



Effect of anti-CD20 antibody-mediated B-cell depletion on susceptibility to *Pneumocystis* infection in mice

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Poster Number: P0322

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Poster Presentation at the 8th Joint ACTRIMS-ECTRIMS Meeting, MS Virtual 2020, September 11–13, 2020

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Disclosures

Guixiang Dai received research support from Novartis

Jay K. Kolls received personal compensation as a one time consultant from phPharma and research support from Novartis

Gisbert Weckbecker is an employee of Novartis

The study was funded by Novartis Pharma AG, Basel, Switzerland

Medical writing support was provided by **Vimal Kumar Muthyala** and **Uma Kundu** (employees of Novartis Healthcare Pvt. Ltd., Hyderabad, India). The final responsibility for the content lies with the authors



Background and objective

- *Pneumocystis* species are heterogeneous atypical microscopic fungi¹
- Immune response against *Pneumocystis* infection is thought to be mediated by B and T cells^{1,2}

Objective

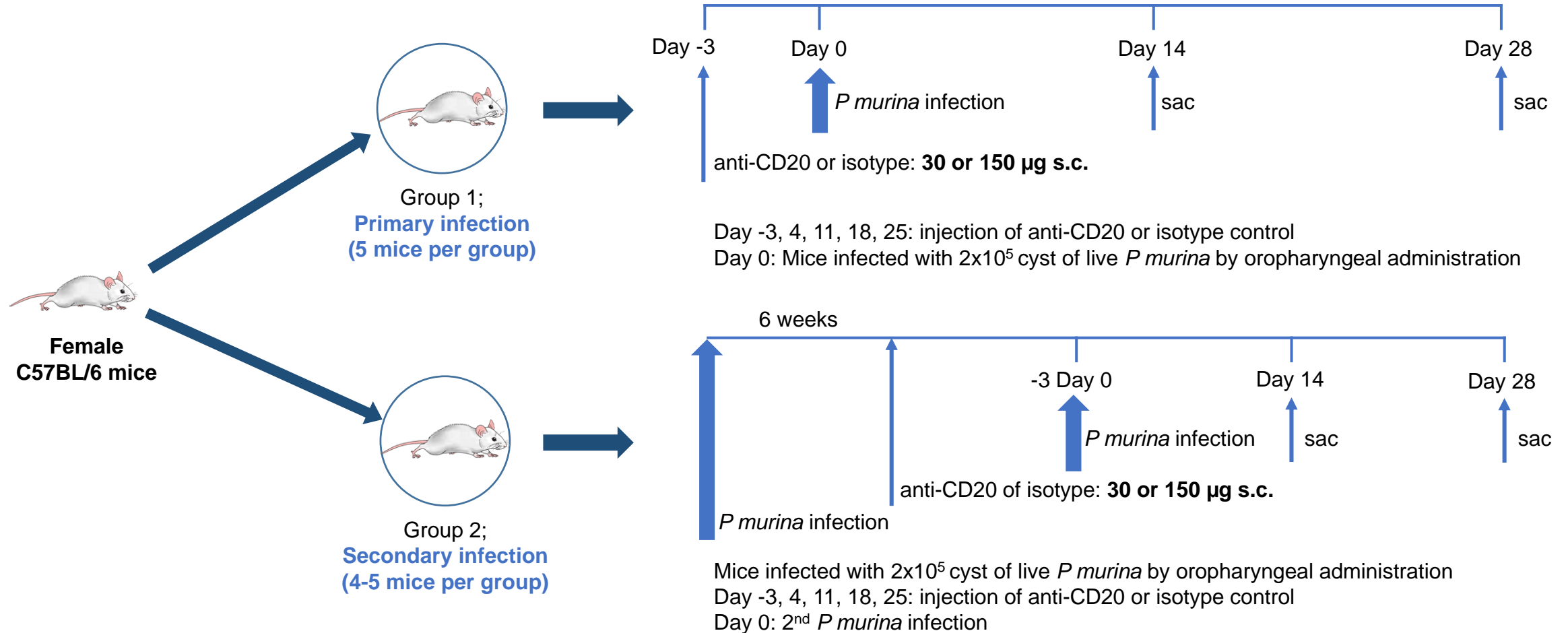
To investigate the effect of subcutaneous (s.c.) anti-CD20 antibody-induced B-cell depletion on T-cell responses and antibody generation against primary and secondary *Pneumocystis* infection in mice

