



Ublituximab, an Optimized Anti-CD20 Monoclonal Antibody, Demonstrates Greater NK-Mediated ADCC Than Rituximab in Waldenstrom's Macroglobulinemia Patients Supporting a Therapeutic Strategy with Ublituximab

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INTRODUCTION

Anti-CD20 monoclonal antibody (mAb) therapy is an important therapeutic option in the treatment of Waldenström's Macroglobulinemia (WM), exhibiting an ORR up to 55% when used as monotherapy (Gertz, *Leuk Lymphoma*, 2004).

NK cells are involved in mAb therapy by an antibody-dependent cellular cytotoxicity (ADCC) mechanism through their FcγRIIIa (CD16) receptor. In this study, we have evaluated the ADCC functional capacities of NK cells in the presence of ublituximab (TGTX-1101 or LFB-R603), an optimized anti-CD20 mAb exhibiting a low fucose content, in comparison to rituximab.

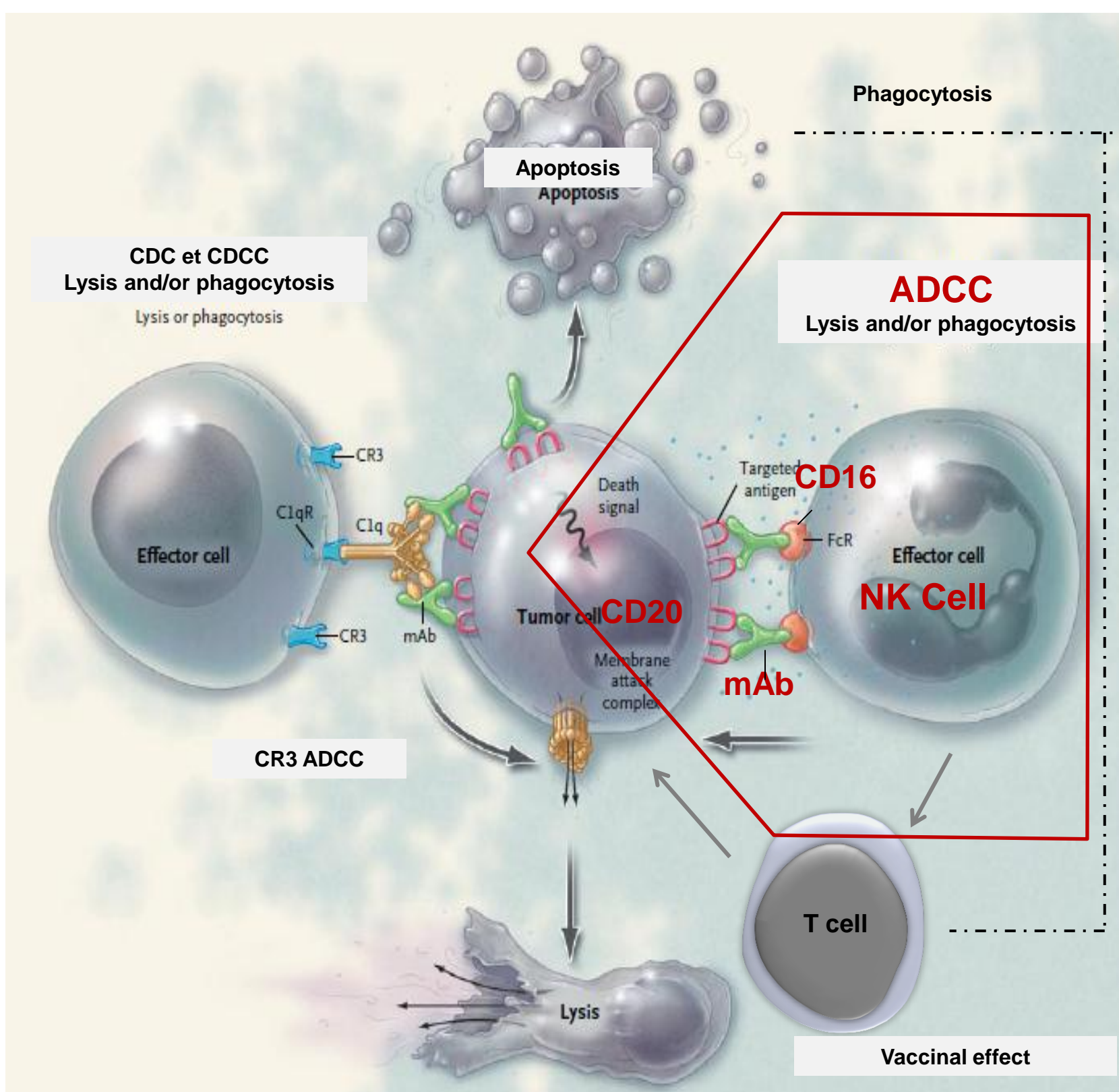


Figure 1:
Potential mechanisms of action of anti-CD20 mAbs

mAbs have several potential mechanisms of action, including antibody-dependent cellular cytotoxicity (ADCC), which involves recruitment of effector cells with FcγR, like NK cells.

