# CD20 re-emergence in a patient with diffuse large B-cell lymphoma who experienced a CD20-negative relapse after glofitamab treatment

Targeting the B-cell surface protein CD20 has played a transformative role in immunotherapy for diffuse large B-cell lymphoma (DLBCL) and other B-cell derived malignancies. While anti-CD20 monoclonal antibodies are integrated into treatment plans, bispecific antibodies (BsAb) co-targeting CD20 and CD3 have recently emerged as promising therapeutic options for patients with refractory or relapsed (R/R) lymphoma.[1] Glofitamab, a BsAb that binds CD20 bivalently and CD3 monovalently, has received approval for the treatment of R/R DLBCL in patients who have received 2 prior lines of therapy.[2] A phase II study in this population showed a 39% complete response rate with 78% of patients maintaining this response at 12 months.[3] While the efficacy of this BsAb is promising, many patients either do not respond or subsequently experience relapse. Several mechanisms at the tumor cell, T-cell, and extrinsic cellular levels may underlie resistance to glofitamab and other bispecific antibodies targeting CD20.[2] Loss of CD20 antigen expression on B cells due to genetic mutations, alternative splicing, or other mechanisms has been observed following CD20-targeted therapies.[4-5] These changes are often perceived as irreversible and the ensuing CD20-negative clonal evolution may compromise subsequent therapies that target the same antigen.

Whether CD20 expression can re-emerge in cases where antigen loss is not genetically determined remains uncertain. We report a case of CD20 re-emergence in a patient at second relapse of non-germinal center B-cell (non-GCB) subtype of DLBCL, who previously experienced loss of CD20 at first relapse post glofitamab therapy. This research was conducted in accordance with the ethical principles of the Declaration of Helsinki and in compliance with applicable institutional regulations and policies. The 71-year-old male patient consented to clinical trial participation, under which the biopsies were conducted. Subsequent biopsies were performed based on clinical indication.

The patient presented with cough and fatigue in 2023. His symptoms progressed to include dysphonia, anorexia, night sweats, and a 10-pound unintentional weight loss. Laboratory work-up was notable for hypercalcemia and computed tomography (CT) of the chest, abdomen, and pelvis revealed splenomegaly, diffuse lymphadenopathy, and bulky bilateral adrenal masses. A core needle biopsy of the left retroperitoneal mass led to a diagnosis of stage IV non-GCB DLBCL. Tumor cells were strongly positive for CD20 (Figure 1A). A positron emission tomography (PET) scan showed diffuse $^{18}$F-fluorodeoxyglucose (FDG) uptake in the bilateral adrenal masses and the portacaval lymph node anteriorly.

In early 2024, the patient was treated on a clinical trial of R-CHOP (rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone) and glofitamab added in cycle 3 based on residual detectable circulating tumor DNA after cycle 1.[6] A total of 6 doses of glofitamab were given over two months. The patient's treatment course was complicated by transient grade 3 cytokine release syndrome, which resolved, but otherwise treatment was well-tolerated.

An interim PET scan after cycle 4 showed reduced bilateral adrenal masses with residual hypermetabolic activity and resolution of his previously hypermetabolic aortocaval lymphadenopathy, consistent with interval treatment response. However, his post-treatment PET scan revealed a new hypermetabolic perirenal soft tissue nodularity adjacent to the left kidney. Pathology from a targeted core needle biopsy performed one week after the last glofitamab dose revealed primary refractory non-GCB DLBCL with complete loss of CD20 expression by immunohistochemistry (intracellular expression) and flow cytometry (cell surface expression) (Figure 1B, C, E).

In late 2024, the patient received bridging therapy with 2 cycles of Pola-ICE (polatuzumab, ifosfamide, carboplatin, and etoposide) and prophylactic high-dose methotrexate with a complete metabolic response and went on to receive lisocabtagene maraleucel. Complete metabolic response was demonstrated on a day-28 PET scan, but relapse of non-GCB DLBCL was noted on day 90 as a hypermetabolic right adrenal mass. Re-emergence of full and homogeneous CD20 positivity was noted on histopathological examination of a core needle biopsy of this site and surface expression was confirmed by flow cytometric analysis of the same sample (Figure 1D, F).