1. Kelly MN and Shellito JE. *Future Microbiol.* 2010;5:43–65.
2. Hoyt TR, et al. *Infect Immun.* 2015;83:743–758.



Methods

Experimental design



Methods

Assessments and statistics

Assessments

- **Flow cytometry** was used to assay T and B cells in the lung at Days 14 and 28 after infection
- **Quantitative PCR** was used to determine lung fungal burden
- Serum IgG, IgE, and IgM levels were measured by **ELISA**

Statistics

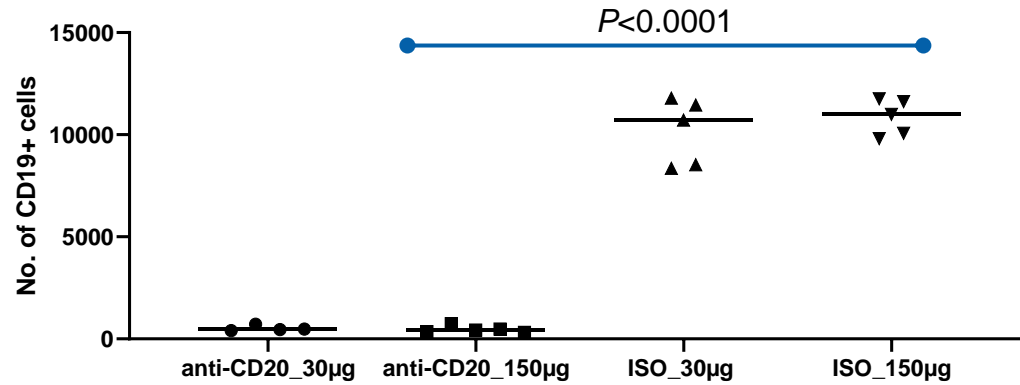
- Graphs were generated and statistical significances were analyzed using GraphPad Prism software
- *P* values of pairwise comparisons between groups of 2 were performed by a simple 2-tailed unpaired Student's *t* test, while groups of 3 or more used 1-way ANOVA with Tukey's multiple comparisons



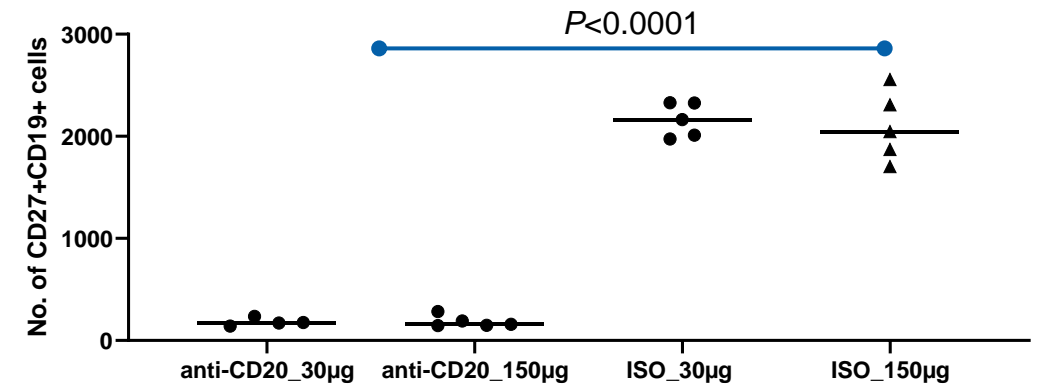
Effect of anti-CD20 antibody treatment on B cells in lungs

