Nanoencapsulated rituximab mediates superior cellular immunity against metastatic B-cell lymphoma in a complement competent humanized mouse model

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Figure S1. Lymphoma progression in NSG xenograft mice. A series of in vivo bioluminescent images of a representative xenograft NSG mouse engrafted with luciferase-labeled 2F7-BR44 cells. 2F7-BR44 cells were transplanted to NSG mice (n=4) via tail vein (2x10⁶/animal). Representative bioluminescent images from the same animal were taken on IVIS Lumina II in vivo imaging system at weeks 1, 2, 3, and 4 post-xenograft. Sensitivity settings were adjusted at each time point to maintain 500-5,000 counts per pixel and assigned the same color scale for all time points.
Figure S2. Anti-lymphoma efficacy of RTX was enhanced in the presence of serum obtained from NSG-Hc1 mice. RTX-mediated cytotoxicity was assessed against 2F7-BR44 cells. 2F7-BR44 cells were cultured in 48-well plates (1x10^5/mL) for 24 hours in the presence of native RTX (20 µg/mL) and 25% volume of mouse serum obtained from NSG or NSG-Hc1 mice, as well as NSG-Hc1 serum with heat inactivation at 56ºC for 30 min. Cells cultured with PBS were used as negative controls. The same volumes of cell culture were taken, and absolute cell numbers of each condition were counted on MACSQuant® Analyzer 10. Experiments were repeated independently three times. Data are shown as means ± SDs. Statistical significance was calculated by one-tailed unpaired t-test with Welch’s correction. ns; not significant.
Figure S3. Cobra venom factor (CVF) treatment efficiently depletes the complement activity in NSG^Hct^ mice. NSG^Hct^ mice (n=4) were treated with 1mg/kg of CVF at Day 0, 6, 13, and 21 via intraperitoneal injection. Blood samples were collected before and 24h later at each treatment. Serum was separated from whole blood by clotting blood at room temperature for 1 hr and centrifuging the clot afterwards. A) Mouse complement concentration was tested by mouse complement C3 ELISA. B) Total complement activity was tested with CH50 (50% hemolytic complement) assay. Serum samples were serially diluted and incubated with red blood cells with hemolysin at 37°C for 30 minutes. The lysis of red blood cells was detected by reading the absorbance of the samples at 540 nm. The %lysis was calculated using the following formula: %lysis=(OD_{540test}-OD_{540blank})/(OD_{540total lysis}-OD_{540blank})x100%. The percentage of lysis was plotted with the serum dilution. The serum dilution required for 50% hemolysis was read out as the CH50 at each time point. The CH50 was normalized to Day 0 (Setpoint) and plotted as normalized CH50 (Test time point/Setpoint). The serum from NSG mice was included as negative control for the CH50 assay, but the complement activity was below the detection limit.
Figure S4. n-RTX\textsuperscript{CXCL13} improved lymphoma suppression at different stages of lymphoma progression in xenograft NSG mice. 2F7-BR44 cells were injected into NSG mice via the tail vein (2x10^6/animal) (n=3). Xenograft mice were treated with native RTX or nanoencapsulated RTX (n-RTX\textsuperscript{CXCL13}) via the retro-orbital vein at week 1 (Group I) or week 2 (Group II) after 2F7-BR44 injection (4 mg kg/day for 5 sequential days). A) Lymphoma progression was monitored weekly by bioluminescence imaging using an IVIS Lumina II In Vivo Imaging system. Sensitivity settings were adjusted at each time point to maintain 250-5,000 counts per pixel and assigned the same color scale for all time points. Boxes containing a red X represent deceased mice. B) BLI values in the whole body were compared between Group I and Group II. Data are shown means ± SDs. Statistical significance was calculated with means at all time points using a p-value. p-values were calculated by Wilcoxon matched-paired signed rank test. ns: not significant.
Figure S5. Two repeated experiments in xenograft NSG-Hc1 mice confirm improved lymphoma suppression by n-RTX<sub>CXCL13</sub>. 2F7-BR44 cells were injected into NSG-Hc1 mice via the tail vein (2x10^6/animal) (n=2). Xenograft mice were treated with native RTX or n-RTX<sub>CXCL13</sub> via the retro-orbital vein at week 1 (Group I) or week 2 (Group II) after 2F7-BR44 injection (4 mg kg/day for 5 sequential days). Lymphoma progression was monitored weekly by bioluminescence imaging using an IVIS Lumina II In Vivo Imaging system. Sensitivity settings were adjusted at each time point to maintain 250-5,000 counts per pixel and assigned the same color scale for all time points. Boxes containing a red X represent deceased mice. BLI values in the whole body were compared between Group I and Group II. Data are shown means ± SDs. Statistical significance was calculated with means at all time points using a p-value. p-values were calculated by Wilcoxon matched-paired test with Spearman’s rank correlation. ns: not significant.
Figure S6. Blood reconstitution of humanized BLT mice. Peripheral blood was collected from NSG-BLT and NSG-\textsuperscript{Hc1}-BLT mice 6 weeks after the humanization surgery. Blood samples were processed and stained with antibodies for monitoring immunophenotyping on flow cytometry.
Figure S7. Control nanocapsules containing Herceptin show no therapeutic efficacy in xenograft humanized mice. Humanized BLT mice of the NSG or NSG-Hc1 strain were administered 2F7-BR44 cells via the tail vein (2x10^6/animal). Xenograft mice were treated with nanoencapsulated Herceptin (n-HER\textsuperscript{CXCL13}) as a control treatment via retro-orbital vein injection (4 mg kg/day for 5 sequential days) at week 2 after 2F7-BR44 injection (red arrows). Lymphoma progression was monitored weekly by bioluminescence imaging using an IVIS Lumina II In Vivo Imaging system. Sensitivity settings were adjusted at each time point to maintain 150-5,000 counts per pixel and assigned the same color scale for all time points. Boxes containing a red X represent deceased mice.
Figure S8. The concentration of native or released RTX from nanocapsules in the plasma of NSG\textsuperscript{Hct1}-BLT mice. NSG\textsuperscript{Hct1}-BLT were administered native RTX (n=4) or n-RTX\textsuperscript{CXCL13} (n=4) via the retro-orbital vein (4 mg kg/day for 5 sequential days). Plasma samples were collected and measured for the concentration of free RTX (native or released from nanocapsules) by ELISA on days 1, 7 and 28. Data are shown as means ± SDs. Statistical significance was determined by one-tailed unpaired t-test with Welch’s correction. ns; not significant.
Figure S9. Experimental scheme of humanized BLT mouse. This figure depicts workflow and timeline of the xenograft and treatment discussed in this article.
Figure S10. n-Saporin<sup>anti-CD7</sup> mediates NK and T cell depletion. The numbers (%) in each panel show the population percentages in human CD45+ population for CD3, CD4, CD8<sup>high</sup>, CD8<sup>dim</sup> and CD19, and in CD3-/CD19- population for CD14 and CD56. n-Saporin and n-Saporin<sup>anti-CD7</sup> were prepared and stored at a concentration of 10ng/μL. 1x10<sup>6</sup> of human peripheral blood mononuclear cells (PBMCs) were treated with 10μL of PBS (untreated), n-Saporin, and n-Saporin<sup>anti-CD7</sup>. 