mAb: monoclonal antibody; FcR: Fc receptor; CD16 = FcγRIIIa

Adapted from
Cheson et al, *NEJM*, 2008

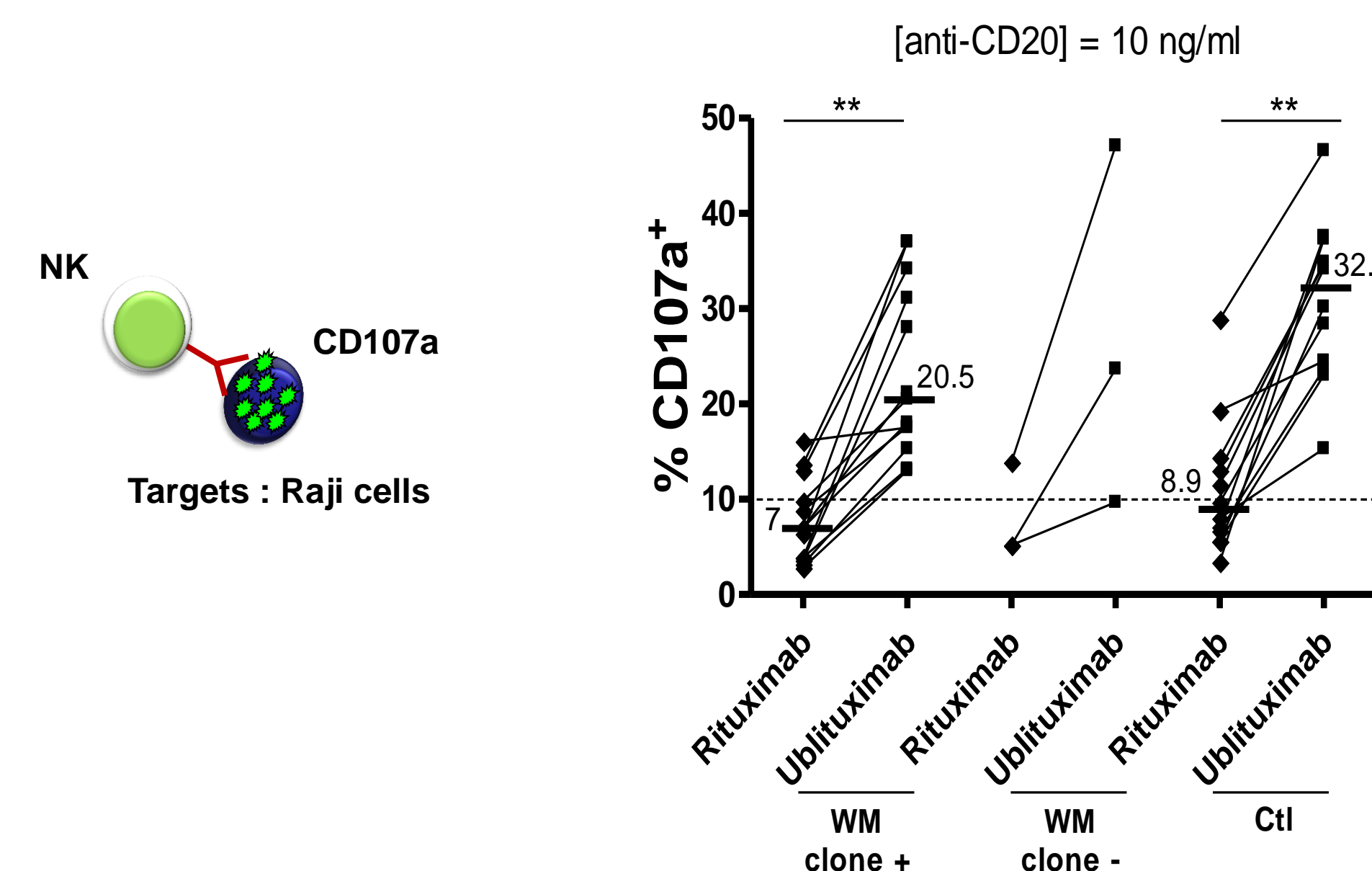
METHODS

Blood samples from 40 untreated or without ongoing treatment WM patients and from 30 age-matched healthy donors (Ctl) were collected to quantify CD16 expression (clone 3G8, Quantibrite®) on NK cells and/or to measure their functional capacities. Patients were divided in two groups relative to the presence (WM clone+) or absence (WM clone-) of blood clonal B cells. NK cell degranulation was assessed by the surface expression of CD107a on NK cells after