 mAb in MCL, we performed experiments using the combination *in vitro* (i). Two well-characterized MCL cell lines, with low sensitivity to ibrutinib (Jeko-1, REC-1) (Additional file 1: Figure S1) were used as target cells, to enable evaluation of the effect of ibrutinib on the cytotoxic activity of peripheral blood mononuclear effector cells (PBMC) and not the direct effect on target cells. PBMC were treated with ibrutinib (0.1/0.5/1/5 μM) or R10 (control) 1 h and incubated with anti-CD20 mAb-coated [rituximab/obinutuzumab (1 μM)] target cells o/n. Target cells were stained with carboxyfluorescein succinimidyl ester (CFSE) at baseline and 7-AAD was used as a marker for the non-viable population (see Additional file 1 for details in Methodology). CFSE and 7-AAD positive (+) cells were measured using flow cytometry, and the fraction of non-viable target cells were calculated from the ratio 7-AAD<sup>+</sup> out of CFSE<sup>+</sup> populations.

Immune-mediated cell death, hereafter named as cell death, was defined as mean value of (7-AAD/CFSE)-ratio of duplicates compared to mean value of control duplicates without ibrutinib from four individual experiments. Student's unpaired t test was performed to identify significant differences. A p-value < 0.05 was considered significant.

We found that ibrutinib inhibits ADCC in a concentration dependent manner as shown in Fig. 1 and Table 1. In samples treated with the type I antibody rituximab, a significant lower cell death was observed at ≥ 0.5 μM

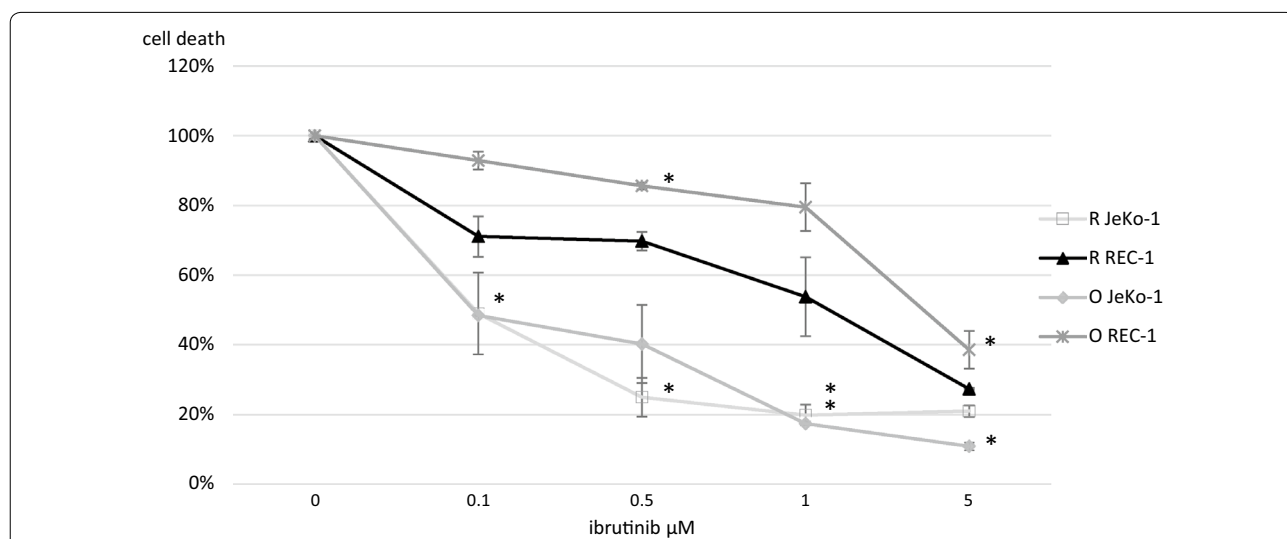
ibrutinib (0.25 ± 0.06, p = 0.024, JeKo-1) and at 5 μM ibrutinib (0.27 ± 0.00, p = 0.001, REC-1). The maximal difference in cell death was observed at 1 μM ibrutinib (0.20 ± 0.03, p = 0.005, JeKo-1). In samples treated with obinutuzumab, a significant lower cell death was observed at 0.1 μM (0.48 ± 0.30, p = 0.004, JeKo-1) and 0.5 μM (0.86 ± 0.01, p = 0.044, REC-1), and maximal reduction in cell death was observed at 5 μM ibrutinib (0.11 ± 0.01, p = 0.008, JeKo-1) (0.39 ± 0.05, p = 0.056, REC-1).

