Effects of targeted therapy on flow cytometric testing in a tertiary care academic center

UC DAVIS HEALTH | Department of Pathology and Laboratory Medicine

Shafagh Parsazad, M.D., Hang Mieu Ha, D.O., Elham Vali Betts, M.D., Ananya Datta Mitra, M.D.

Introduction

Background: Therapeutic monoclonal antibodies (mAb) and adoptive immunotherapies are increasingly used in treating hematolymphoid neoplasms.

Diagnostic Challenge: These agents can interfere with flow cytometry assays by:

- Downregulating or causing loss of target antigens
- Competing with diagnostic antibodies for the same epitope
- Inducing lineage switching in malignant cells

Problem Statement: Despite recognition of these effects in the literature, no standardized guidelines exist for interpreting flow cytometry following therapeutic mAb or immunotherapy.

Objective: To review representative cases from our institutional experience and highlight strategies to mitigate these diagnostic limitations.

Methods

- **Design:** Retrospective case review (2016–2025).
- **Data Source:** Institutional hematopathology database.
- **Case Selection:**
  - ✓ B-ALL post-therapy: Relapsed/refractory B-ALL cases following
    - Anti-CD19 CAR T-cell therapy (CART)
    - Blinatumomab (Bispecific T-cell Engager, BiTE)
  - ✓ Plasma cell myeloma post-therapy: Cases following daratumumab (anti-CD38) treatment
- **Analysis:** Reviewed antigen expression profiles (CD19, CD38) by flow cytometry and correlated with clinical and morphologic data.

Acknowledgments
Special thanks to the Flow Cytometry Laboratory and Hematopathology teams for data collection and analysis.

Results

B-Lymphoblastic Leukemia (B-ALL)

Therapy	Total Cases Reviewed	CD19-Negative Relapse	Percentage
Blinatumomab (BiTE)	99	4	4.0%
CAR T-cell (anti-CD19)	8	1	12.5%

Plasma Cell Myeloma

Therapy	Total Cases Reviewed	Dim/Negative CD38 Relapse	Percentage
Daratumumab (anti-CD38)	35	17	48.6%

Key Observations:

✓ Loss of **CD19** expression observed in relapsed B-ALL post targeted therapy

✓ **Dim to negative CD38** plasma cell relapses occurred after daratumumab

✓ False light chain (kappa) restriction identified, though this is a known diagnostic pitfall

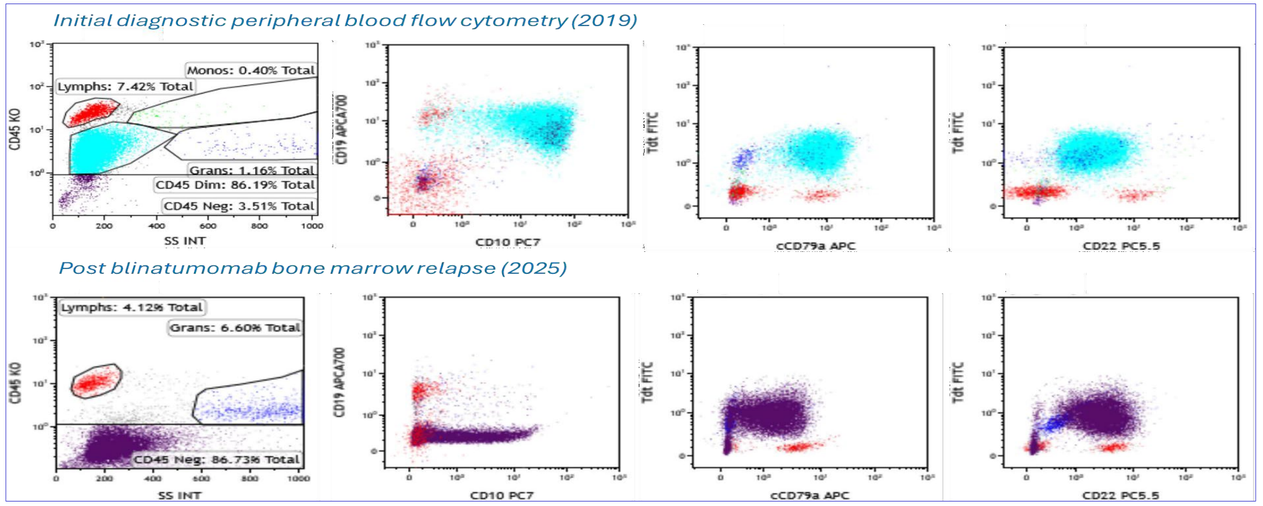

Figure: The top panel shows initial peripheral blood flow cytometry at diagnosis, where the blasts are CD45 dim and positive for CD19, CD10, TDT, CD22, and CD79a. Bottom panel shows bone marrow flow cytometry at relapse where blasts are essentially CD45 negative, CD10 negative, CD19 negative but retained other B cell markers (CD79a and CD22) and TDT.

Discussion

- Flow cytometry remains a powerful tool for detecting minimal residual disease (MRD) and distinguishing normal from neoplastic populations
- **Target antigen loss** following therapy significantly impacts MRD interpretation:
  - **CD19 loss** compromises detection of B-ALL clones post anti-CD19 therapy
  - **CD38 downregulation** may mask residual plasma cells, leading to potential under-diagnosis or misclassification
  - Consistent with our findings, reports in B-ALL and myeloma describe CD19-negative or antigen-modulated relapses after CD19-targeted therapy and daratumumab-related CD38 masking/downregulation, both of which can reduce the sensitivity of standard flow-based MRD assays
- **Clinical Implication:** Awareness of therapeutic history is essential for accurate gating and data interpretation

Conclusions

- Targeted therapies such as BiTE, CAR T-cell, and anti-CD38 antibodies alter surface antigen expression, pose diagnostic challenges in flow cytometry
- Awareness and correlation with clinical and morphologic findings are critical to prevent misinterpretation
- Recommended strategies:
  - Incorporate therapy-specific gating algorithms
  - Use additional markers (e.g., CD22, CD24, CD138, cytoplasmic kappa/lambda) to confirm lineage
  - Maintain close communication between clinicians, technologists, and hematopathologists
  - Careful review of patient's treatment plan
  - Comparison the original diagnostic flow cytometry to compare the markers expressed

Reference: (Mejstríková et al., 2017; Mikhailova et al., 2022; Oberle et al., 2017; Ng et al., 2021).