incubation of PBMC with or without Raji CD20+ target cells in the presence of anti-CD20 mAbs at 10 and 1,000 ng/ml. ADCC experiments were performed using a chromium assay with purified NK cells and autologous B cells or Raji target cells, in the presence of anti-CD20 mAbs at 1 and 100ng/ml.

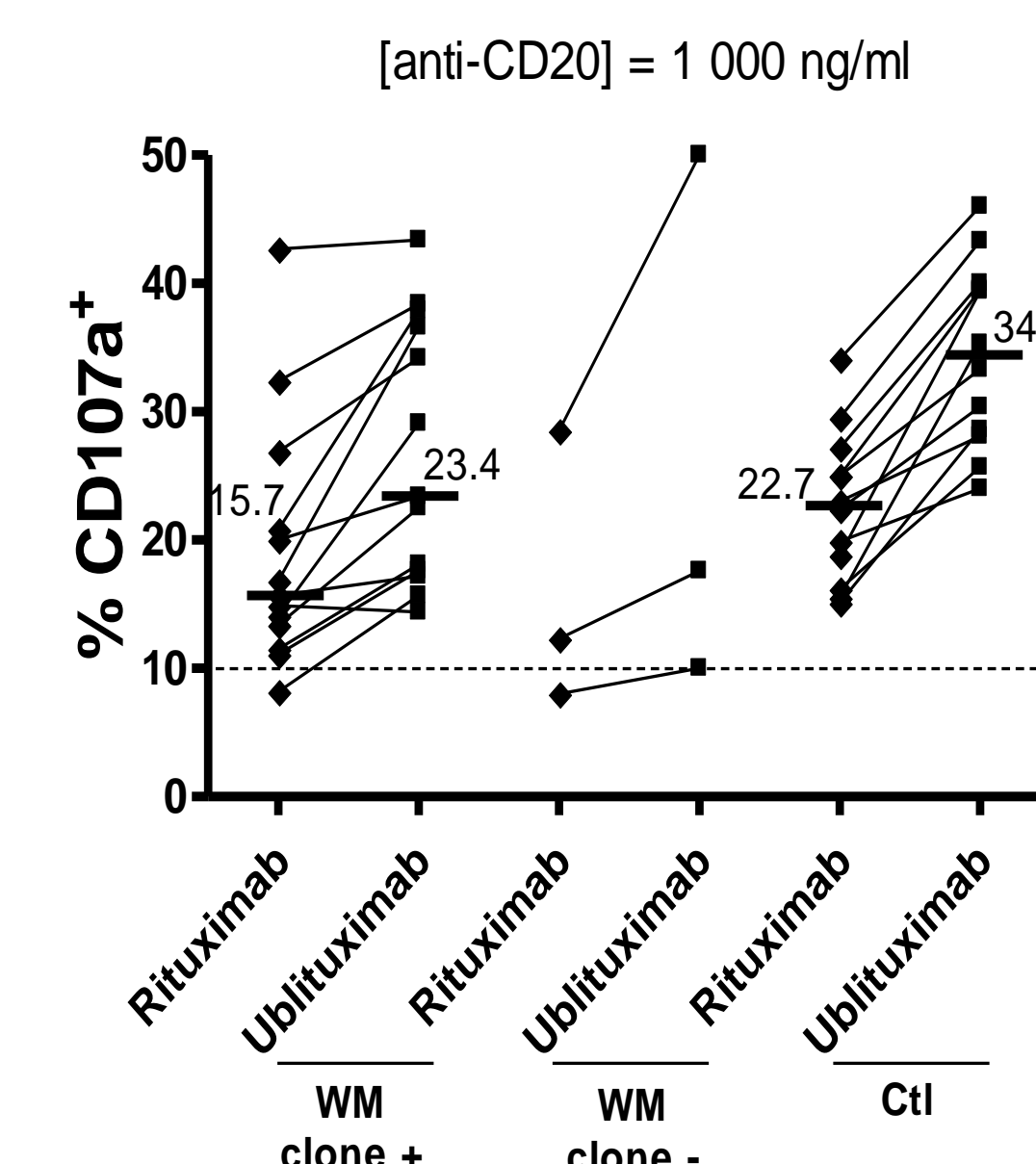
Statistical analyses were performed with Prism 5 software. Intergroup comparisons were assessed with the nonparametric Kruskal-Wallis test, with the Dunn's postanalysis test. Significance defined by *P* less than 0.05 with a two-tailed test. **P* < 0.05, ***P* < 0.01