To investigate whether the immune modulator lenalidomide could revert the repressing effect of ibrutinib on PBMC (ii), the protocol used in (i) was extended by pre-treatment of PBMC with lenalidomide

**Table 1 Cell death in MCL cell lines, exposed to anti-CD20 antibody and PBMC pre-treated with ibrutinib**

Ibrutinib (μM)	Cell death (± SD)	p-value	Cell death (± SD)	p-value
JeKo-1		Rituximab (1 μM)		
0.1	0.49 ± 0.12	0.145	Obinutuzumab (1 μM)	
0.5	0.25 ± 0.06	0.023*	0.48 ± 0.00	0.003*
1	0.20 ± 0.03	0.024*	0.40 ± 0.11	0.118
5	0.21 ± 0.02	0.012*	0.17 ± 0.06	0.005*
REC-1		Rituximab (1 μM)		
0.1	0.71 ± 0.06	0.126	Obinutuzumab (1 μM)	
0.5	0.70 ± 0.03	0.056	0.93 ± 0.03	0.222
1	0.54 ± 0.11	0.152	0.86 ± 0.01	0.044*
5	0.27 ± 0.00	0.001*	0.79 ± 0.07	0.204
			0.39 ± 0.05	0.056

\* p < 0.05, student's t-test, compared to control



**Fig. 1** Ibrutinib interferes with immune mediated cell death induced by anti-CD20 antibody in MCL cell lines. Cell death (%) ± standard deviation in MCL cell lines (JeKo -1, REC-1), opsonized with 1 μM anti-CD20 mAb (rituximab, obinutuzumab) and co-cultured with PBMC pretreated with ibrutinib (0-5 μM). Results were compared by unpaired student's t-test. \* = p < 0.05. R rituximab, O obinutuzumab

(0/0.01/0.05/0.1/1 μM) 2 h prior to the addition of ibrutinib (1 μM). As in previously described experiments, cell death was lower by pretreatment of PBMC with ibrutinib, but not affected by addition of lenalidomide (Additional file 1: Figure S2 and Table S1). Hence, lenalidomide failed to revert the inhibitory effect of ibrutinib in this experimental set-up. Of note, although there were a trend towards increased cell death at higher concentrations in JeKo-1, we did not observe a significant increased cell death by pretreatment of PBMC with lenalidomide compared to controls (Additional file 1: Figure S3).

Third (iii), we compared the potency of type I versus type II anti-CD20-mediated ADCC on MCL in vitro, by comparing cell death, measured by 7-AAD<sup>+</sup>/CFSE<sup>+</sup>-ratios of samples with and without anti-CD20 mAb in three individual experiments. We found a significant higher cell death after treatment with obinutuzumab compared to rituximab in one out of two cell lines (Fig. 2 and Table 2).

In summary, the results of our study demonstrate an inhibitory effect of ibrutinib on immune mediated cell death induced by type I or II anti-CD20 monoclonal antibody treatment in MCL in vitro, not overcome by pre-sensitizing effector cells with the immune modulator lenalidomide. Our findings suggest a more pronounced immune mediated cell death induced by a type II anti-CD20 mAb obinutuzumab than the type I CD20 targeting antibody rituximab.

### Discussion

Ibrutinib is an attractive component in combinatory regimens in MCL, based on its high anti-proliferative activity and favorable toxicity profile. However, little is known about how ibrutinib interferes with the immune-mediated activity induced by anti-CD20 targeted treatment in MCL, which was the focus of this study.

