Effect of anti-CD20 antibody-induced B-cell depletion on the susceptibility to *Streptococcus pneumoniae* infections

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Disclosures

Giuseppe Ercoli, Elisa Ramos-Sevillano, Milda Folkmanaite, Geraldine Cambridge, and Jeremy Brown have nothing to disclose.

Maria Leandro received personal compensation from Genentech - Fees for consulting meeting on obinutuzumab trial in lupus nephritis.

Gisbert Weckbecker is an employee of Novartis.

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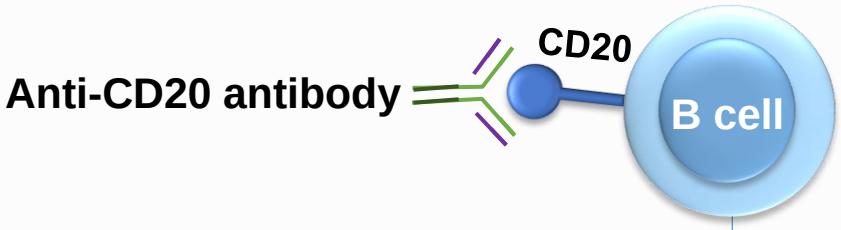
Background and objective

B-cell depletion with anti-CD20 therapies

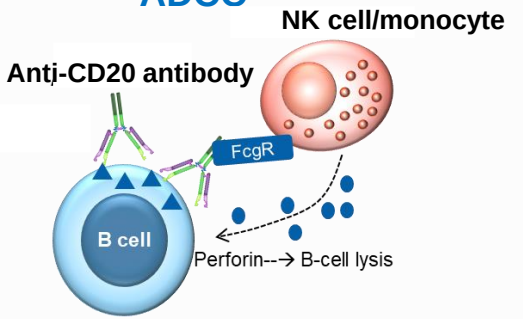
- Anti-CD20 therapy results in depletion of B cells and is effective in the treatment of B-cell malignancies and autoimmune disorders[1]

Mechanism of action of anti-CD20 therapies[2]

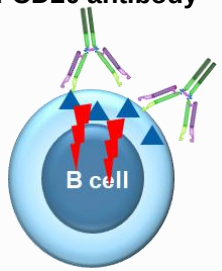


Anti-CD20 antibody binds to CD20 antigens expressed on the surface of B cells

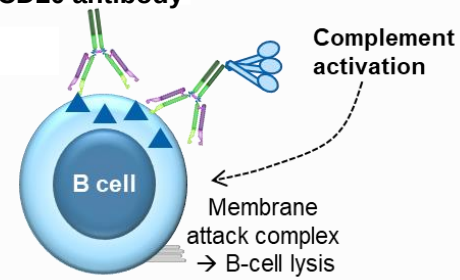
ADCC



Direct cell death



CDC



Objective

To investigate the effect of B-cell depletion on antibody-mediated immunity to *Streptococcus pneumoniae* in mice

ADCC, antibody-dependent cell-mediated cytotoxicity; CD, cluster of differentiation; CDC, complement-dependent cytotoxicity; FcgR, Fc gamma receptor; NK, natural killer

1. Montalvao F, et al. *J Clin Invest*. 2013;123: 5098–5103; 2. Klein C, et al. *mAbs*. 2013;5:22–33..

Methods

- In this analysis, a single dose of the anti-CD20 antibody (mIgG1) was administered to mice via two different routes of administration (i.v. or s.c.) to investigate the effect of B-cell depletion on the antibody-mediated immunity to *Streptococcus pneumoniae*

Animal	C57BL/6 female mice, aged 6 weeks
B-cell depletion	• B cells were depleted by administration of 50 µg/mouse of anti-CD20 (mIgG1, n=8 per group) either via i.v. or s.c. route of administration • Mice without B-cell depletion received the same concentration of isotype control antibody (s.c.)
Vaccination	• Mice were vaccinated with pneumococcal 13-valent conjugate vaccine Prevnar13® (20 µL/mouse i.p.) – **One-dose vaccination study:** One dose of Prevnar13 – **Two-dose vaccination study:** Two doses of Prevnar13 • Control animals (neither depleted nor vaccinated) received PBS (i.p.)