RESULTS

Degranulation

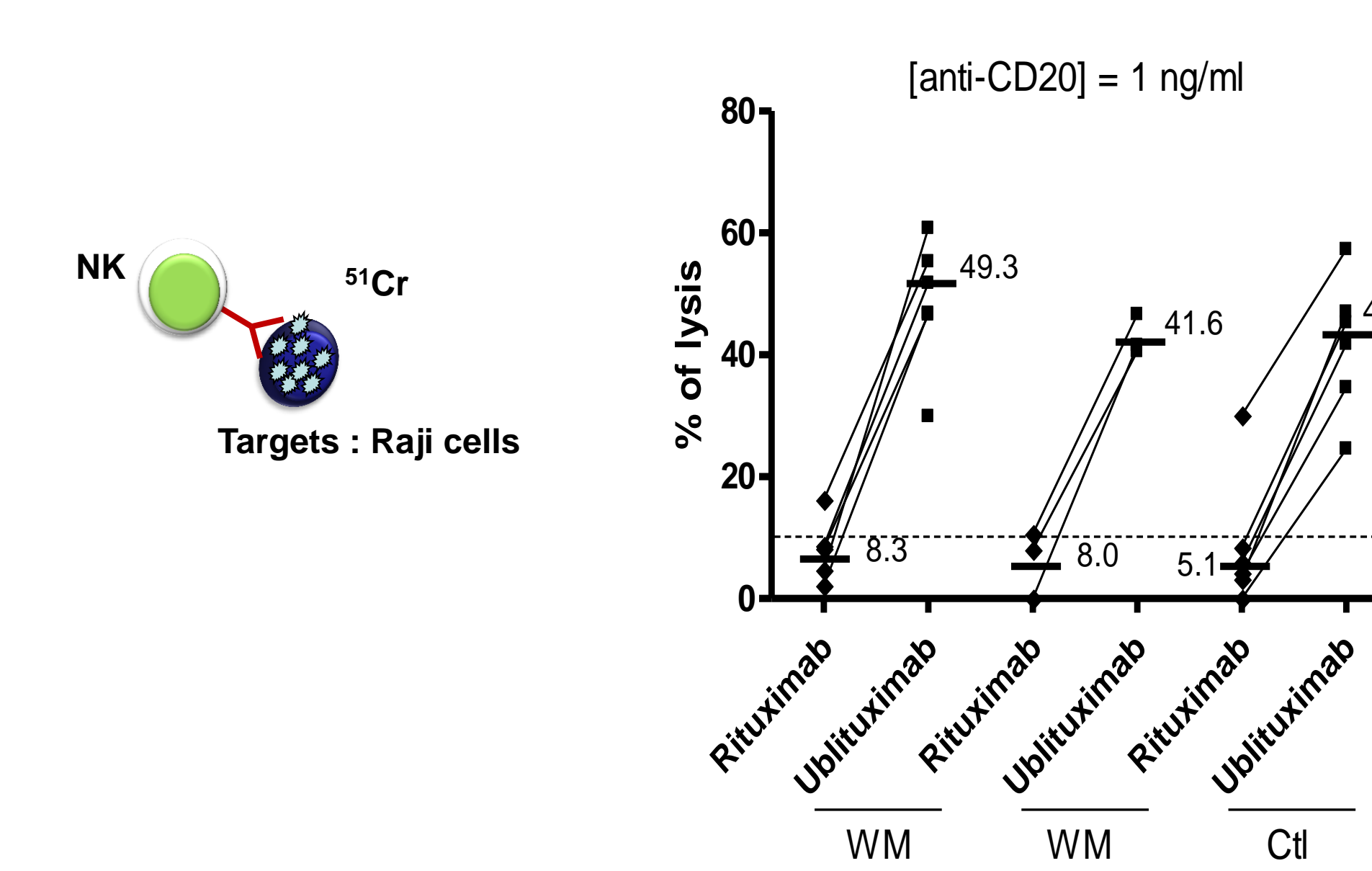


IN PRESENCE OF RAJI CELLS

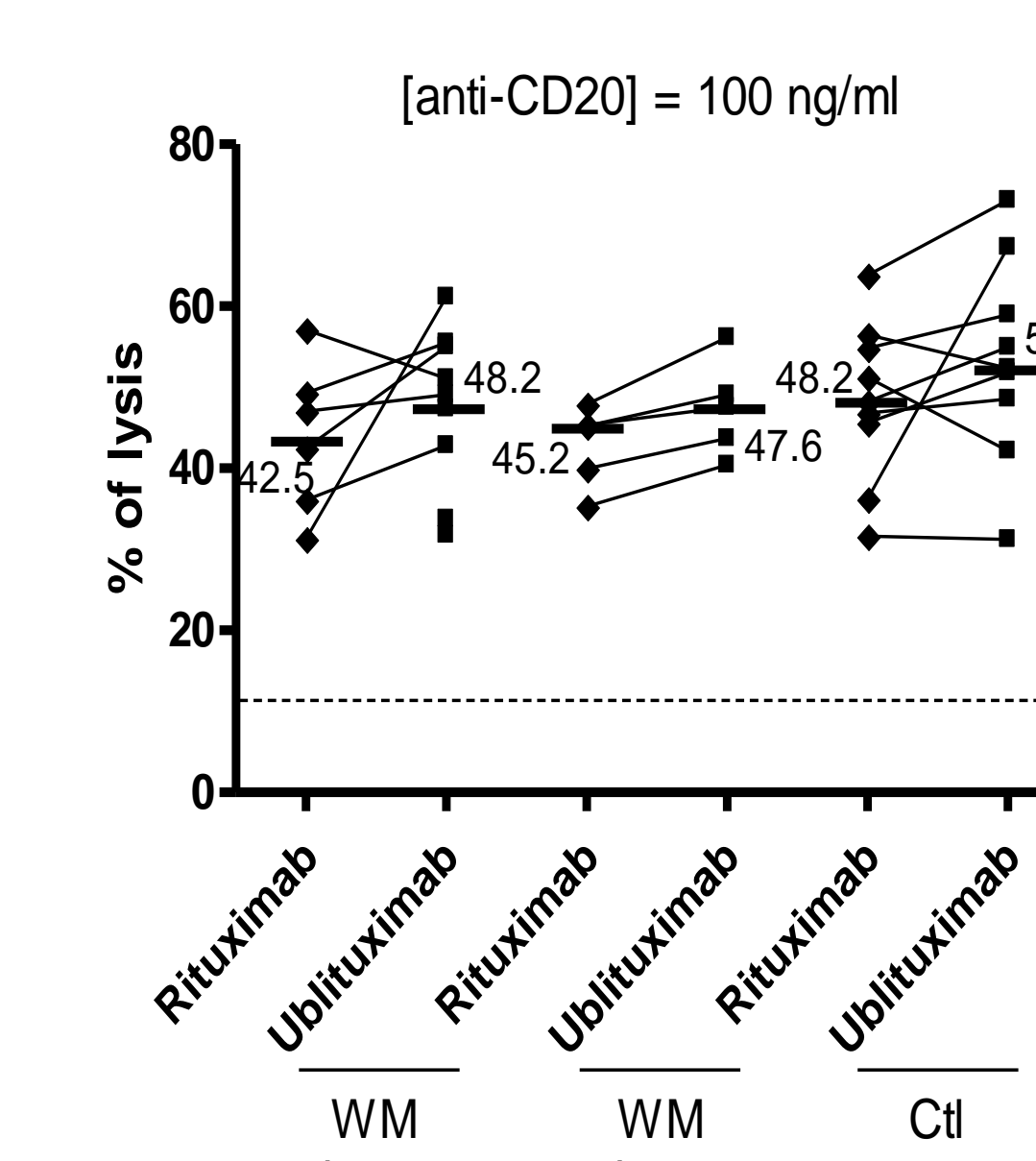


In the presence of Raji cells, at low concentration, a significantly greater amount of CD107a expression was observed with ublituximab compared to rituximab (*P* < 0.01), regardless of patient's groups. In contrast, at the highest concentration, similar effects were obtained with both anti-CD20 mAbs.