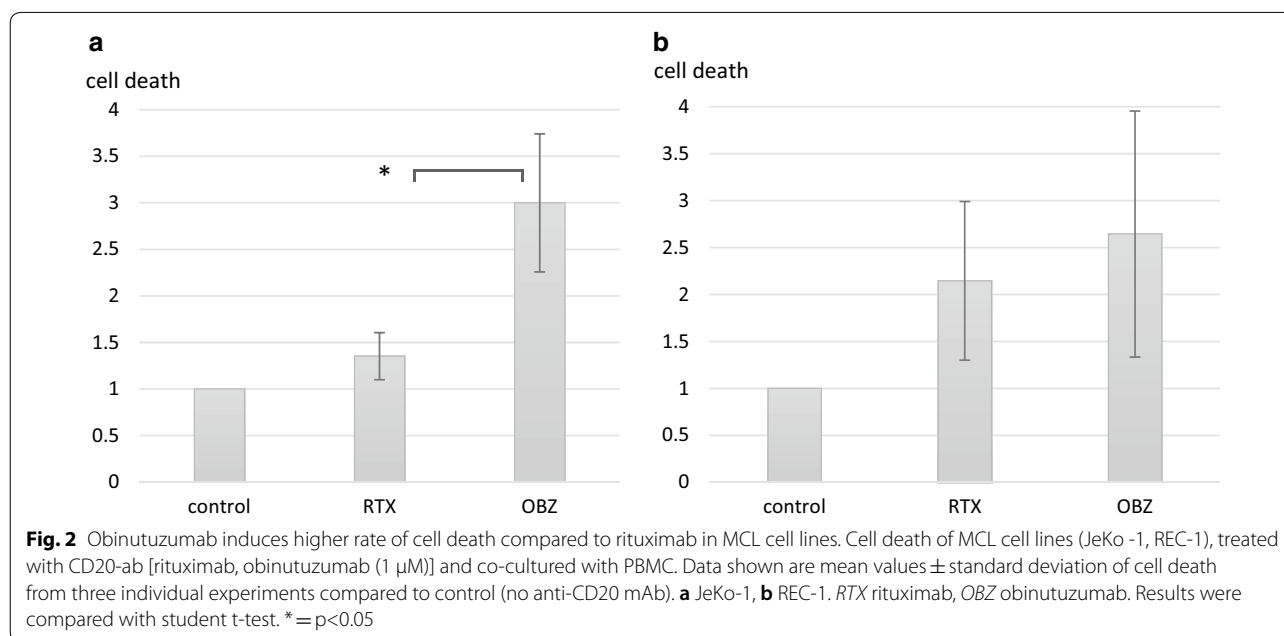
We observed a reduced immune mediated cell death induced by rituximab at physiologically relevant concentrations of ibrutinib (0.1 μM), being within the range of plasma concentration (0.07–0.2 μM) reported in patients receiving 420–840 mg daily ibrutinib [4, 5].

Our results are in line with previous data on in vitro models in CLL [2, 3, 6], which may be explained by affinity of ibrutinib to interleukin-2-inducible T-cell kinase (ITK), expressed in NK cells, but could also be related to reduced CD20-expression [7, 8]. Interestingly, Kohrt et al. demonstrated a superior tumor control by sequential administration of anti-CD20 mAb and ibrutinib compared to concomitant administration of these drugs

**Table 2 Cell death induced by rituximab and obinutuzumab in MCL**

	Rituximab		Obinutuzumab		Rituximab vs obinutuzumab
	Cell death (%)	p-value	Cell death (%)	p-value	p-value
JeKo-1	1.31 ± 0.26	0.024*	3.00 ± 1.02	0.012*	0.030*
REC-1	2.15 ± 0.85	0.029*	2.64 ± 1.31	0.038*	0.493

\* p < 0.05



in ex vivo models in CLL that would be interesting to explore in MCL [2].

We used two cell lines with low and intermediate sensitivity to ibrutinib to minimize the dose-dependent toxic effect on target cells by ibrutinib per se, although a direct cytotoxic effect could not be neglected at 5  $\mu\text{M}$  in one of the cell lines (REC-1), thus possibly overestimating cell death. PBMC was incubated with ibrutinib for 1 h, identical to previous reported works which should be sufficient to reach occupancy of target binding [3]. The concentration of anti-CD20 mAb was based on a pre-study where we observed immune mediated cell death at 1  $\mu\text{M}$ , in line with previous ADCC in vitro assays in NHL [2, 3]. Although being lower than the serum maximal concentration ( $C_{\text{max}}$ ) of 3.2  $\mu\text{M}$  in patients after repeated doses of rituximab, 1  $\mu\text{M}$  should thus be adequate to evaluate the hypothesis of the actual work [9].

The use of PBMC instead of purified NK cells, being more alike the in vivo situation, required a high ratio of effector: target cells (100:1) resulting in a low count of target cells in flow cytometry, being a limitation of the study.

