Leveraging Donor-Derived, in vitro PBMC Assay to Model B cell Depletion and Test Efficacy of B Cell–Targeted Agents for Non-Hodgkin's Lymphoma Indications

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Background

- The current landscape of B-NHL treatment involves several B cell targeting therapeutics, such as Mosunetuzumab, Epcoritamab and Glofitamab, which have shown efficacy in the clinic.
- These therapeutics drive distinctive on-target activity but also enhance CRS and ICANS in patients[1].
- T-cell redirecting therapies, including current anti-CD3xCD20 Bispecific antibodies, can be effective as monotherapies or in combinations with chemotherapy or immunomodulatory agents, with the potential to increase the curative fraction in B-NHLs.
- Evaluating drug efficacy of B cell targeting therapeutics preclinically, using in vitro co-culture assays, can greatly enhance our understanding of these treatments, optimize their use, and potentially improve patient outcomes.

Therapy	Focus	Currently Approved	Future Landscape
T cell engaging/derived	**CART cells:** Engineered T cells targeting B cell antigens	Axicabtagene ciloleucel (2L+), Tisagenlecleucel (3L+), Lisocabtagene maraleucel (2L+)	Autologous CAR T: IMPT-314 (3L+), JNJ-90009530; Allogeneic CAR T-cells
	Bispecific Antibodies: CD3 engagers linking T cells to B cell targets (e.g. CD19, CD20)	Epcoritamab (3L+), Glofitamab (3L+)	Mosunetuzumab (3L+), Odronextamab (3L+), TNB-48 (1L+), Epcoritamab (1L+), Glofitamab + pola-R-CHP (1L+)
	Trispecific Antibodies: Potential for deeper/more durable responses vs CD3 bispecifics		P1T565 (3L+), JNJ-8543
NK/Myeloid engaging/derived	**Monoclonal Antibodies:** Fc-mediated engagement of NK/myeloid cells; enhanced ADCC/ADCP; cytotoxicity to malignant B cells	Rituximab (R-CHOP; 1L), Polatuzumab vedotin (pola-R-CHP; 1L, pola-BR; 3L+), Tafasitamab (tafa-LEN; 2L+), Loncastuximab (3L+)	Tafasitamab-LEN + R-CHOP (1L); Tafasitamab-LEN + rituximab + acalabrutinib (1L, chemotherapy-free)
	NK cells: unmodified NK, CAR NK **CAR Macrophages** Attractive cellular immunotherapy options; ADCC		CAR NK cells: AFM13 Ongoing development of CAR macrophage platforms that redirect macrophages, guide antigen-dependent phagocytosis, potentiate T-cell activity
	CD47/SIRPα axis: Innate immune checkpoint; promotes anti-tumor phagocytosis		Magrolimab (2L+), Lemzoparlimab (3L+), TTI-621 (2L+), TTI-622 (2L+), AO-176, Ligufalimab, Evorpacept, IMM01
Combination Strategies	**B cell surface marker combinations**	CD19: Tafasitamab, Loncastuximab tesirine; CD20: Rituximab; CD79b: Polatuzumab vedotin; CD20/CD3: Glofitamab, Mosunetuzumab, Epcoritamab, Odronextamab	Combining immunotherapies; combining immunotherapies with other modalities
	Immune Checkpoint Inhibition	Alternative checkpoints under exploration: TIGIT, LAG-3, TIM-3	Further trials across lines of therapy
	Small Molecules	Immunomodulators: lenalidomide; BTK inhibitors: ibrutinib	Next-generation CELMoDs; broader targeted therapy combinations

Figure 1. Emerging therapeutic landscape of NHL

Abstract

We present an optimizable, donor-derived PBMC assay to model B-cell depletion and evaluate B-cell targeted agents in vitro for Non-Hodgkin Lymphoma. Freshly isolated PBMCs preserve the circulating immune compartment, with primary B cells as targets and endogenous NK or T cells as effectors based on therapeutic mechanism. A flow cytometry-based readout (looking at phenotypes of B/T/NK cells) was employed to track pharmacodynamic responses, with CD22 expression used to quantify B cells, minimizing interference with CD20-binding agents. Rituximab (anti-CD20) was used as a positive control and presented here as proof of concept for assay validation. By maintaining native effector:target ratios, the assay enables dose response assessment, comparative screening, and early efficacy readouts in a more physiologically relevant context. Across donors, it supports exploration of mechanisms of action and correlations between NK and T cell mediated depletion, helping prioritize candidates and de-risk translational efforts.