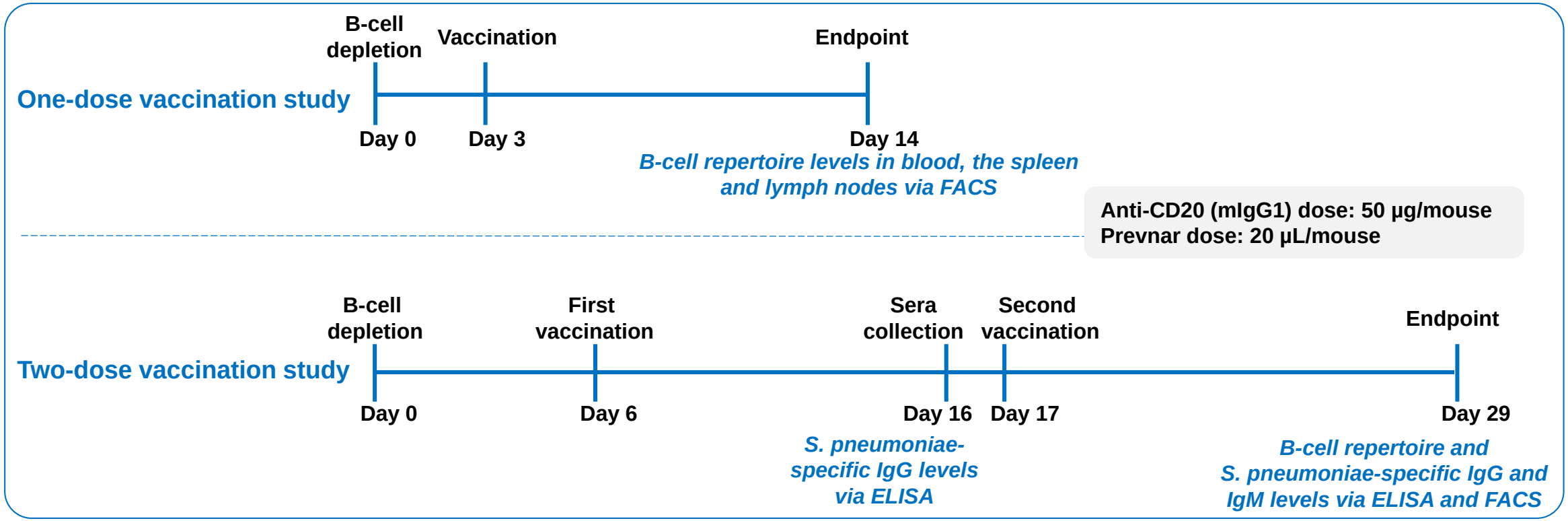
Treatment groups

Depleted (i.v.) (N=8)	**Anti-CD20 antibody (i.v.) + Vaccinated (i.p.)**
Depleted (s.c.) (N=8)	**Anti-CD20 antibody (s.c.) + Vaccinated (i.p.)**
Non-depleted (N=8)	**Isotype control antibody (s.c.) + Vaccinated (i.p.)**
Control (N=4)	**Isotype control antibody (s.c.) + Non-vaccinated (PBS; i.p.)**

i.p., intraperitoneal; i.v., intravenous; PBS, phosphate buffer saline; s.c., subcutaneous

Methods

B-cell depletion and vaccination study design



Assessments

- Serum pneumococcal-specific IgG levels were measured at Day 16 (after the first dose of the vaccine) and Day 29 (endpoint) by whole-cell ELISA on pneumococcus (TIGR4 strain)-coated plates
- Pneumococcal bacteria were incubated with mice sera (Day 29) and the antibody binding on the Pneumococcal surface was measured by flow cytometry (FACS); pneumococcal-specific IgG and IgM levels were measured by a serum deposition assay