In the present study, the inhibitory effect of ibrutinib was not reverted by pre-treatment of PBMC with lenalidomide. Lenalidomide has been reported to potentiate anti-CD20 induced ADCC in vitro but not in in vivo xenograft model [10, 11]. We did not observe a significant higher cell death in control samples with lenalidomide and anti-CD20 mAb compared to anti-CD20 mAb which should be emphasized. A shorter incubation time and no addition of interleukins such as IL-2 and IL-12 may explain a less pronounced activation of effector cells and potential synergistic actions in our set-up. We included concentrations of lenalidomide 0.01–1  $\mu\text{M}$ , being within the range of dose-dependent cytotoxic effect of lenalidomide in previous in vitro experiments and close to the serum concentration of 1.7  $\mu\text{M}$  at oral daily dose of 25 mg [9, 12]. In our experiment, controls included series with lenalidomide-treated PBMC and non-exposed target cells. In these samples, a markedly lower cell death was observed for lenalidomide compared to samples with anti-CD20 mAb (data not reported). Altogether, this clearly demonstrate that treatment with monoclonal antibody is responsible for the major cytotoxic activity in MCL and that immune modulator such as lenalidomide may increase cell death to a limited extent.

Clinical trials have demonstrated that the combination of lenalidomide and rituximab (R2) is active in MCL with a response rate higher than 50% even in patients with relapsed disease [13, 14]. However, pretreatment of lenalidomide to rituximab does not seem to restore sensitivity in rituximab-refractory MCL, according to results from the phase II trial by Chong et al. [15]. Furthermore,

ibrutinib-lenalidomide-rituximab was associated with high response rate in relapsed/refractory MCL including patients with poor prognostic risk factors as reported from a Nordic trial [12, 16]. Altogether, the synergistic effects of lenalidomide on anti-CD20 mAb activity in MCL seems to be restricted to anti-CD20-sensitive cases. To further evaluate the role of lenalidomide when combined with rituximab and a BTK-inhibitor such as ibrutinib, it would be valuable to explore how immune mechanisms including NK cell activation status are affected during this combination and if an alternative approach, i.e. by sequential administration, would overcome possible counteracting effects.

Our results suggest that obinutuzumab may have a more pronounced capacity of inducing ADCC in MCL compared to rituximab. These results confirm previous in vitro studies in CLL and other non-Hodgkin lymphoma models [3, 17]. Ongoing clinical trials evaluating the combination of obinutuzumab and ibrutinib are underway and will, together with further preclinical investigations, reveal which is the optimal anti-CD20 targeting agent in MCL [18].

Being a strict in vitro study, our study has several limitations. The results need to be confirmed by other methods for evaluating immune mediated cell death as well as by in vivo experiments to support the hypothesis.

In summary, we show that ibrutinib inhibits the immune mediated response induced by anti-CD20-mAb with markedly lower cell death at concentrations comparable to in vivo serum levels, not overcome by addition of a potential immune sensitizer such as lenalidomide. Although a type II anti-CD20 mAb may have a stronger capacity of inducing immune mediated cell death in MCL, the inhibitory effect of ibrutinib on effector cells seems to be relevant irrespectively if combined with a type I or II anti-CD20 mAb. Besides confirming studies on in vivo models, future studies on sequential administration of ibrutinib and anti-CD20-mAbs in MCL as well as exploration of more selective BTK-inhibitors with less off-target binding may reveal how these optimally could be combined in vivo, with respect to efficacy, potential synergism and toxicity of each compound.

## Conclusion

The findings of our study strongly indicate that the inhibitory effect of ibrutinib on immune-mediated cell death induced by anti-CD20 mAb due to unwanted off-target binding is applicable even in MCL. The inhibitory effect of ibrutinib on effector cells seems to be strong enough to withstand either a more potent activator of effector cells such as a type II anti-CD20 mAb like obinutuzumab or by pretreatment of lenalidomide, a potential sensitizer to anti-CD20mAb. In the light of the high activity of these

agents in MCL, sequential administration of the compounds as well as exploring more selective BTK-inhibitors could be of relevance in future design of regimens.

## Additional file

**Additional file 1.** Supplementary tables and figures & Methodology and Material.

## Abbreviations

7-AAD: actinomycin D; ADCC: antibody-dependent cellular cytotoxicity; BTK: Bruton's Tyrosine Kinase; CFSE: carboxyfluorescein succinimidyl ester; CLL: chronic lymphocytic leukemia; ITK: interleukin-2-inducible T cell kinase; mAb: monoclonal antibody; MCL: mantle cell lymphoma; NK cell: natural killer cell; PBMC: peripheral blood mononuclear cell.

## Acknowledgements

The authors specially thank the Departure of Transfusion Medicine, Skåne University Hospital and all healthy donors for support.

## Authors' contributions

AA-L contributed in Conceive and Design of study; collected and interpreted data and was responsible for manuscript writing. CF: contributed in design of study, collected data and contributed in analysis and interpretation of data. SE was the major contributor of conceive and design of the study and contributed in analysis and interpretation of data. MJ: contributed in conceive and design of study, interpretation of data and manuscript writing. All authors read and approved the final manuscript.

