Sequential loss of tumor surface antigens following chimeric antigen receptor T-cell therapies in diffuse large B-cell lymphoma

Diffuse large B-cell lymphoma (DLBCL) is the predominant subtype of Non-Hodgkin lymphoma (NHL) in adolescents and adults. Originating from a B-cell lineage, the neoplastic cells typically express pan-B- cell antigens including CD19, CD20 and CD22.[1] DLBCL can be highly curable, particularly with localized disease.[2] Treatment with multi-agent chemotherapy +/- rituximab yields event-free survival rates of greater than 85% in all children and in adults with early stage disease only. However, for patients with refractory or relapsed disease, or adults with high stage disease, outcomes are poor and response to chemotherapy based salvage attempts are limited.

Chimeric antigen receptor (CAR) T-cell therapy using an anti-CD19 binding domain has been shown to be effective in adults with lymphoma[3] and may represent an alternative treatment strategy in pediatric lymphoma, although experience in this younger age group is limited. Loss of the target antigen, as a mechanism of tumor escape following immunotherapy,[4-7] is an increasingly recognized phenomena which has limited the efficacy of immunotherapy in leukemia, however little is known about antigen loss in lymphoma.[3,8,9] We present a case of a pediatric patient with multiply relapsed advanced stage DLBCL who developed sequential antigen loss disease following sequential CAR immunotherapy. This case provides a proof of concept of antigen loss as a mechanism for relapse following immunotherapy in lymphomas, and highlights the need for repeat biopsy and flow cytometric analysis in guiding sequential immunotherapeutic interventions.

Case: A 12-year-old male with no prior medical history presented with a 3-month history of right thigh pain, bilateral neck lymphadenopathy, and a lower abdominal mass. Imaging studies demonstrated multiple conglomer-

