Advancing B-Cell Monitoring in B-Cell-Depleting Therapies for Autoimmune Disorders

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CellCarta

INTRODUCTION

Emerging B-cell–depleting therapies (BCDT)—including T-cell engagers, myeloid cell engagers, and CAR-T approaches—are transforming autoimmune disease (AID) treatment. Flow cytometry is a central biomarker platform in BCDT clinical trials to monitor of B-cell depletion and immune reconstitution, supporting pharmacodynamic assessment and evaluation of long-term immune reset. However, the lack of standardized definition for B-cell aplasia and the increasing complexity of multiparametric gating strategies for therapies targeting multiple B-cell epitopes can affect data comparability and interpretation across studies and therapeutic programs.

SCIENTIFIC & REGULATORY STRATEGY

Here we present our strategy to address gaps in B cell monitoring for BCDT clinical trials:

- How do we enable highly-sensitive, standardized, yet versatile B cell count measurement to track depth and duration of peripheral B cell aplasia across diversifying therapeutic modalities?
- How do we enable the use of peripheral B cell immune reset assessment as a surrogate readout for deep tissue depletion, therapeutic efficacy and predictor of long-term outcome?

PERIPHERAL B CELL ENUMERATION WITH A WHOLE BLOOD TBNK+CD20 ASSAY

- Commercially available TBNK kits traditionally used to track peripheral B cell counts rely solely on CD19 for the identification of B cells.
- To address clinical trials involving BCDT targeting CD19, we validated a **TBNK+CD20** enumeration assay in whole blood samples collected in **Cyto-Chex**™ blood collection tubes.
- The assay is deployed globally across four analytical labs and can support **secondary endpoint** use in clinical trials.

Table 1. Panel composition.

CD20	V450
CD19*	APC
CD4*	PE-Cy7
CD45*	PerCP-Cy5.5
CD16*/CD56*	PE
CD3*	FITC
CD8*	APC-Cy7

*Markers part of the BD Multitest™ 6-color TBNK reagent.

Table 2. Summarized performance parameters for B cell absolute count readouts.

Subset	Intra-Assay Precision (% CV)	Inter-Assay Precision (% CV)	LLOQ* (cells/µL)	Stability (RT and 2-8°C)
Total B cells	< 10%	<10%	1.4	168h
Total CD19+ B cells			1.4	
Total CD20+ B cells			2.1	
CD20+ CD19+ B cell			2.1	

*LLOQ determined according to CLSI H62 guidelines on minimum gated-events cut-off and precision.

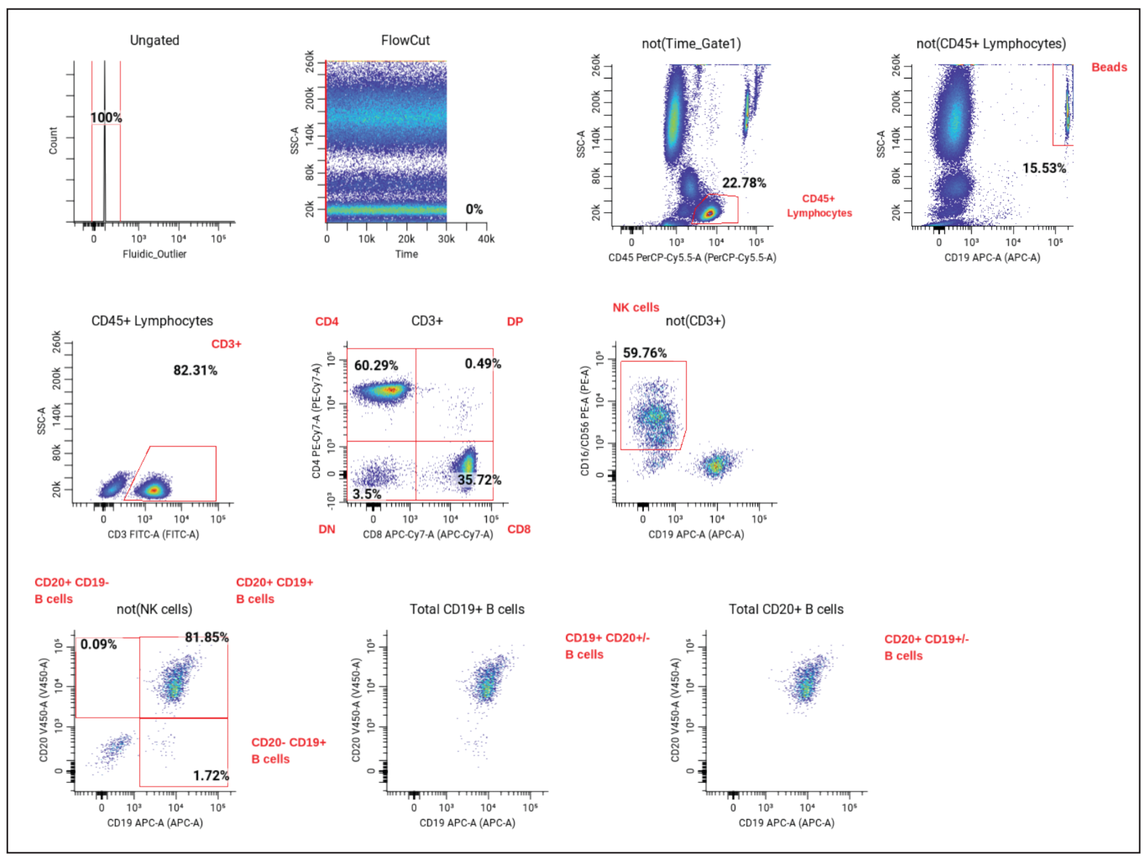


Figure 1. Gating strategy.

HIGH-SENSITIVITY B CELL ENUMERATION WITH A SINGLE-SITE IVD TBNK+CD20 ASSAY

- B cell aplasia threshold is not regulated/standardized and is dependent on the analytical sensitivity of the B cell enumeration assay used.
- To enable deeper measurement, we are currently optimizing the TBNK+CD20 enumeration assay to provide higher sensitivity in B cell enumeration.
- In parallel, we are aligning with the FDA on the proposed regulatory path and analytical validation plan to obtain single-site IVD regulatory clearance.

Technical Method Optimization *PoC LLOQ < 0.5 cell/µL (done)* → **Stage 1** *Initiation & Planning* → **Stage 2** *Development* → **Stage 3** *Design Verification* → **Stage 4** *Clinical Deployment*

510(k) FDA Pre-Submission *Regulatory alignment (ongoing)*

510(k) submission

HIGH-SENSITIVITY ALTERNATIVE B CELL GATING AND B CELL IMMUNE RESET ASSAYS

- To provide usage versatility across emerging therapeutic targets, we will validate a high-sensitivity B cell enumeration assay incorporating alternative B cell markers for flexible gating.
- To enable early evaluation of therapeutic efficacy and prediction of long-term outcome, we will also validate a high-sensitivity B cell immune reset assay.

Alternative B Cell Markers ← **B Cell Core Panel** → **B Cell Immune Reset Markers**

NETWORK WITH ME

CONCLUSION

Our approach towards deeper, broader and standardized quantification of peripheral B-cell dynamics can significantly strengthen pharmacodynamic assessment and efficacy prediction in B-cell–depleting therapeutic trials for autoimmune diseases across B cell targets and therapeutic modalities.