Primary *Pneumocystis* infection

CD19+ cells

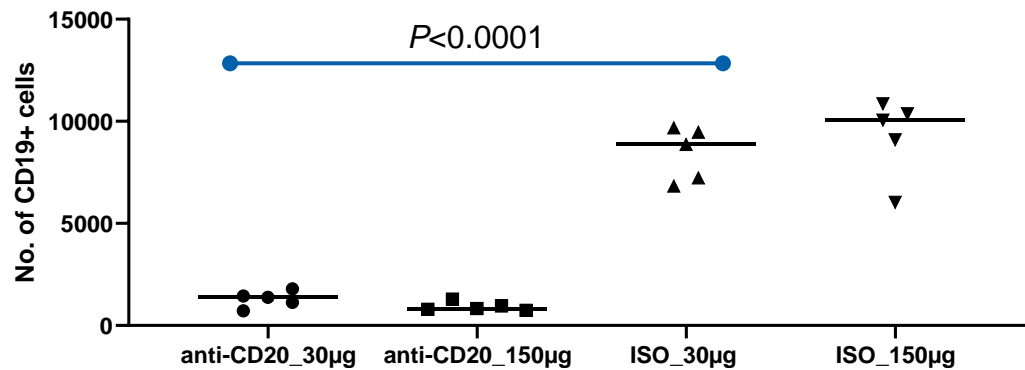


CD27+CD19+ cells

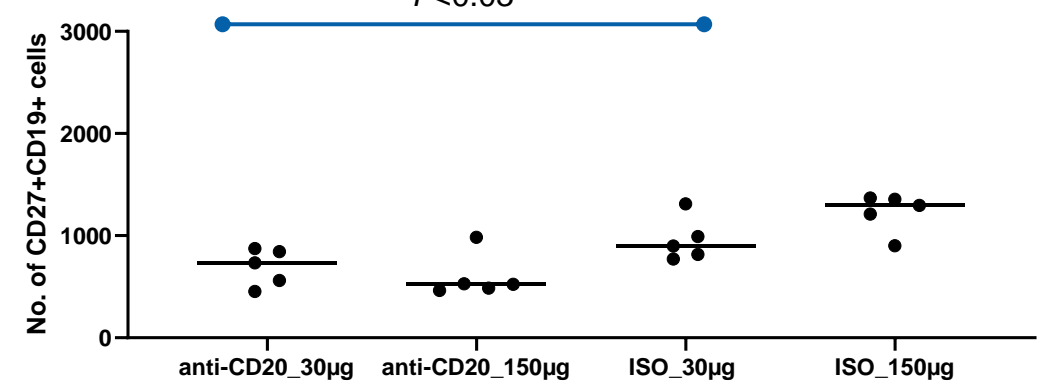


Day 14

CD19+ cells



CD27+CD19+ cells



Day 28

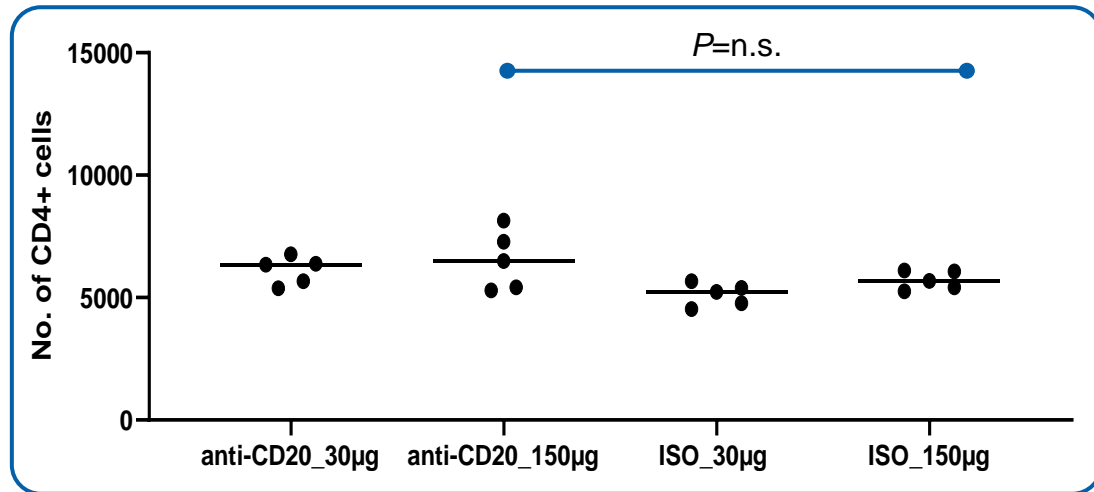
Anti-CD20 antibody treatment depleted both CD19+ and CD27+CD19+ cells, in the lung at Days 14 and 28