Methods

Objective

- Optimize an in vitro co-culture assay that can aid in screening drug efficacy of B cell targeting therapeutics prior to in vivo studies
- Test and quantify B cell depletion in response to drug compounds on freshly isolated donor PBMCs
- Use flow cytometry to immunophenotype B cell, NK cell and T cell populations in assay samples

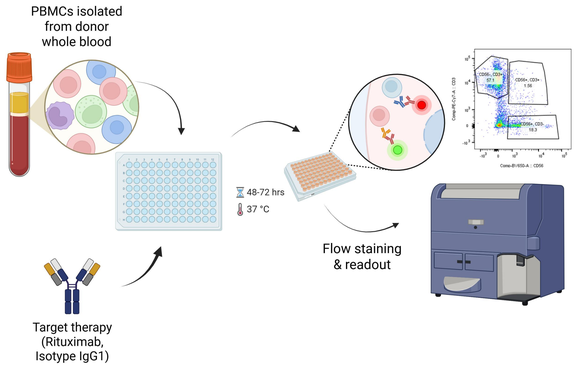

Figure 2. B cell Depletion Assay Schematic

Proof-of-Concept Drug

Rituximab, standard-of-care monoclonal αCD20 antibody[2,3] for NHL Indications

- Targets CD20 present on healthy and malignant B cells, mediating B cell depletion
- Fc portion from human IgG1 increases t1/2 of the drug
- Anti-tumor activities include Complement Dependent Cytotoxicity (CDC) and Antibody Dependent Cellular Cytotoxicity (ADCC, mediated by Fc-γ-receptor-bearing immune cells, including CD16 on NK cells)

Assay Workflow

- PBMCs, freshly isolated from healthy donors, were used in the assay, treated with Rituximab or fucosylated IgG1 (isotype control, Figure 2)
- Several B cell markers were tested to accurately enumerate B cells in vitro (Figure 3A)
- Timepoints and working doses for B cell depletion readout were optimized (Figure 3B, and Figure 4)
- Effector cell activation and proliferation were monitored and analyzed using flow cytometry (Figure 5,6)

Results

Figure 3. Validation of assay compatible B cell marker and timepoints for the B cell depletion assay

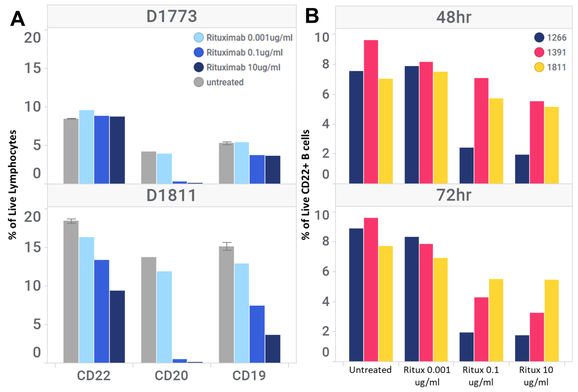

Figure 3A. Cryopreserved healthy donor PBMCs (D1773, D1811) were thawed and plated at 0.2e6/well, treated with 0.001,0.1, 10 ug/ml concentrations of rituximab and incubated for 48hrs at 37C following which they were stained with CD19, CD20 and CD22 antibodies and analyzed on flow cytometer. **3B**. Cryopreserved healthy donor PBMCs (D1266, D1391, D1811) were thawed and plated 0.2e6/well, treated with 0.001,0.1, 10 ug/ml concentrations of rituximab and incubated for 48, 72hrs at 37C following which they were stained with CD22 antibody and analyzed on flow cytometer

Figure 4. Dose response curve for Rituximab observed at 48hr showcases B cell depletion