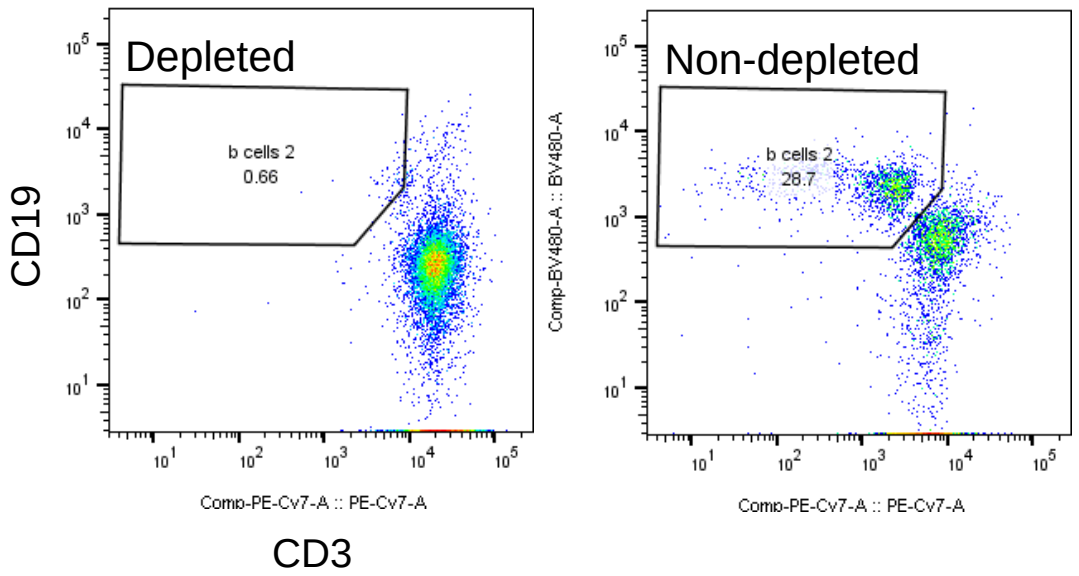
ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorting; Ig, immunoglobulin; i.p., intraperitoneal; i.v., intravenous; PBS, phosphate buffer saline; s.c., subcutaneous

