

CD20-Negative Primary Bone Marrow Large B Cell Lymphoma: A Report of Two Cases with Review of Literature

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Abstract



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Keywords

- ▶ CD20-negative lymphomas
- ▶ primary bone marrow lymphomas
- ▶ plasmablastic lymphomas

We describe diagnostic and therapeutic challenges in this rare entity of CD20-negative B cell lymphomas. They comprise only 1 to 2% of all B cell lymphoma with four subcategories. However, with an increasing understanding of these cases, novel entities have been recognized. Workup, treatment, and follow-up of two CD20-negative B cell lymphoma cases, including a novel entity, have been described. A novel case of triple expresser CD20-negative B cell lymphoma and plasmablastic lymphoma has been diagnosed. The absence of CD20 leads to the abolishment of the targeted drug (rituximab) from the treatment regimen. These cases render the importance of extensive immunohistochemistry and fluorescence in situ hybridization workup for diagnosis of this rare entity. Treatment also remains a challenge as immunotherapy with CD20-targeted drugs cannot be given.

Introduction

CD20 is a glycosylated phosphoprotein, commonly expressed on most mature B cell neoplasms. A small subset of 1 to 2% B cell lymphomas is CD20 negative.¹ The most common CD20-negative lymphomas are plasmablastic lymphoma, primary effusion lymphoma, large B-cell lymphoma arising from human herpesvirus-8 (HHV8)-associated multicentric Castleman's disease, and anaplastic lymphoma kinase positive (ALK+) large B cell lymphoma.² Due to its CD20 negativity, it poses a diagnostic and therapeutic challenge and are usually resistant to the efficacious rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) chemo-

therapy regime and have inferior overall survival rates. We hereby discuss two case reports of this novel entity, CD20-negative B cell lymphomas that were primary bone marrow lymphomas as well.

Case Report

Case Report 1

A 35-year-old female presented to a tertiary care center with a history of weakness for the last 2 months along with recent onset fever and mild headache for the last 3 to 4 days. On general and systemic examination, there was only pallor with no icterus, any neurological deficit, no palpable

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lymphadenopathy, or hepatosplenomegaly. Complete blood count (CBC) revealed severe anemia for which the patient was further investigated. Peripheral smear examination along with bone marrow aspiration and biopsy and radiology was done.

CBC showed severe anemia requiring transfusion with hemoglobin of 3.6 gm/dL, normal total leucocyte count (4,700 cells/ μ L), and mild thrombocytopenia (105,000/ μ L). Peripheral smear revealed leucoerythroblastic picture. Bone marrow aspiration was diluted; however, imprint smears were cellular with 70.0% large abnormal cells seen with blastoid morphology (–Fig. 1). Normal marrow components were reduced with few megakaryocytes seen. Myeloperoxidase stain was negative. Bone marrow biopsy showed hypercellular marrow with overall cellularity of approximately 95 to 100% with near total replacement of normal hematopoietic cells by abnormal looking cells. The bone marrow aspirate sample for flow cytometry was diluted, which showed no evidence of abnormal cells with normal B lymphocytes (Cd19-positive B cells) showing polyclonal kappa/lambda, T lymphocytes, granulocytes, and monocytes identified. In view of morphological presence of abnormal cells, immunohistochemistry (IHC) was advised. On IHC, these cells were positive for CD45, CD138, PAX5, CD79A, BCL2, Ki 67 (55–60%) and negative for CD20, CD30, MPO, ALK, BCL6, EMA, cMYC, PanCK, MUM1, GATA3, CD5, SYNAPTOPHYSIN, CD10, EBV, CD56, and HHV-8. To exclude multiple myeloma, serum-free light chain ratio revealed normal kappa:lambda ratio (1.48) with borderline raised beta-2 microglobulin (1,810, range: 700–1,800 ng/mL). FISH for BCL2, BCL6, and cMYC were negative. Enzyme-linked immunosorbent assay for human immunodeficiency virus and EBERish were negative. Positron emission tomography-computed tomography (PET-CT) scan showed diffuse uptake in bone marrow with no lymphadenopathy/organomegaly or any other focal lesion.