Subsequently, the patient underwent palliative radiation therapy of the right adrenal gland to acutely control disease progression and received an alternative investigational regimen containing the anti-CD20 antibody obinutuzumab. After 4 cycles, the patient achieved a complete metabolic response with an $^{18}$FDG-PET FDG-PET demonstrating a Deauville score of 2.

CD20 expression guides treatment options for patients with R/R B-cell lymphoma. Thus, patterns of expression are critical to understanding the long-term implications of immunotherapy. In patients with R/R B-cell non-Hodgkin lymphoma, a high rate of CD20 expression loss has been

described after receiving glofitamab therapy.[7] This is congruent with the patient's initial relapse with loss of CD20 expression after treatment which included glofitamab. While CD20 re-expression has been previously documented after

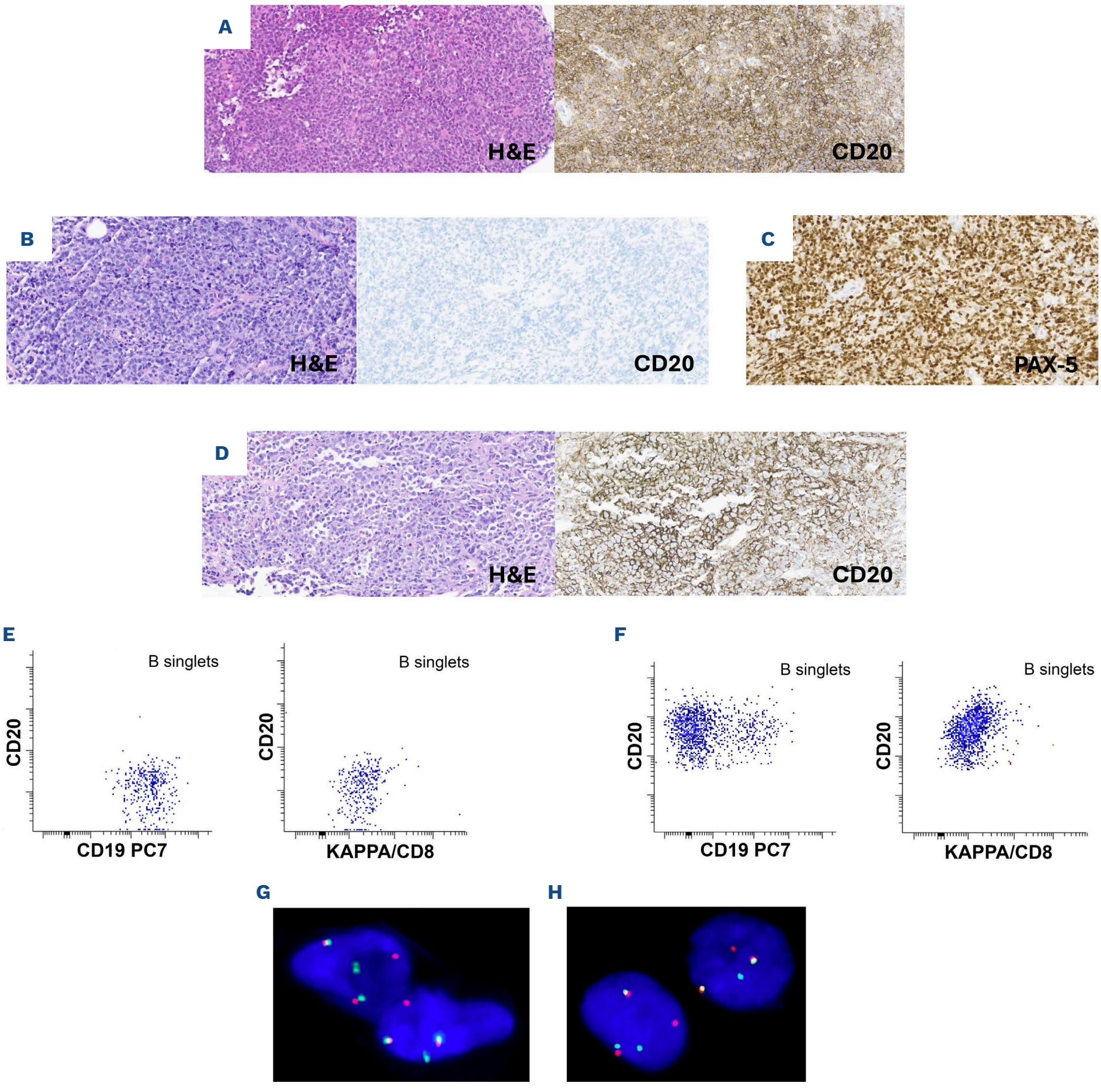



**Figure 1. CD20 stainings in core biopsies, flow cytometry, and FISH analysis at diagnosis and relapse timepoints.** Immunohistochemistry images and flow cytometry show (A) CD20 expression in the left retroperitoneal mass at initial non-germinal center B-cell subtype of diffuse large B-cell lymphoma diagnosis, (B) CD20 loss in the left perirenal mass at first relapse, (C) PAX-5 expression confirming B-cell lineage at first relapse, (D) CD20 re-emergence in the right adrenal mass at second relapse, (E) abnormal B-cell population negative for CD20, dim-positive for CD19, and κ-restricted at first relapse, and (F) abnormal B-cell population expressing CD20, variable CD19 expression (negative to dim), and κ-restricted at second relapse. Hematoxylin and eosin stained images corresponding to the CD20 stains are shown. Images taken at 400x magnification. FISH testing confirms BCL2 translocations in (G) the first relapse (CD20-negative) and (H) the second relapse (CD20-positive). BCL2 break part probes (Abbott Molecular, Des Plains, IL, USA) 5' and 3' probes are labeled in spectrum orange and green, respectively. Both cells show two normal fusions and one split 5' and 3' signal.

rituximab treatment for DLBCL, antigen expression evolution after BsAb is less well-defined.[8] This is the first reported case of strong, clinically actionable CD20 re-emergence in a patient following antigen loss after glofitamab treatment. Prior work has described the finding of a minute CD20-positive malignant subclone (in a predominantly CD20-negative relapse) in one patient with relapse after CD20-directed BsAb.