## Funding

This work was supported by ALF (Medical Training and Research Agreement) funds, Faculty of Engineering, Lund University and BioCARE.

## Availability of data and materials

The datasets included in the current study are available from the corresponding author upon reasonable request.

## Ethics approval and consent to participate

The study was conducted according to protocols approved by local institutional review board in accordance with the Declaration of Helsinki. PBMC was collected from healthy donors, approved by Department of transfusion Medicine, Skåne University Hospital Lund, ref 2015:23.

## Consent for publication

Not applicable.

## Competing interests

AA-L; CF; SE: no competing interests; Mats Jerkeman: Research support from Celgene, Janssen, Gilead, Abbvie.

## Author details

<sup>1</sup> Department of Oncology, Skåne University HOSPITAL, Lund University, Lasarettsgatan 23, 221 85 Lund, Sweden. <sup>2</sup> Department of Immunotechnology, Lund University, Lund, Sweden.

Received: 11 June 2019 Accepted: 27 July 2019

Published online: 06 August 2019

## References

- Dreyling M, et al. Newly diagnosed and relapsed mantle cell lymphoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2017;28(suppl\_4):iv62–71.
- Kohrt HE, et al. Ibrutinib antagonizes rituximab-dependent NK cell-mediated cytotoxicity. *Blood*. 2014;123(12):1957–60.
- Da Roit F, et al. Ibrutinib interferes with the cell-mediated anti-tumor activities of therapeutic CD20 antibodies: implications for combination therapy. *Haematologica*. 2015;100(1):77–86.
- Byrd JC, et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. *N Engl J Med*. 2013;369(1):32–42.
- Advani RH, et al. Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B-cell malignancies. *J Clin Oncol*. 2013;31(1):88–94.
- Duong MN, et al. Effect of kinase inhibitors on the therapeutic properties of monoclonal antibodies. *MAbs*. 2015;7(1):192–8.
- Berglöf A, et al. Targets for Ibrutinib Beyond B Cell Malignancies. *Scand J Immunol*. 2015;82(3):208–17.
- Bojarczuk K, et al. B-cell receptor pathway inhibitors affect CD20 levels and impair antitumor activity of anti-CD20 monoclonal antibodies. *Leukemia*. 2014;28(5):1163–7.
- Liston DR, Davis M. Clinically relevant concentrations of anticancer drugs: a guide for nonclinical studies. *Clin Cancer Res*. 2017;23(14):3489–98.
- Hernandez-Ilizaliturri FJ, et al. Immunomodulatory Drug CC-5013 or CC-4047 and rituximab enhance antitumor activity in a severe combined immunodeficient mouse lymphoma model. *Clin Cancer Res*. 2005;11(16):5984–92.
- Zhang L, et al. Synergistic antitumor effects of lenalidomide and rituximab on mantle cell lymphoma in vitro and in vivo. *Am J Hematol*. 2009;84(9):553–9.
- Wu L, et al. lenalidomide enhances natural killer cell and monocyte-mediated antibody-dependent cellular cytotoxicity of rituximab-treated CD20+ tumor cells. *Clin Cancer Res*. 2008;14(14):4650–7.
- Wang M, et al. Lenalidomide in combination with rituximab for patients with relapsed or refractory mantle-cell lymphoma: a phase 1/2 clinical trial. *Lancet Oncol*. 2012;13(7):716–23.
- Ruan J, et al. Initial treatment with lenalidomide plus rituximab for mantle cell lymphoma: 5-year follow-up and correlative analysis from a multicenter phase II study. *Blood*. 2017;130(Suppl 1):154.
- Chong EA, et al. Combination of lenalidomide and rituximab overcomes rituximab resistance in patients with indolent B-cell and mantle cell lymphomas. *Clin Cancer Res*. 2015;21(8):1835–42.
- Jerkeman M, et al. Ibrutinib, lenalidomide, and rituximab in relapsed or refractory mantle cell lymphoma (PHILEMON): a multicentre, open-label, single-arm, phase 2 trial. *Lancet Haematol*. 2018;5:e109–16.
- Mossner E, et al. Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell-mediated B-cell cytotoxicity. *Blood*. 2010;115(22):4393–402.
- Parrott M, et al. A systematic review of treatments of relapsed/refractory mantle cell lymphoma. *Clin Lymphoma Myeloma Leuk*. 2018;18(1):13–25.

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.