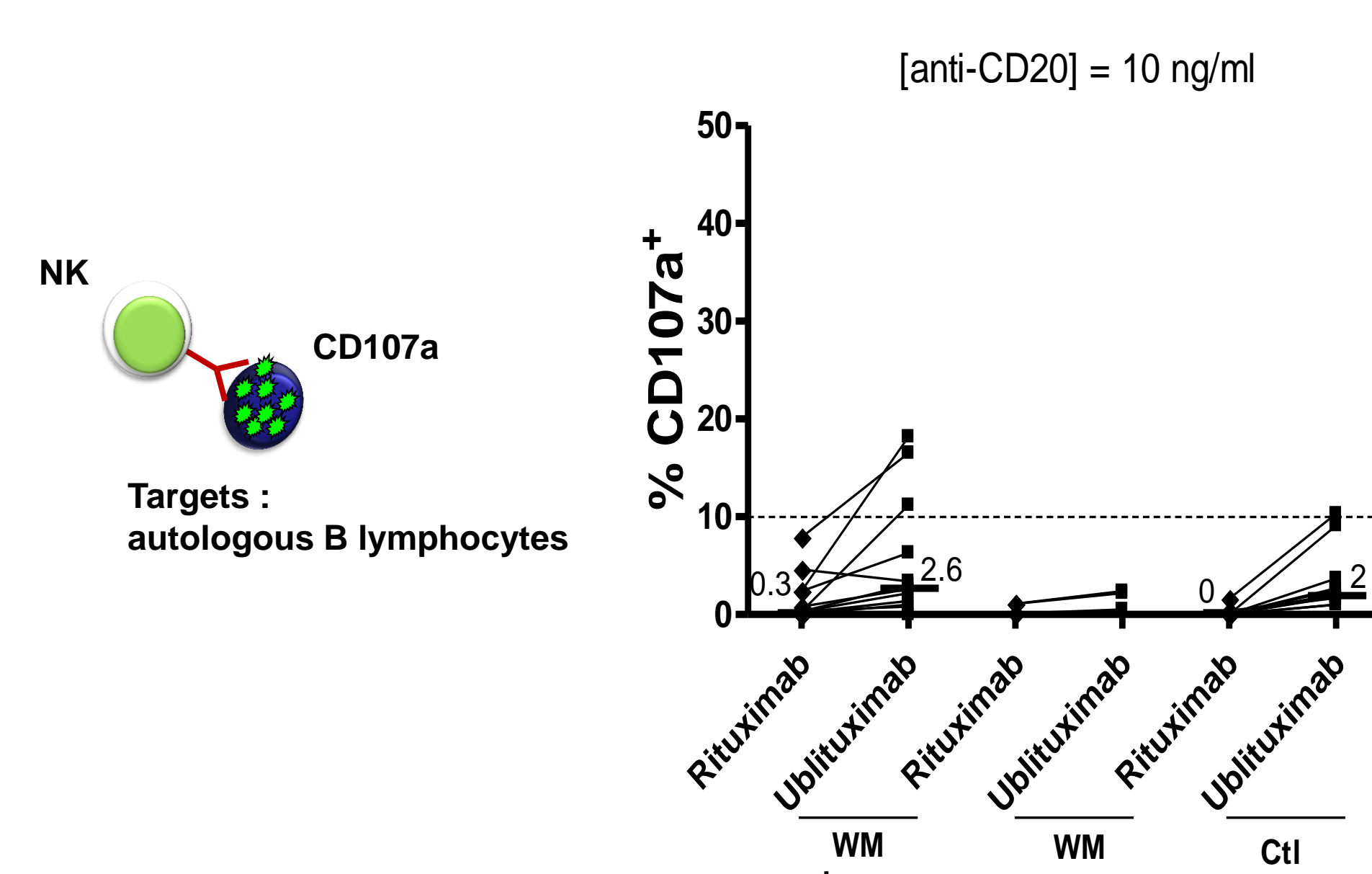
ADCC



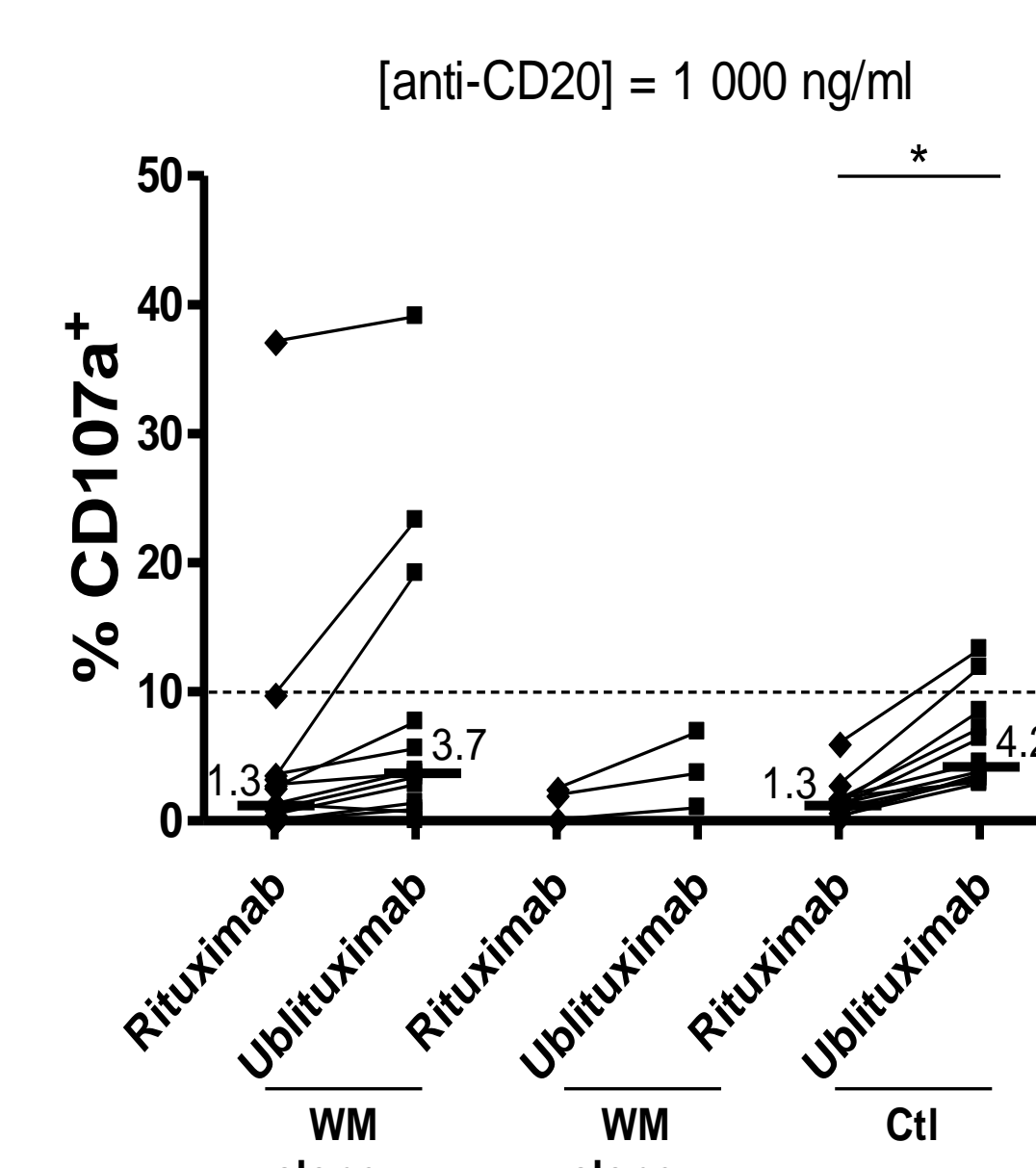
These results were confirmed by ADCC. In the presence of Raji cells, a high level of ADCC (>40%) was detected at low concentration of ublituximab and remained stable at 100ng/ml. In contrast, with rituximab the highest concentration was necessary to reach similar efficacy.