[9] This illustrates heterogeneity and biological evolution conferring survival advantage as one explanation for a CD20-positive relapse, but it remains unclear whether and how complete re-emergence of CD20 expression can occur. While our clonal analysis of longitudinal evolution of CD20 expression is limited by potential site-specific variations resulting from different biopsy locations and unsuccessful molecular testing at all timepoints due to insufficient material, FISH analysis demonstrated *BCL2* (18q21.3) rearrangements in 90% of cells within the second (CD20-negative) and third (CD20-positive) biopsies (Figure 1G, H). This suggests a clonal relationship between samples at successive time points and raises the question of whether a CD20-negative relapse always indicates a uniform, permanent loss of the antigen. This question is highly relevant because, while CD20-negative relapse has been reported after rituximab-containing therapy, this is believed to be a relatively uncommon phenomenon[10] when contrasted with the frequency of CD20 loss following BsAb therapy.[11]

Finally, it should also be noted that immunohistochemistry antibodies like L26 detect epitopes in the cytoplasmic portion of the CD20 protein. A positive antibody stain indicates that the CD20 gene, *MS4A1*, is transcribed and translated, but does not indicate if the protein is transported or stabilized on the plasma membrane. In our patient, CD20 results were consistent between immunohistochemistry and flow cytometry for all biopsies, suggesting both initial complete loss and subsequent surface, clinically targetable CD20 re-expression.

As CD20 loss remains a major limitation of BsAb therapy, our report highlights the possibility of expanding therapeutic options and reconsidering CD20-directed immunotherapy after prior antigen loss and the importance of performing a biopsy at the time of relapse, when feasible, to guide treatment strategies.

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**Contributions**

PG and FL conceived the study, interpreted the data, and wrote the manuscript. AA collected and analyzed the data. ZE, MLP, MC, AM, HZ, PKG, AD and GS interpreted the data and managed the patient. YZ performed cytogenetic analyses. All authors approved the final version of the manuscript for publication.

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**Data-sharing statement**

The data supporting the findings of this study are not available due to confidentiality and privacy restrictions involving research participants.

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