Effect of anti-CD20 antibody treatment on T cells at Day 14

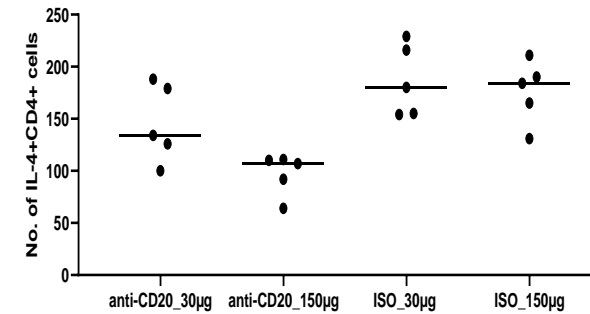
Primary *Pneumocystis* infection

CD4+ T cells

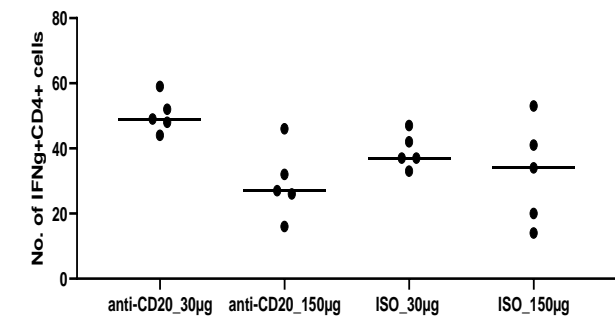


CD4+ T cell subsets

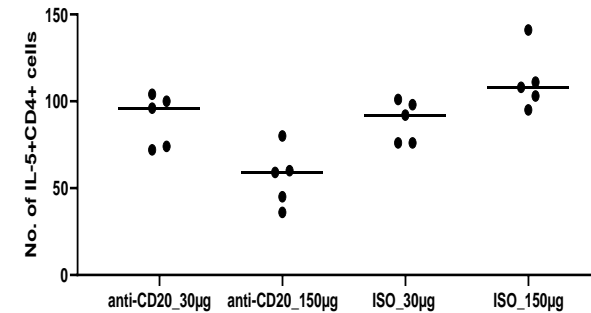
IL-4+CD4+



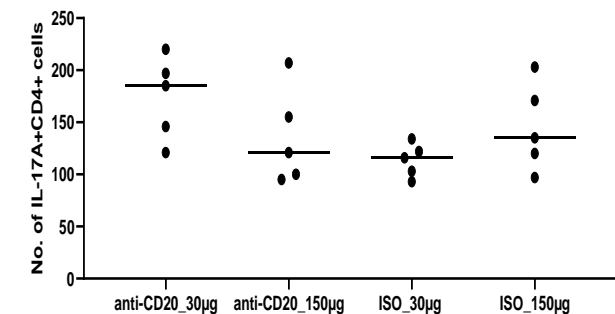
IFNγ+CD4+



IL-5+CD4+



IL-17A+CD4+



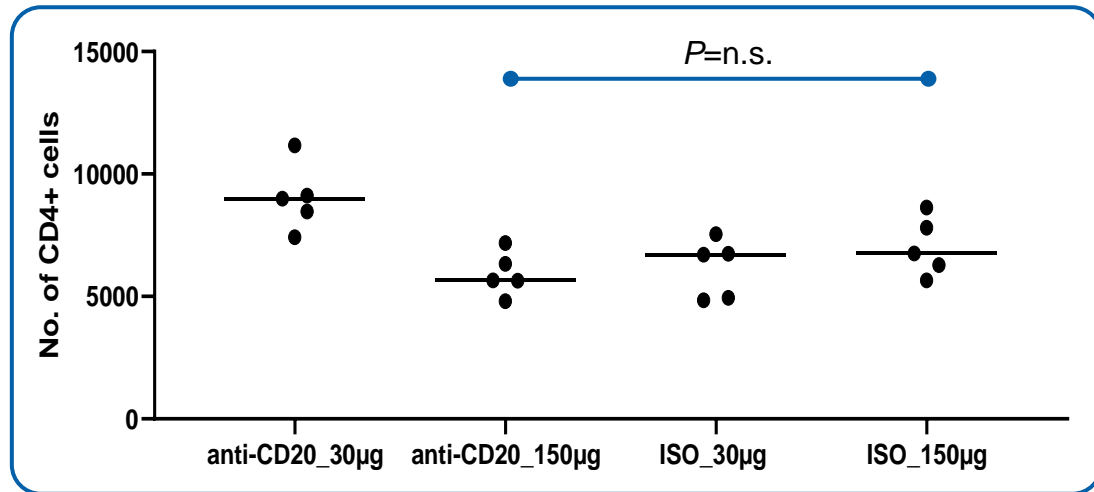
No significant differences in the number of lung CD4+, IFNγ+CD4+, IL-4+CD4+, IL-5+CD4+ and IL-17A+CD4+ cells between depleted and control mice after infection at Day 14



