Profiling of donor-specific immune effector signatures in response to rituximab in a human whole blood loop assay using blood from CLL patients

Eltahir M[1,2*], Fletcher E[3*], Dynesius L[3], Jarblad JL[3], Lord M[1], Olsson I[1], Zekarias M[1], Yu X[4], Cragg MS[4], Hammarström C[5], Hamberg Levedahl K[6,7], Höglund M[6], Ullenhag G[2,8], Mattsson M[2,6], Mangsbo SM[1]

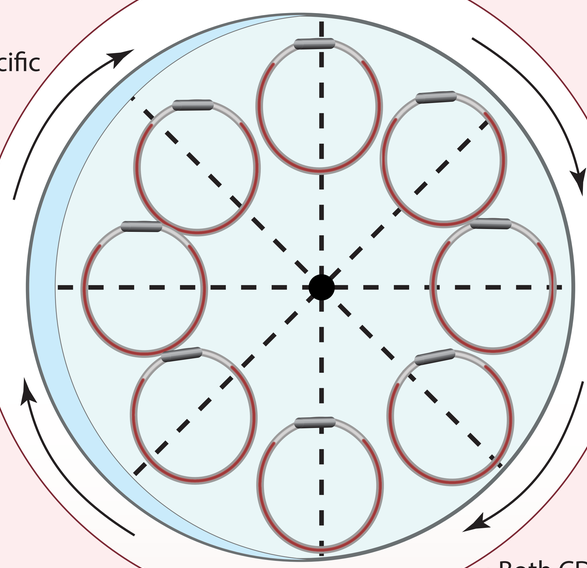
1. Department of Pharmaceutical Biosciences, Uppsala University, Sweden. 2. Department of Immunology Genetics and Pathology, Uppsala University, Sweden. 3. Immuneed AB, Sweden. 4. Antibody and Vaccine Group, Centre for Cancer immunology, University of Southampton, UK. 5. Clinical Trial Consultants AB, Sweden 6. Department of Haematology, Uppsala University Hospital. 7. Department of Public Health and Caring Sciences, Uppsala University, Sweden 8. Department of Oncology, Uppsala University Hospital, Sweden.* Shared co-authorship

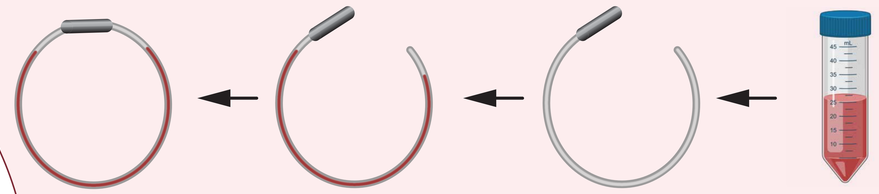
1.Introduction & aim

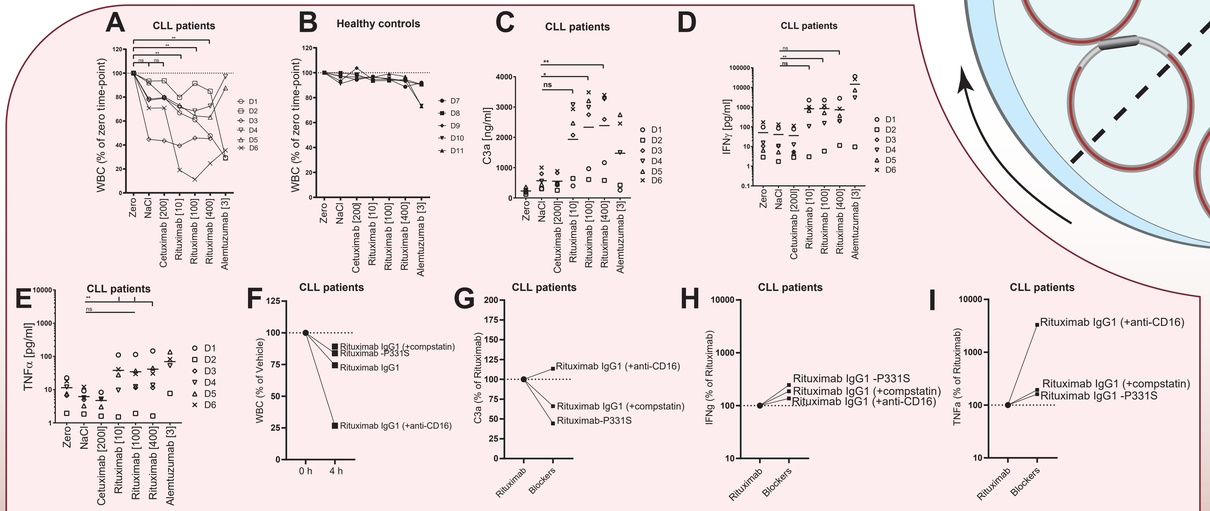
- Rituximab is widely used in the treatment of hematological malignancies, including chronic lymphoid leukemia (CLL)
- Rituximab induces cell lysis via several mechanisms, including complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) are the main mechanisms
- Some patients, especially those with high tumor burden, develop cytokine release syndrome (CRS) upon rituximab treatment
- CRS risk assessment Methods are limited
- We implemented the human whole blood "loop" system to study patient-specific CRS and immune responses to rituximab in blood-derived from CLL patients.

2. Methods

- Fresh human whole blood was collected from CLL patients or healthy controls
- The blood was added to surface heparinized PVS tubing
- Each loop was treated with the one therapeutic agent of interest
- The PVC tubings were connected via a metal connector to form a loop
- The blood loops were attached to a rotating wheel and incubated with the treatments for 4-6 hours at 37C
- Blood was collected and analyzed for cell counts, plasma cytokines and plasma complement split product



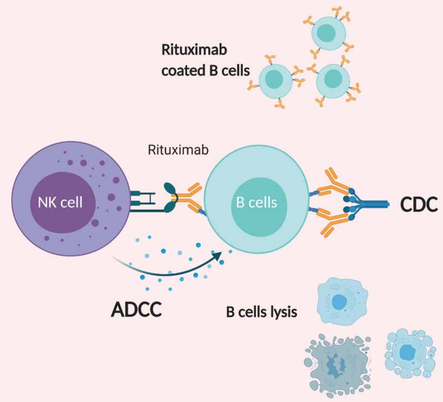




Results. Rituximab depletes white blood cell count in CLL blood but not healthy blood **(A-B)**. Rituximab also activates complement **(C)** and induce cytokine release **(D-E)** in CLL blood in the blood loop system. Blocking of rituximab induced complement- (compstatin/ rituximab P331S) or NK-mediated cytotoxicity (anti-CD16) alters the CLL B cell killing **(F)**, complement activation **(G)**, and cytokine release **(H-I)**.

3. Results

- CLL derived patient blood was explored in a unique whole blood loop assay
- The CLL specific blood loop assay recapitulate target-specific CRS
- Both CDC and ADCC profiling is possible in the CLL specific blood loop model
- There are donor-specific responses to rituximab infusion in terms of MOA. Some patients have a CDC-dominant response. Others have ADCC-dominant response
- ADCC or CDC block skews the immune response towards one another and alters B cell depletion



4. Conclusions