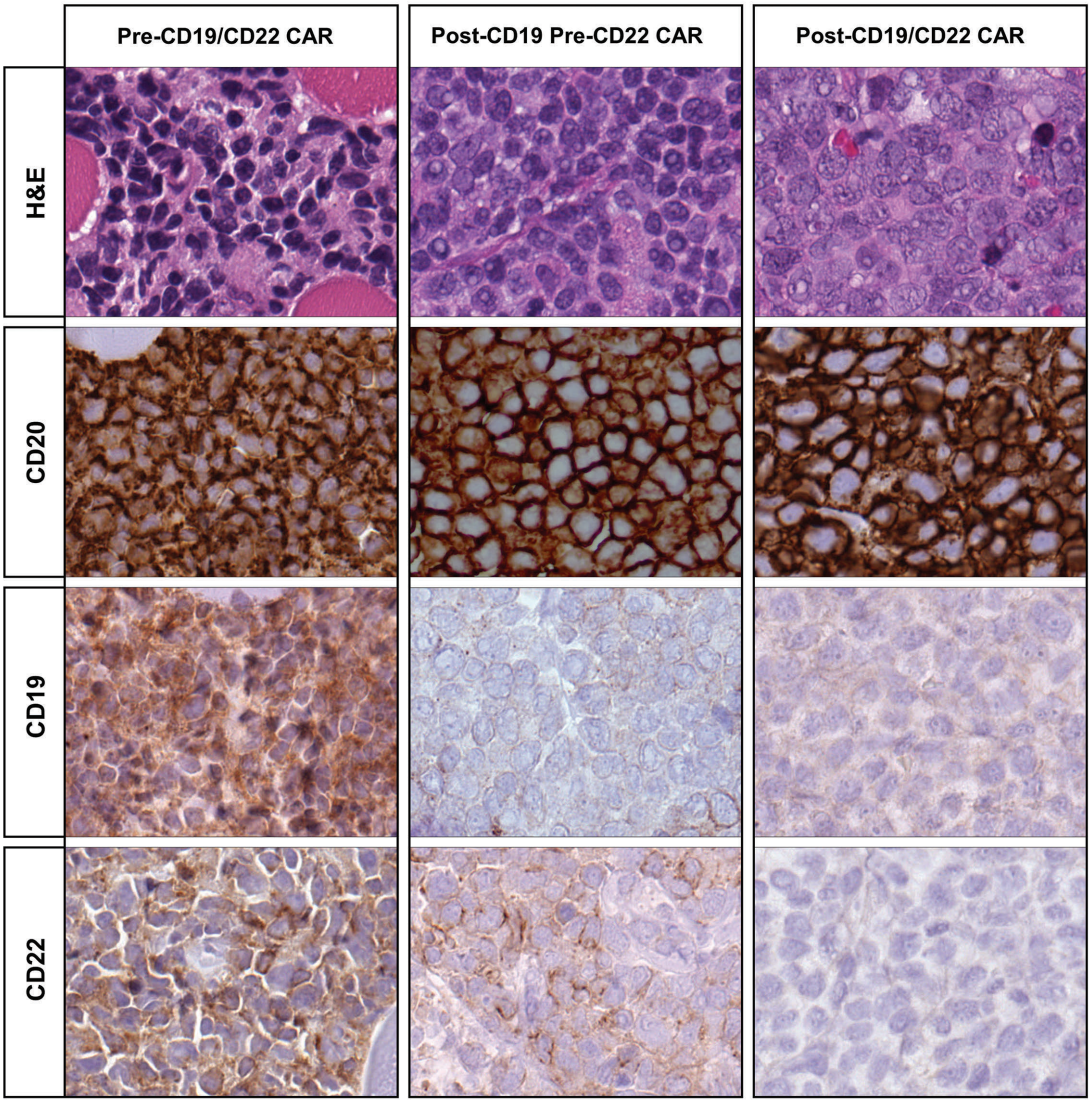

Figure 1. Histology of patient tumor sample with H&E, CD20, CD19 and CD22 staining at three varying time points. Pre-CAR therapy; Post-CD19 CAR therapy/Pre-CD22 CAR therapy and Post-CD22 CAR therapy demonstrating sequential loss of antigen expression over time.

ate masses in his neck, abdomen and retroperitoneum, with parameningeal extension into his lumbar spine. A biopsy of the cervical mass was performed and demonstrated a diffuse infiltrate by large atypical lymphoid cells with expression of CD20, CD19, CD22, and BCL2. This evaluation produced an initial diagnosis of stage IV DLBCL, germinal center B-cell subtype with CNS involvement. The patient received treatment on a pediatric protocol, which included a debulking pre-phase with cyclophosphamide, vincristine, and prednisone (COP), induction therapy with two cycles of R-COPADM (COP in addition to rituximab, doxorubicin, and high-dose methotrexate (HD MTX)), and two consolidation cycles with R-IT-CYVE (rituximab, intrathecal chemotherapy, high-dose cytarabine, and etoposide) with one consolidation cycle also including HD MTX. Restaging after his second consolidation phase showed persistent disease. The patient was taken off study and received three sequential salvage attempts using R-ICE (rituximab, ifosfamide, carboplatin, etoposide), R-GDP (rituximab, gemcitabine, dexamethasone, and cisplatin), and vincristine, irinotecan, and temozolomide, to which his disease remained refractory. Given the chemotherapy refractory nature of his disease, the patient was then referred for CD19 CAR T-cell therapy which utilized a FMC63 scFv-28z CD19 construct and he was treated on