Effect of anti-CD20 antibody treatment on T cells at Day 28

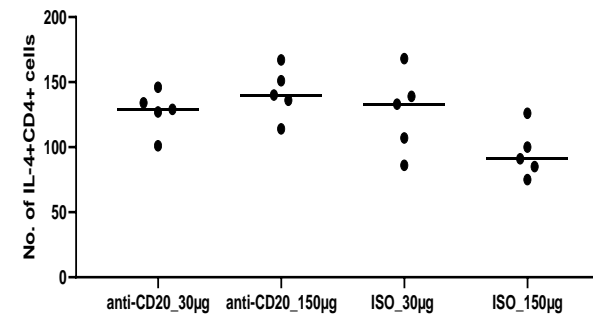
Primary *Pneumocystis* infection

CD4+ T cells

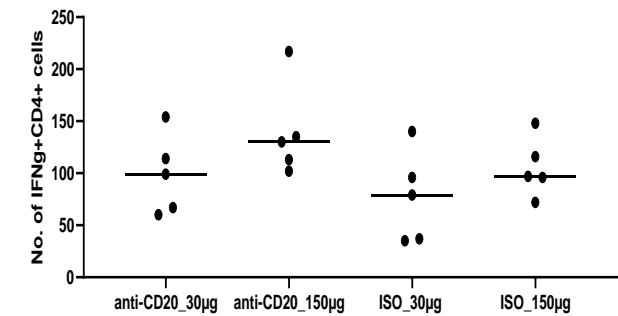


CD4+ T cell subsets

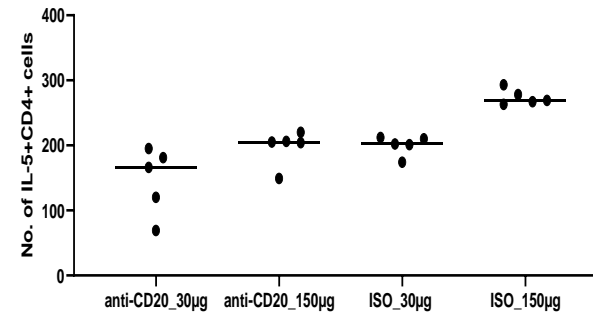
IL-4+CD4+



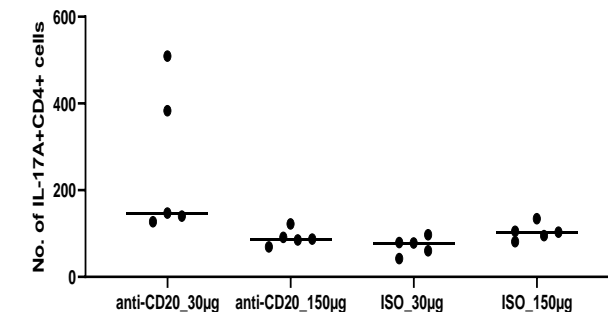
IFNγ+CD4+



IL-5+CD4+