Degranulation

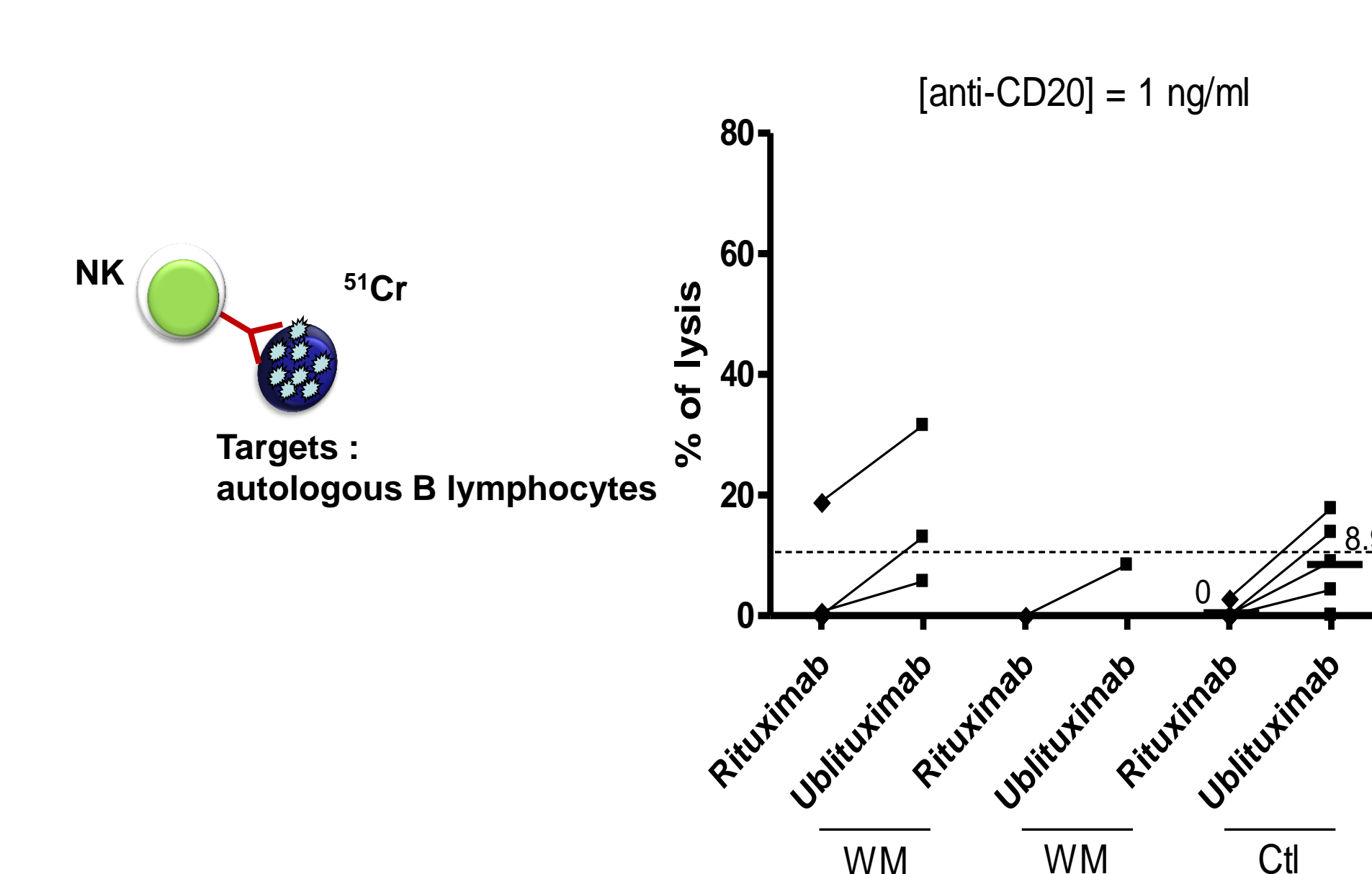


IN THE PRESENCE OF AUTOLOGOUS B CELLS



In the presence of autologous B cells, degranulation assays revealed that none of the NK cells from WM clone- patients exhibited degranulation, irrespective of the anti-CD20 mAb or its concentration. More importantly, NK cells of 3/8 WM clone+ patients exhibited CD107a+ NK cells in the presence of both concentrations of ublituximab. In contrast, with rituximab only 1/8 patients expressed CD107a+ NK cells, and only at the highest concentration. Of note, similar frequency and cell-surface expression level of CD16 on NK cells were detected in both patient groups.

ADCC



Importantly, these data were confirmed by ADCC. In the presence of autologous purified B cells from WM clone- patients, absence or low levels of ADCC were detected, irrespective of the concentration and the anti-CD20 mAb used. Interestingly, in WM clone+ patients, ADCC was detected in all of the 7 tested patients with ublituximab, but only in 2/7 patients with rituximab, and at the highest concentration.

CONCLUSION

These results show that, as previously described in CLL, ublituximab is more efficient than rituximab in inducing ADCC at low doses, in the presence of Raji cells. More importantly, our results suggest that ublituximab could be more efficient than rituximab both to induce NK cell degranulation and ADCC in the presence of autologous peripheral tumor cells. These findings highlight a new putative role of this optimized anti-CD20 mAb in the control of WM, and prompt further investigations in a large cohort of WM patients. A Phase I/II trial with single agent ublituximab in patients with rituximab relapsed / refractory NHL, including WM patients is currently ongoing.