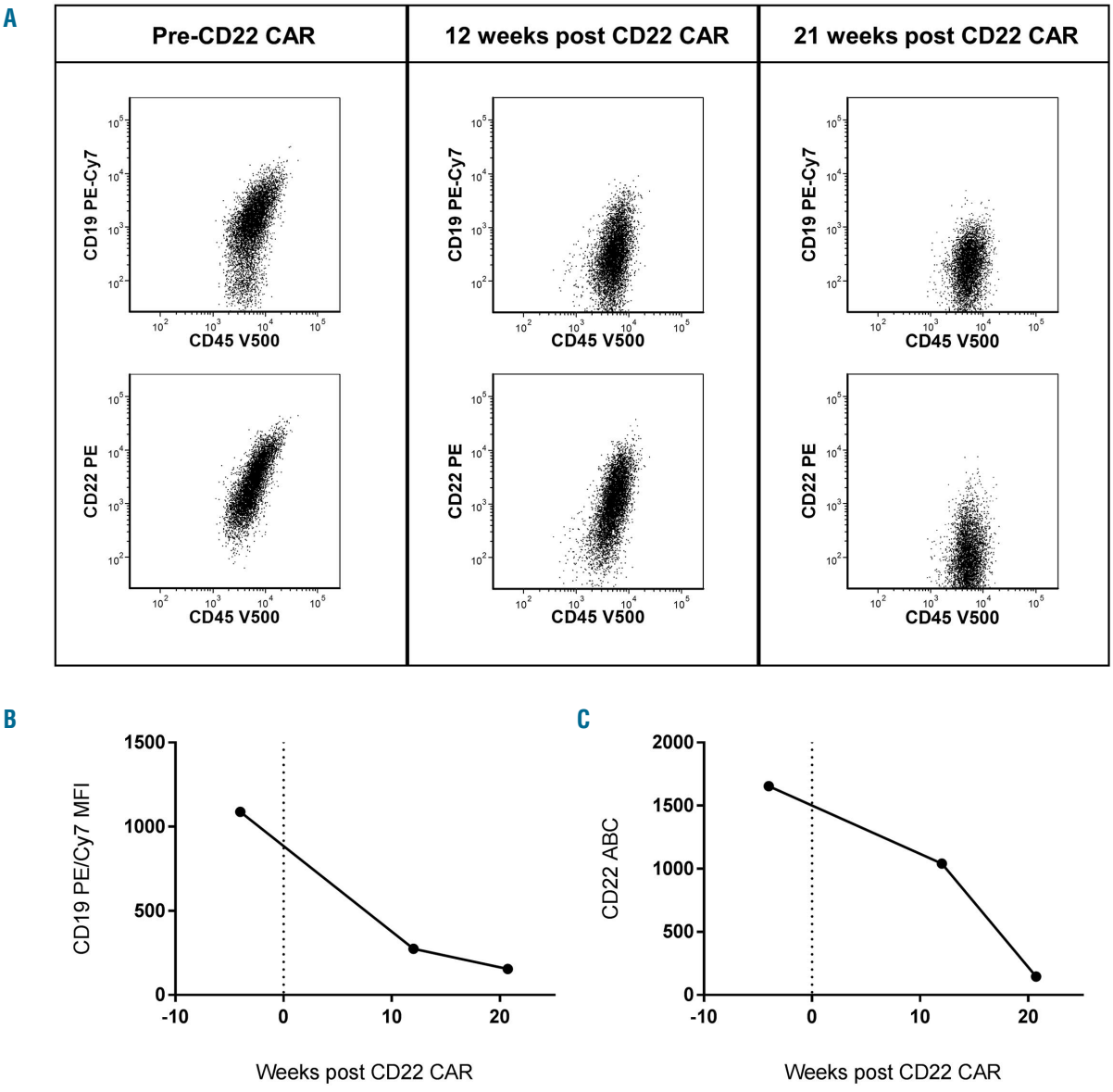

Figure 2. CD19 and 22 expression levels by flow cytometry. A. Flow Cytometry of the patient's diffuse large B-cell lymphoma demonstrates expression of CD19 and CD22 prior to treatment, with decreased levels of both CD19 and CD22 at 12 weeks and 21 weeks post CD22 CAR therapy, including emergence of true antigen negative populations. At the pre-CD22 CAR time point, $CD19^+$ is seen on 91% of cells (9% CD19 negative) and continues to progressively decline. By the 21-week post CD22 CAR time point, only 13% and 11% of neoplastic cells were $CD19^+$ and $CD22^+$, respectively. B. CD19 mean fluorescent intensity (MFI) by flow cytometry is decreased post-CD22 CAR compared to pre-treatment CD19 MFI. C. CD22 antigen binding capacity (ABC) by flow cytometry is decreased post CD22 CAR compared to pre-treatment CD22 ABC.