IL-17A+CD4+



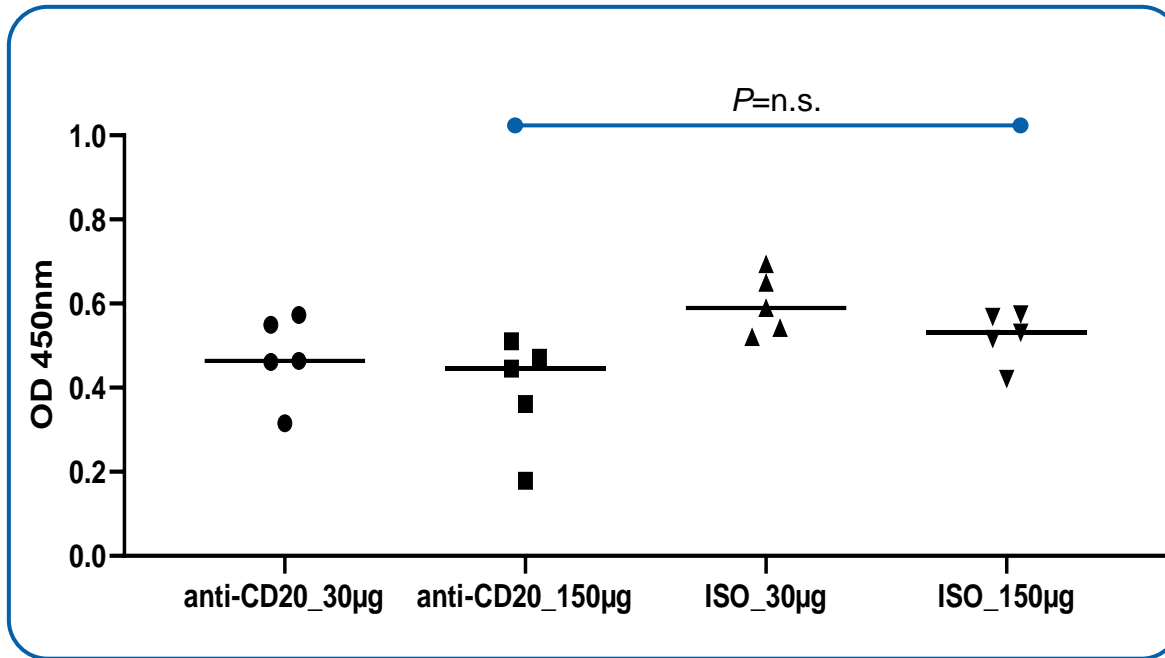
No significant differences in the number of lung CD4+, IFNγ+CD4+, IL-4+CD4+, IL-5+CD4+ and IL-17A+CD4+ cells between depleted and control mice after infection at Day 28



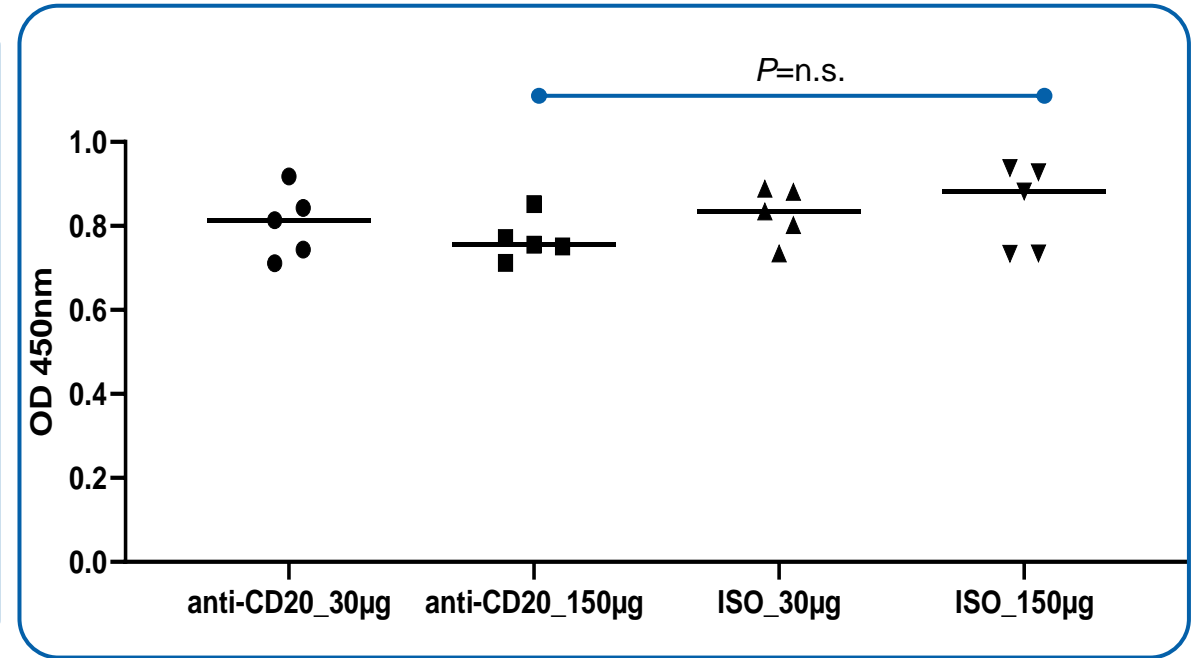
Effect of anti-CD20 antibody treatment on IgG in sera