One-dose vaccination study

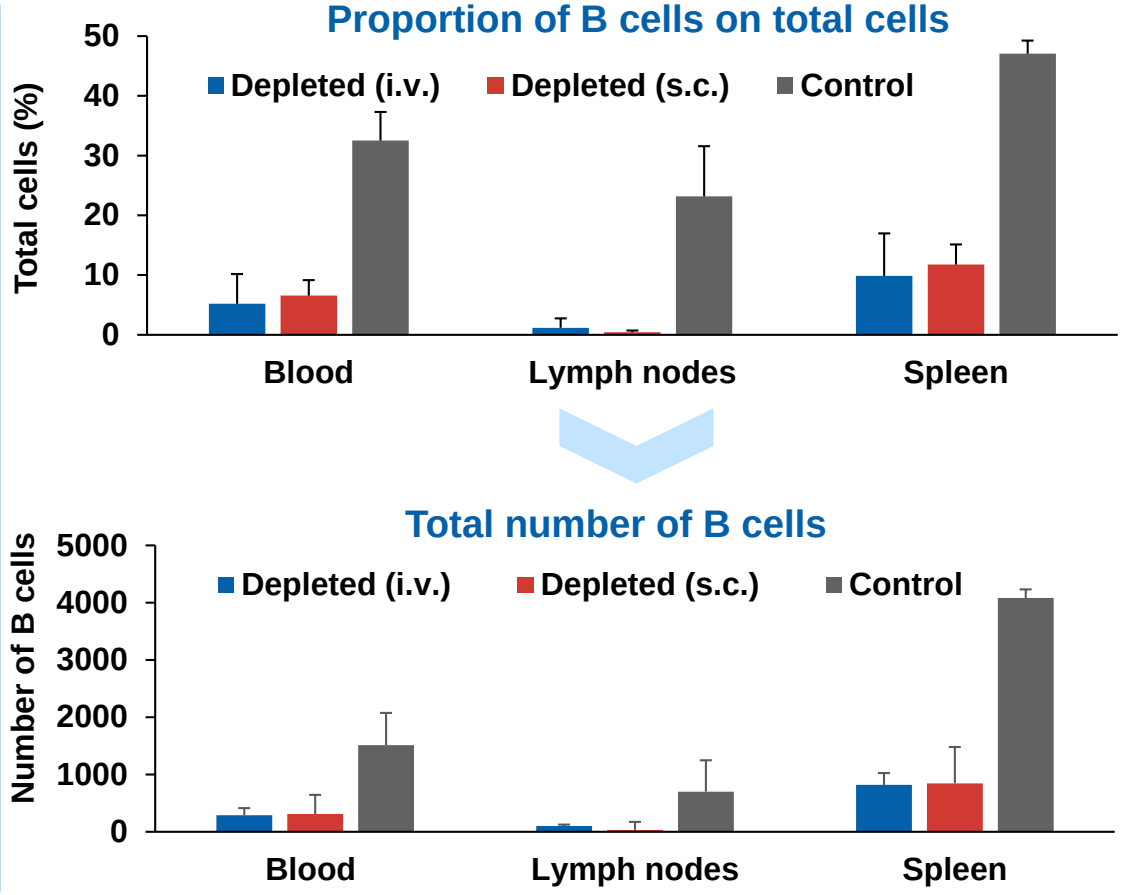
Total B-cell population at Day 14

- Blood, the spleen and lymph nodes were analyzed to observe the effect on the B-cell repertoire levels at Day 14

B-cells gating: CD19+
CD3-
CD11b-
Ly6G/C-



Spleen homogenates – Day 14



After 14 days of anti-CD20 antibody treatment, the number of remaining B cells was around 20% compared to untreated groups

