Role of NK-mediated ADCC in WM patients: evidence supporting a therapeutic strategy with ublituximab, an optimized anti-CD20 monoclonal antibody

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Background: Anti-CD20 monoclonal antibody (mAb) is an important therapeutic option in Waldenström Macroglobulinemia (WM), with an ORR up to 55% when used in monotherapy. NK cells are involved in mAb therapy by an antibody-dependent cellular cytotoxicity (ADCC) mechanism through their FcγRIIIa (CD16). In this study, we have evaluated the ADCC functional capacities of NK cells in presence of ublituximab (TGTX-1101 or LFB-R603), an optimized anti-CD20 mAb exhibiting a low fucose content, in comparison to rituximab. Methods: Blood samples from 37 previously untreated or without ongoing treatment WM patients 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 w/o Raji CD20+ target cells in the presence of anti-CD20 mAbs at 10 and 1,000ng/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. Results: In presence of Raji cells, 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. These results were confirmed by NK degranulation: at low concentration, significant amount of CD107a expression was observed with ublituximab, compared to rituximab (P<0.0001), regardless of patient's groups, while at the highest concentration, similar effects were obtained with both anti-CD20 mAbs. In 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 dose. Of note, similar frequency and cell-surface expression level of CD16 on NK cells were detected in both patients' groups. Importantly, these data

were confirmed by ADCC. In the presence of purified B cells from WM clone- patients, absence or low levels of ADCC were quantified, whatever the concentration and the anti-CD20 mAbs used. Interestingly, in WM clone+ patients, ADCC was detected in all of the 5 tested patients with ublituximab, but only in 2/5 with rituximab 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 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.