Primary *Pneumocystis* infection

Day 14



Day 28



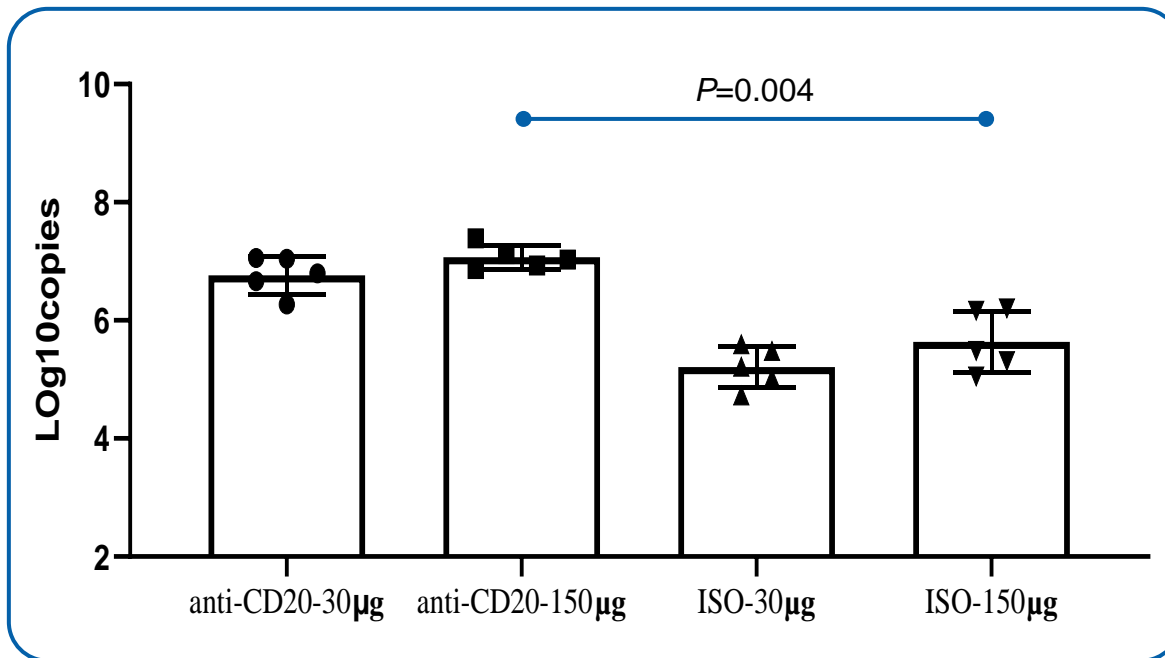
Anti-CD20 antibody treatment did not alter antigen-specific serum immunoglobulin levels compared with control mice



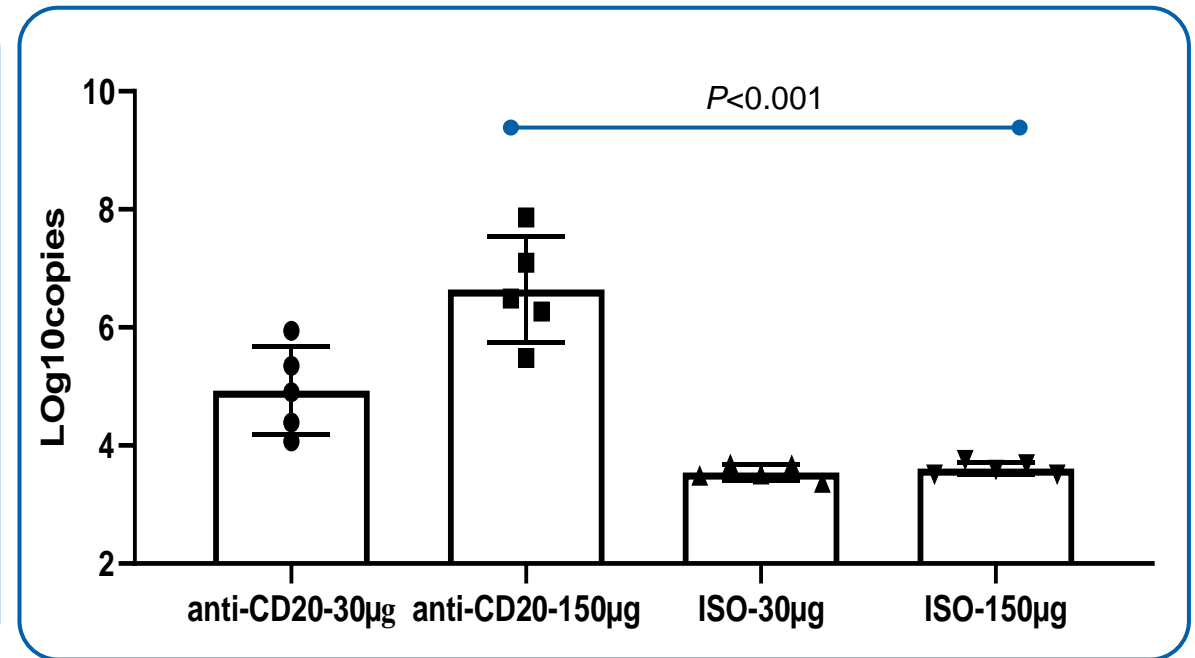
Effect of anti-CD20 antibody treatment on lung fungal burden