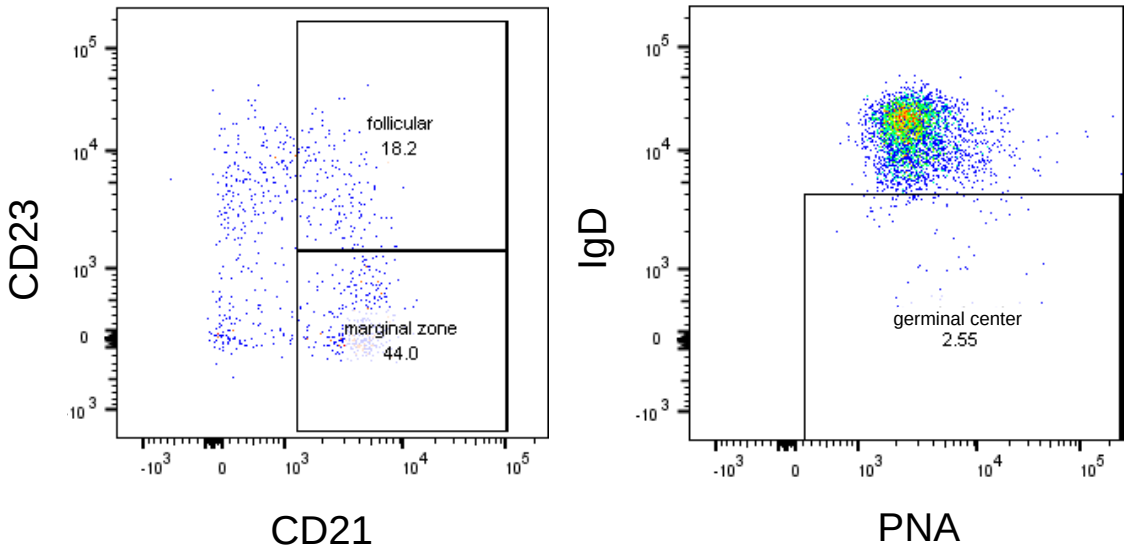
i.v., intravenous; s.c., subcutaneous

One-dose vaccination study

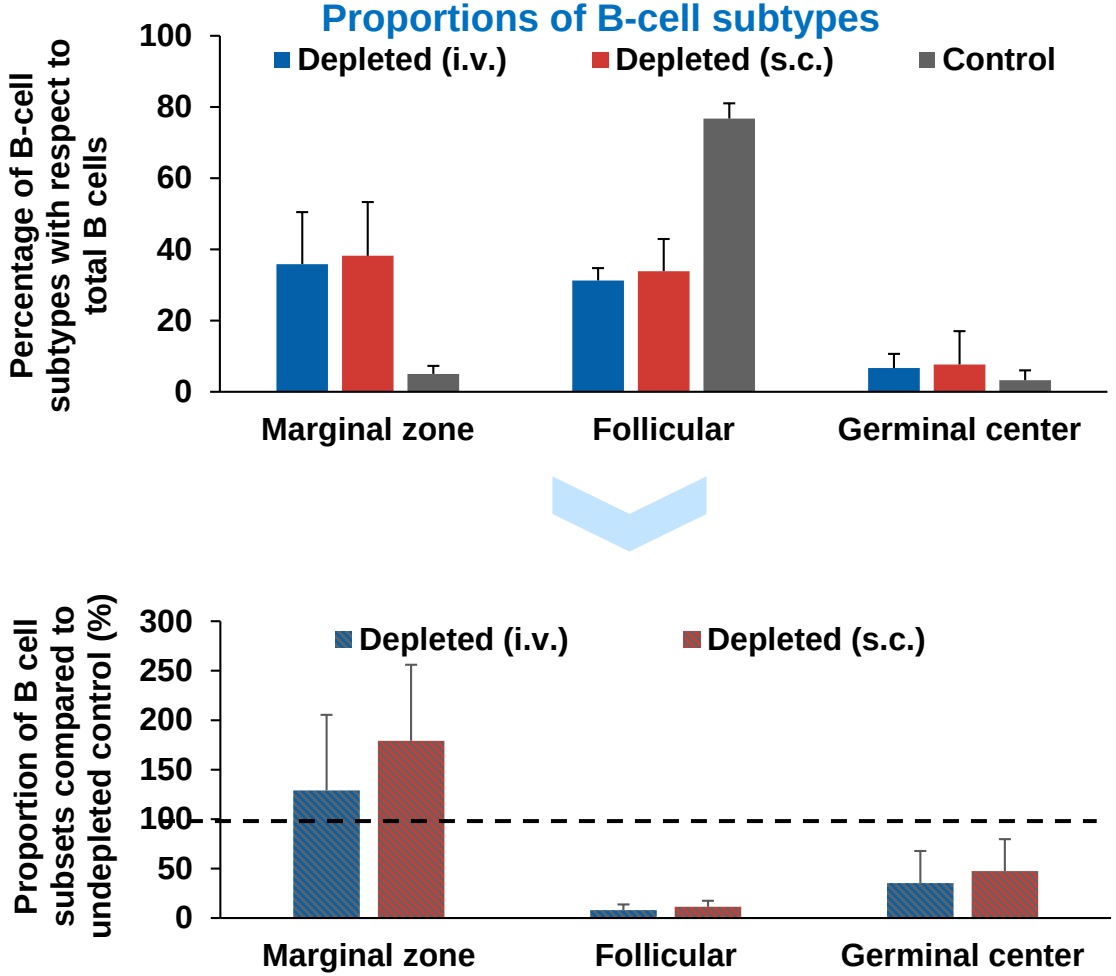
B-cell subtypes at Day 14

B cells subtypes gating:

Marginal zone	CD23-CD21+
Follicular	CD23+IgD+
Germinal center	PNA+IgD-



Spleen homogenates – Day 14



A marked decrease in the follicular B cell-subtypes was observed in both i.v. and s.c. anti-CD20 treatment groups

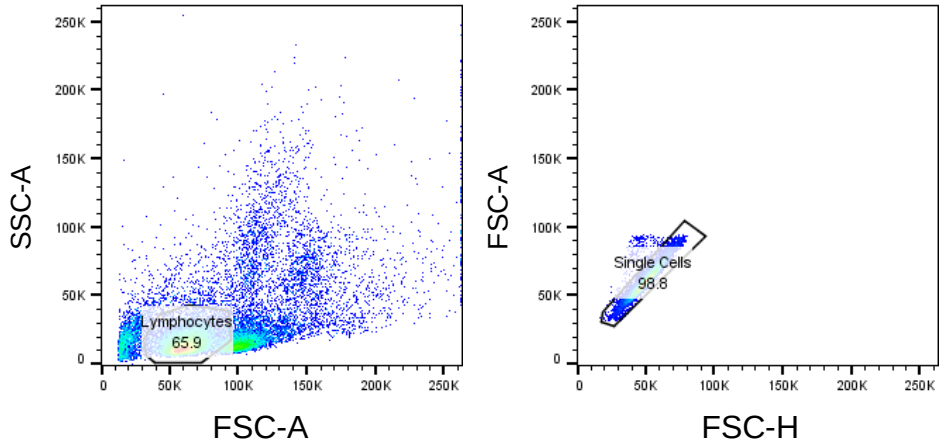
Ig, immunoglobulin; i.v., intravenous; s.c., subcutaneous

