Supplemental experimental methods:

Cell culture

Raji and WIL2 NS cells were cultivated in Dulbecco's modified Eagle's medium containing 10% fetal calf serum (FCS) and N-acetyl-L-alanyl-L-glutamine (2 mM). Z138 cells and SU-DHL4 cells were cultivated in RPMI1640 containing 10% FCS and N-acetyl-L-alanyl-L-glutamine (2 mM). All cell culture material was purchased from Invitrogen. For time-lapse confocal microscopy studies, Z138 cells were purified using Ficoll gradient centrifugation to remove any dead cells present in the culture (Ficoll-Paque^{TM} PLUS, # 17-1440-02, Amersham Bioscience). Purified cultures were then transferred to eight-well chamber slides (Lab-Tek^{TM} # 155405, Thermo Fisher) that were previously coated with 0.005% poly-L-ornithine (Sigma-Aldrich #P4957) for 15 min and washed two times with H_2O. A total of 10^6 Z138 cells were plated in 500 µL in each well in phenol red-free media. Cells were incubated with GA101, rituximab or ofatumumab at a final concentration of 5-20 µg/mL. Finally, 500 µL of Annexin V/PI staining mixture was carefully added (Annexin V FLUOS Staining Kit, Roche Applied Science). Chamber slides were then transferred to the stage top incubator of the confocal microscope. Experiments were started after allowing the cells to settle for about 15–20 min.

Assessment of direct cell death

Phosphatidylserine exposure and cell death were assessed by FACS analysis of Annexin V- (Annexin V FLUOS, Roche Applied Science, #11828681001) and PI-stained cells. Briefly, 1 × 10^5 target cells/well (190 µL/well) were seeded in 96-well plates and incubated with mAb (10 µg/mL) for 24 h (untreated samples were used as negative control). Cells were then washed with Annexin V binding buffer (10 mM HEPES/NaOH, pH7.4, 140 mM NACl, 2.5 mM $CaCl_2$), stained with Annexin V FITC for 15 min at room temperature in the dark, then washed again and re-suspended in Annexin V binding buffer (200 µL/well) containing PI (0.3 µg/mL, Sigmal Aldrich, #P4864). Samples were analyzed immediately on a BD FACSCanto^{TM} II.

Assessment of ADCC

Z138, Raji and SU-DHL4 target cells were harvested, washed, re-suspended in AIM V® medium (Life Technologies), and plated at a concentration of 3 × 10^4 cells/well. The respective antibody dilutions were added to cells and incubated for 10 min before addition of the effector cells (peripheral blood mononuclear effector cells, PBMCs). Effector (E) and target (T) cells were then incubated for 4 h at 37°C at an E:T ratio of 25:1 (triplicates for all

samples). Lactate dehydrogenase (LDH) release was measured using the LDH Cytotoxicity Detection Kit (Roche Applied Science, #11644793001). ADCC was calculated using the following formula:

$$\text{Percentage ADCC} = \left(\left[\frac{\text{sample release} - \text{spontaneous release}}{\text{maximal release} - \text{spontaneous release}} \right] \right) \times 100.$$

Spontaneous release (corresponding to target cells incubated with effector cells without antibody) was defined as 0% cytotoxicity; maximal release (corresponding to target cells lysed with 2% Triton X-100) was defined as 100% cytotoxicity. The average percentage of ADCC and standard deviations of the triplicates of each experiment were calculated.

Assessment of CDC

Target cells were plated in AIM V® medium at a density of 5 × 10^4 cells/well in flat-bottomed 96-well plates. Diluted antibody was added to cells 10 min before the addition of Low-Tox® Rabbit Complement preparation (Cedarlane Laboratories Ltd). CDC was estimated by measuring LDH release in cell supernatants 2 h after incubation at 37°C in 5% CO_2.

CDC was calculated using the following formula:

$$\text{Percentage CDC} = \left(\left[\frac{\text{sample release} - \text{spontaneous release}}{\text{maximal release} - \text{spontaneous release}} \right] \right) \times 100.$$

Spontaneous lysis (corresponding to LDH released by target cells in the absence of antibody) was defined as 0% CDC; maximal lysis (corresponding to target cells lysed with 0.66% Triton X-100) was defined as 100% CDC. The average percentage of CDC and standard deviations of the triplicates of each experiment were calculated.

Whole-blood B-cell depletion

Fresh blood from healthy volunteers was collected in heparin-containing syringes and subsequent B-cell depletion was performed and evaluated using established methods (1). In some cases, blood was centrifuged (600×*g*, 15 min). The plasma was collected and heat-inactivated (56°C, 45 min), cooled to 37°C, and added back to the blood sample. Control plasma samples that were not heat-inactivated were kept at 37°C before they were pooled back to the blood sample.

Subsequently, blood aliquots (280 µL/well) were placed in deep-well 96-well plates, supplemented with antibody dilutions (20 µL/well) and incubated for 24 h at 37°C in 5% CO_2

in a humidified cell incubator. After incubation, 35 µL blood aliquots were removed and incubated with anti-human CD45-PECy5, anti-human CD3-FITC, or anti-human CD19-PE (all BD Biosciences) for 15 min at room temperature (in the dark) to estimate the total, T- and B-lymphocyte populations, respectively. Subsequently, 200 µL/well of FACS lysis solution (BD Biosciences) was added to deplete erythrocytes and to fix cells prior to flow cytometry. Results were evaluated by gating 2×10^4 CD45-positive cells and determining the CD3-positive T-cell and CD19-positive B-cell populations therein.

B-cell depletion was evaluated using antibody-untreated samples as a 100% control and the following formula:

$$100 - \left(\left[\frac{100}{\text{B/T cell ratio in control}} \right] \right) \times \left(\left[\text{B/T cell ratio in sample containing antibody} \right] \right).$$

The average B-cell depletion and standard deviations of the triplicates of each experiment were calculated.