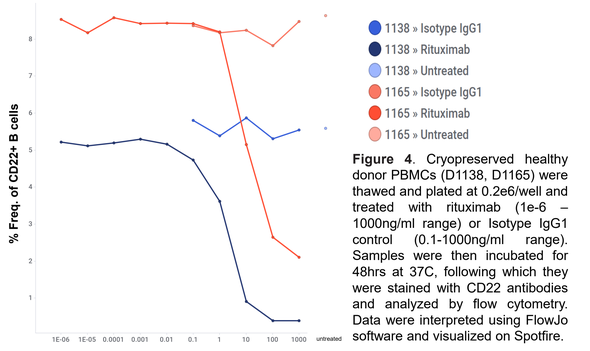

Figure 4. Cryopreserved healthy donor PBMCs (D1138, D1165) were thawed and plated at 0.2e6/well and treated with rituximab (1e-6 – 1000ng/ml range) or Isotype IgG1 control (0.1-1000ng/ml range). Samples were then incubated for 48hrs at 37C, following which they were stained with CD22 antibodies and analyzed by flow cytometry. Data were interpreted using FlowJo software and visualized on Spotfire.

Figure 5. Rituximab increases NK cell activation and proliferation compared to untreated control

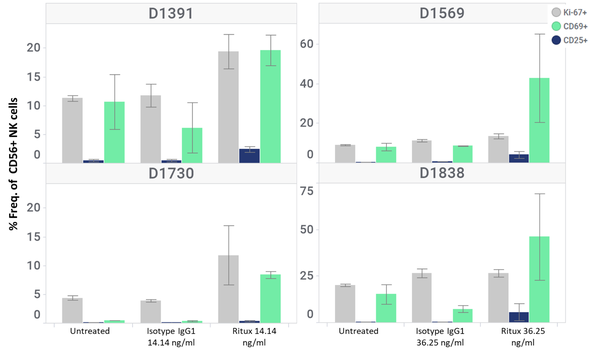

Figure 6. Rituximab increases T cell activation and proliferation compared to untreated control

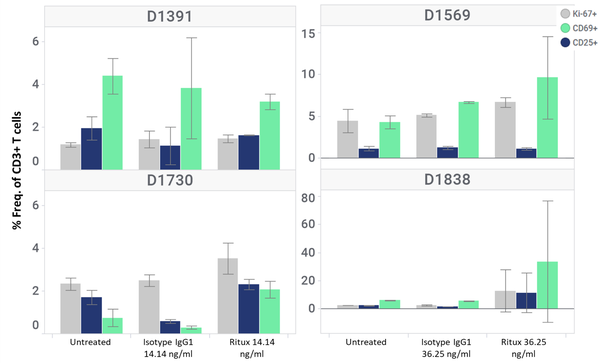

Figure 5,6. Healthy donor PBMCs (D1391, D1569, D1730, D1836) were freshly isolated using leucosep protocol and plated at 0.2e6 cells/well. Samples were then treated with 14.135 ng/ml (EC75) and 36.245ng/ml (EC90) of rituximab or IgG1 Isotype control and incubated for 48hrs at 37C. Samples were then stained with a customized flow panel including enumeration markers for B/NK/T cell as well as activation (CD25, CD69) and proliferation (Ki-67) markers, later analyzed by flow cytometry. Data were interpreted using FlowJo software and visualized on Spotfire.

Discussion

We have developed an in vitro, flow cytometry-based assay to monitor B cell depleting agents. The assay:

- preserves donor-specific effector:target ratios, enabling comparative dose response and early efficacy readouts;
- is configurable: timepoints, drug concentrations, and biomarker panels are tunable;
- is compatible with PBMCs, whole blood, or isolated immune subsets;
- is amenable to high-throughput screening.
- is reliable, as evidenced by the data with rituximab (shown to drive strong NK-cell activation/proliferation at 48hrs with modest T-cell changes, consistent with an ADCC-dominant mechanism);
- does not, however, capture prolonged exposure or in vivo microenvironmental effects.

References

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2. *Oncogene* **22,** 7359–7368 (2003). https://doi.org/10.1038/sj.onc.1206939
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