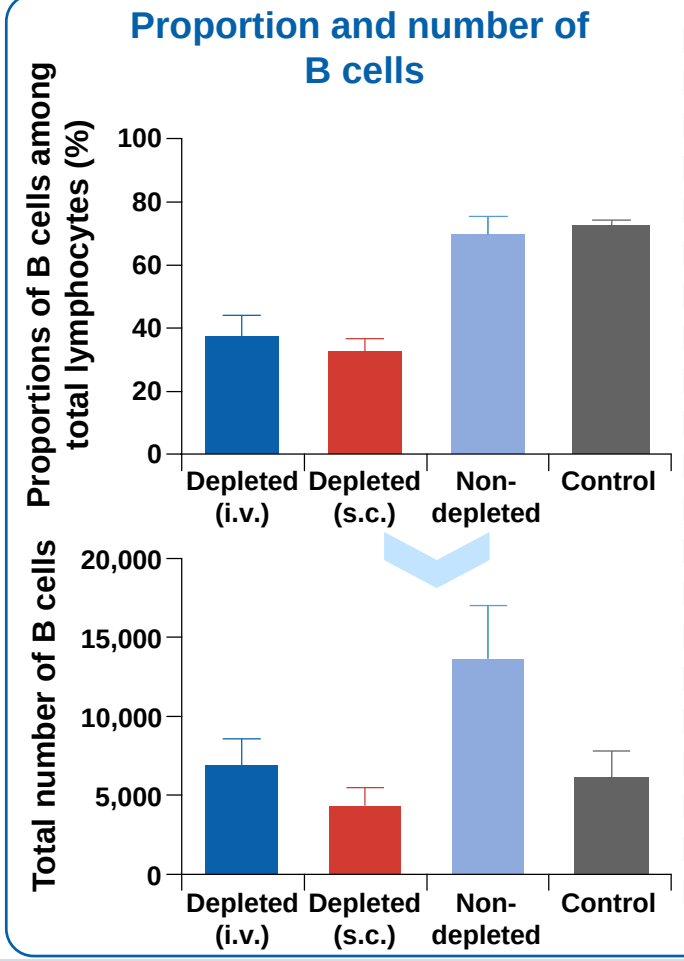
Two-dose vaccination study

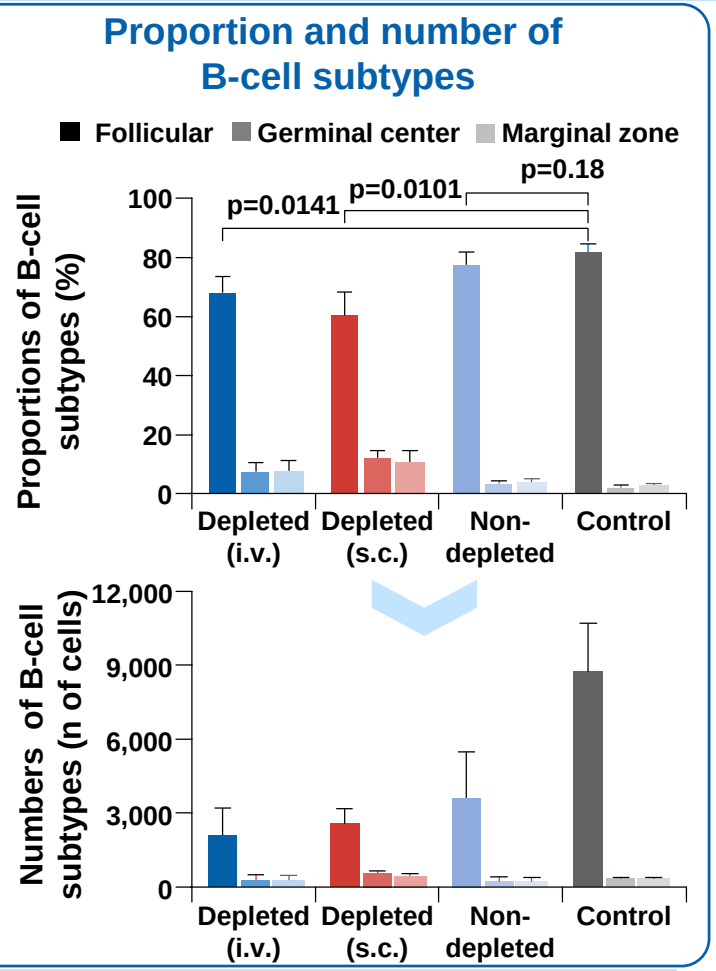
B-cell depletion at Day 29

Spleen homogenates

Flow cytometric gating strategy	
Lymphocyte subset	**Gating strategy**
B cells	**CD19^{+}**
Marginal zone	**CD19^{+}CD23^{+}CD21/CD35^{++}**
Germinal center	**CD19^{+}PNA^{+}CD95^{++}**
Follicular	**CD19^{+}CD23^{++}CD21/CD35^{+}**