study (*clinicaltrials.gov identifier 01593696, National Cancer Institute IRB approved*). Following infusion, he developed grade 2 cytokine release syndrome (CRS) with fever and hypotension,[10] and day 28 response evaluation demonstrated a partial remission. At two months post-infusion, however, he experienced disease progression evidenced by an enlarging right gluteal mass. Biopsy of the mass demonstrated variable loss of CD19 expression in the neoplastic cells by IHC staining (Figure 1) and flow cytometry showed dim CD19 expression compared to prior, with no evidence of CD19 CAR T cells in the biopsy specimen. Initial signs demonstrated the emergence of CD19 negative disease with evidence of 9% of DLBCL cells being CD19 negative. Therefore, the immunotherapy was shifted to treatment with anti-CD22 CAR T-cell therapy utilizing a 4-1BB based CD22 CAR construct (*clinicaltrials.gov identifier 02315612, National Cancer Institute IRB approved*), and again he developed grade 2 CRS, characterized by fever and hypotension.[10] His initial day 28 restaging evaluations showed stable disease; however, at his two-month follow up evaluation, there was concern for disease progression at the original site of disease only, with concurrent loss of CAR-T cells. Subsequently, a second infusion of CD22 CAR T cells, at the same dose as the original infusion, was performed to bolster the response given the lack of detectable circulating CD22 CAR T cells. Unfortunately, progressive disease was noted at the day 28 restaging following re-infusion, which included development of new liver lesions, increasing PET avidity of the original sites of disease, and multiple new areas of recurrence. Biopsy of the liver lesions confirmed involvement by DLBCL with ongoing evolution in the loss of CD19 and new demonstration of loss of CD22 as shown by IHC and flow cytometry (Figures 1 and 2).

Molecular analysis (at diagnosis, post CD19 CAR T cell, and post CD22 CAR T-cell therapy) was limited due to small sample size and viability, however, genome and transcriptome sequencing provided some insight into mechanisms for therapy resistance. First, the patient was found to have a somatic, homozygous TP53 p.R174Stop mutation in all samples analyzed. Although unknown at the time of therapy, this mutation was present at diagnosis. Although the impact of TP53 mutations on response to immunotherapy is unknown, we postulate that this could have served as a driving event in the clonal expansion of a pre-existing CD19 negative and CD22 negative population. Copy number variations at disease presentation were stable throughout the treatment course. These included single copy gains of the proximal aspect of chr 1q and two copy gains of proximal parts of chr 13q and 18q. Additionally, single copy losses were noted on the distal aspect of 1q and 13q. Transcriptome analysis showed stable transcript levels of CD19 and CD22 RNA after the CD19 CAR T-cell therapy and coding region mutations in CD19 or CD22 were not found at any of the surveyed time points. The RNA quality was insufficient for conclusive interpretation in the post CD22 CAR T-cell sample. Furthermore, the RNA quality limited a detailed isoform analysis of the antigens. Altogether, these data reinforce the high-risk potential of TP53 mutations, even in the setting of CAR T-cell therapy. Additionally, these data imply that the post-therapy loss of CD19 and CD22 proteins may be due to a post-translational mechanism rather than modulation of the expression of the genes. Although we could not identify the mechanism leading to antigen loss in this report, alternate splicing of CD19 mRNA resulting in isoforms which escape the CAR T-cell motif, which has been described by others, could be one possibility.[7] Mechanisms for alternations in CD22 expression are being explored.

Durable remissions have recently been reported after CD19 CAR T-cell therapy in cases of chemotherapy refractory DLBCL,[3,8] making CAR T-cell therapy an important new therapeutic option for relapsed and advanced stage lymphoma. However, as seen in patients with leukemia who receive CD19 directed immunotherapy, antigen loss may prevent durable remissions.[11,12] In this case, re-biopsy of the target lesions was instrumental in guiding further management. Although radiographic studies allow for quantification of tumor response,[13] they are limited in their ability to provide details on tumor evolution, which is of increasing importance in the era of targeted therapies. By obtaining multiple tissue samples, the treatment team was able to serially assess antigen expression and site density by IHC and flow cytometry, which further guided therapeutic choices. Concurrent molecular analysis, although retrospectively performed in this case, provided insight into the chemotherapy refractory nature of this patient's disease, further supporting the need for novel approaches in treatment of this patient. Antigen loss as a mechanism of relapse in lymphoma is less frequently reported than in leukemia; however, a recent report concerning primary mediastinal large B-cell lymphoma showed that after CD19 CAR therapy and treatment with rituximab, loss of both CD19 and CD20 was seen. In that case, loss of DNA repair proteins in addition to clonal evolution of the lymphoma due to CAR immune pressure was the suggested mechanism.[9] Multi-targeted immunotherapy may be necessary to overcome such tumor immune evasion mechanisms. This case serves as proof-of-concept of the potential for antigen loss in lymphoma following targeted immunotherapy and with the growing field of novel targeted immunotherapy, highlights the importance for sequential tissue evaluation through the course of treatment.

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doi:10.3324/haematol.2017.183459

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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