Primary *Pneumocystis* infection

Day 14



Day 28



Although anti-CD20 antibody-treatment impaired fungal clearance at Day 14 post-infection, fungal burden in the lungs was substantially reduced at Day 28 in both B-cell depleted and control mice

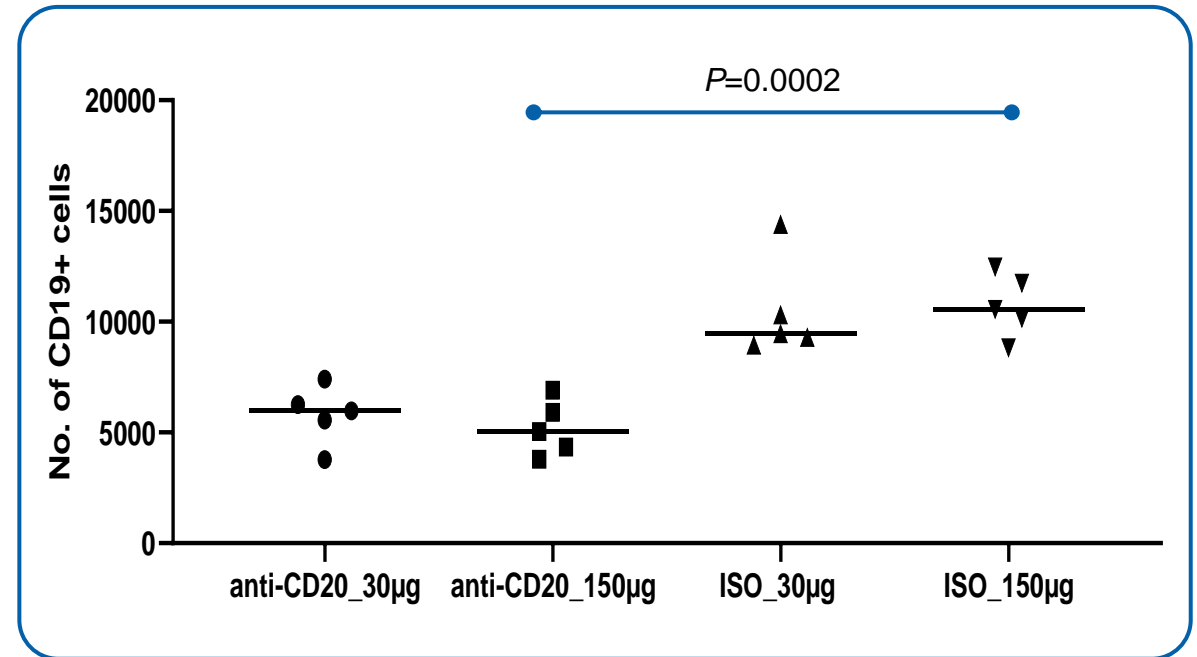


Effect of anti-CD20 antibody treatment on B cells

Secondary *Pneumocystis* infection

- Anti-CD20 antibody treatment partially depleted CD19+ but not other measured cell subsets including CD27+CD19+
- No significant differences in the number of lung CD4+, IFN γ +CD4+, IL-4+CD4+, IL-5+CD4+ and IL-17A+CD4+ cells between depleted and control mice after secondary infection
- Anti-CD20 antibody treatment did not alter antigen-specific serum immunoglobulin levels compared with control mice 14 days after re-infection
- The lung fungal burden was comparable between depleted and control mice 14 days after re-infection

CD19+ cells



Conclusions

Subcutaneous anti-CD20 antibody treatment may delay fungal clearance but it does not impair the ability of the host to clear *Pneumocystis* infection, irrespective of primary or secondary infection

Thank you