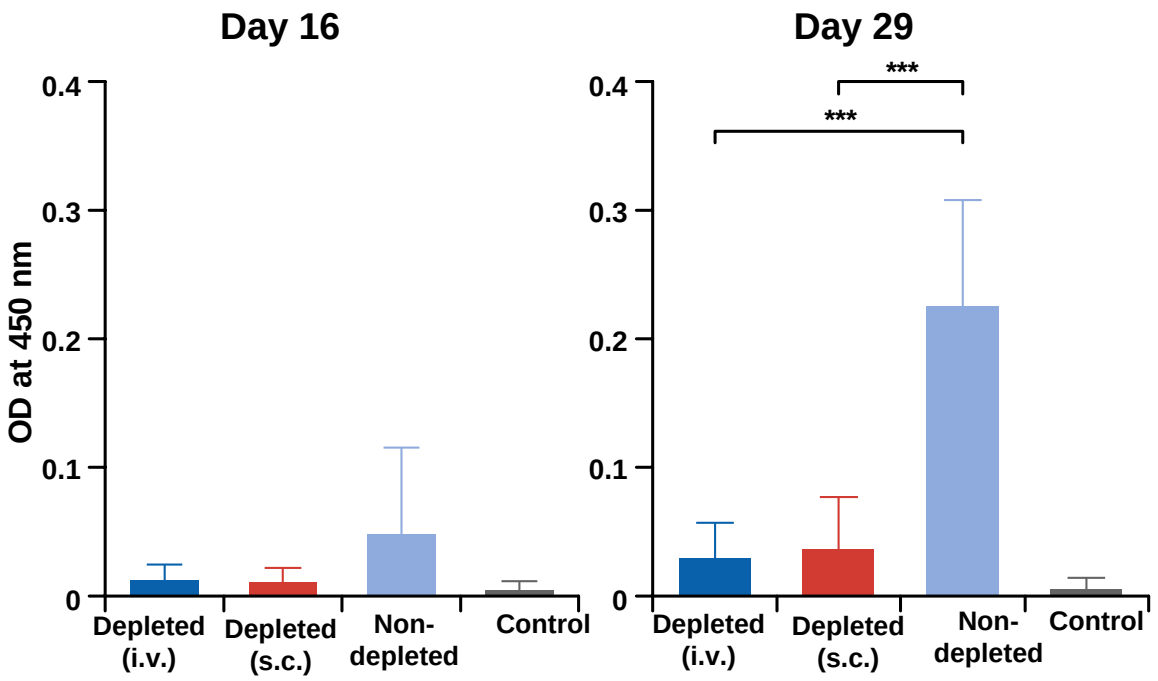
- **Only 60% of the B-cell population was reconstituted after 4 weeks of anti-CD20 treatment**
- **No significant differences in the B-cell subtypes were observed between the s.c. and i.v. anti-CD20 treatment groups**

FSC-A, forward scatter area; FSC-H, forward scatter height; i.v., intravenous; s.c., subcutaneous; SSC-A, side scatter area

Two-dose vaccination study

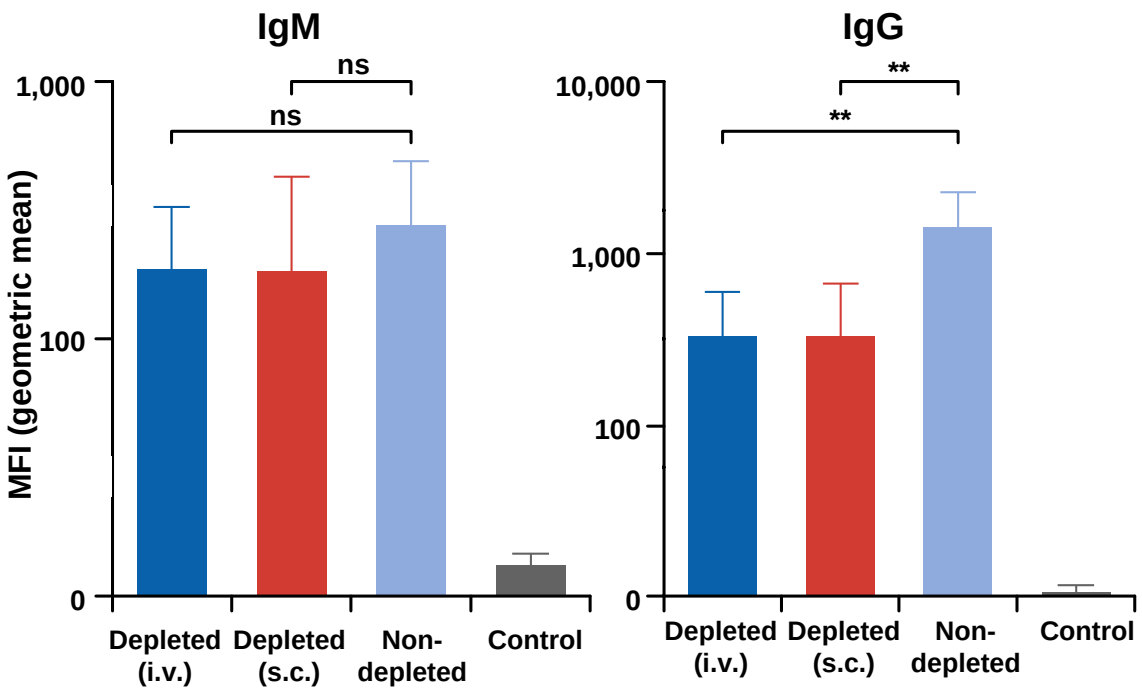
Pneumococcal-specific immunoglobulin levels (IgG/IgM)

Pneumococcal-specific IgG levels (ELISA)



- The level of IgG against pneumococcus in anti-CD20 treated mice (i.v. and s.c.) was comparable to the vaccinated group after the first dose of the vaccine (Day 16)
- No significant difference in the IgG level was observed between the s.c. and i.v. anti-CD20 treatment groups

Pneumococcal-specific IgG and IgM levels at Day 29 (FACS)



- Antibody deposition on the bacterial surface suggested a lower level of IgG binding to pneumococcus in the anti-CD20 treated groups (depleted samples) compared to the vaccinated samples; the IgM levels, however, were comparable

**p<0.01; ***p<0.0001;ELISA, enzyme linked immunosorbent assay; FACS, fluorescence-activated cell sorting; Ig, immunoglobulin; i.v., intravenous; OD, optical density; MFI, mean fluorescence intensity; ns, not significant; s.c., subcutaneous

Conclusions

- A marked decrease in the follicular B-cell subtypes was observed with anti-CD20 treatment, while the marginal zone and germinal center B-cell subtypes appeared to be less affected
  - The preservation of marginal zone and germinal center B-cell subtypes may play a role in preserving a rapid first-line and selective/diverse immune response to infections, respectively
- When administered at the same dose, the route of administration of anti-CD20 antibody does not influence the non-depleted B-cell populations
- The B-cell population was not fully reconstituted after 4 weeks of anti-CD20 treatment, demonstrating a prolonged pharmacodynamics effect
- B-cell depletion reduced the pneumococcal-specific IgG levels, while a reduction in IgM levels was much lower
  - Further clinical validation is warranted to understand the impact of B-cell depletion on the production of antigen specific IgG and IgM in response to vaccinations or infections

Thank you for your attention!

Ig, immunoglobulin