Final diagnosis was of bone marrow involvement by high-grade CD20-negative B-cell lymphoma. Possibility of plasmablastic lymphoma was favored. As no lymph nodes and no hepatosplenomegaly were present with PET-CT showing isolated diffuse uptake in bone marrow, a diagnosis of primary bone marrow lymphoma was established.

The patient was treated with three cycles of dose-adjusted EPOCH (infusional etoposide, vincristine and doxorubicin along with bolus cyclophosphamide and prednisone) and is doing well with no transfusion requirement.

Case Report 2

A 19-year-old male presented with a short history of fever for the last 2 weeks. On examination, mild hepatosplenomegaly was seen. PET-CT scan showed no lymphadenopathy with increased uptake in marrow and a diagnosis of primary bone marrow lymphoma was favored radiologically. CBC revealed moderate anemia with hemoglobin of 7.0 gm/dL, low total leucocyte count (1,530 cells/ μ L), and moderate thrombocytopenia (40,000/ μ L). Ninety-three percent of abnormal cells were seen in the peripheral smear. Bone marrow aspiration was cellular with more than 90.0% large abnormal cells with blastoid morphology. Normal marrow components were suppressed. Flow cytometry (–Fig. 2) was performed on

this bone marrow sample that revealed CD20-negative, CD10-positive, and aggressive B cell neoplasm. Due to CD20, kappa, lambda negativity and moderate-to-bright CD45 expression, IHC on bone marrow trephine biopsy was advised for definite exclusion of leukemia. Bone marrow trephine biopsy was hypercellular for age with overall cellularity of more than 95%. Hematopoietic elements of the marrow were diffusely replaced by sheets of abnormal cells that were larger than lymphocytes, monomorphic in appearance, with open chromatin and distinct nucleoli. There was near complete absence of normal marrow components. On IHC, the abnormal cells expressed CD19, CD79a, PAX5, CD10, BCL2, BCL6, cMYC with proliferation index of 60 to 70% (ki 67) and were negative for CD20, CD34, TdT, CD138, CD30, and ALK-1. Cytogenetics and molecular studies were not performed due to financial constraints. A diagnosis of high-grade B-cell lymphoma (CD20 negative) with expression of BCL2, BCL6, and cMYC rearrangement (Triple-Expressor lymphoma) was rendered.

This patient was given acute lymphoblastic leukemia like protocol and is doing well. Two time points minimal residual disease (post-induction and post-consolidation) are negative.

Discussion

CD20 is encoded by the membrane-spanning 4A gene located on chromosome 11q12.2.³ It is a transmembrane polypeptide with 297 amino acid residues and plays a role in the differentiation, maturation, and activation of B cells. A chimeric CD20 monoclonal antibody called rituximab was developed in 1997 for the treatment of CD20 expressing B cell malignancies.⁴ It destroys B lymphoid malignancies through complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity. Henceforth, R-CHOP comprising of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone became the mainstay for management of diffuse large B cell lymphoma. Now newer anti-CD20 drugs have been developed and approved by Food and Drug Administration called ofatumumab and obinutuzumab for the treatment of B cell malignancies.^{5,6} It has been contemplated that those genetic mutations of MS4A1 lead to conformational changes in the protein and hence the CD20-negative phenotype. The loss of CD20 expression is associated with aggressive disease. It poses a diagnostic and therapeutic dilemma and more studies are required to establish the standard of care in this group of patients.

CD20-negative B cell non-Hodgkin lymphoma is frequently associated with atypical cell morphology, extranodal involvement, aggressive clinical behavior, resistance to standard chemotherapy, and poor survival rates.⁷ The most common CD20-negative lymphomas include plasmablastic lymphoma, primary effusion lymphoma, large B-cell lymphoma arising from HHV8-associated multicentric Castleman's disease, and ALK+ large B cell lymphoma. In addition to these, CD20 positive lymphoma can also relapse as CD20-negative lymphoma after CD20 antibody (rituximab) therapy. We report a plasmablastic lymphoma and a triple expressor CD20-negative large cell lymphoma primarily involving the bone marrow.

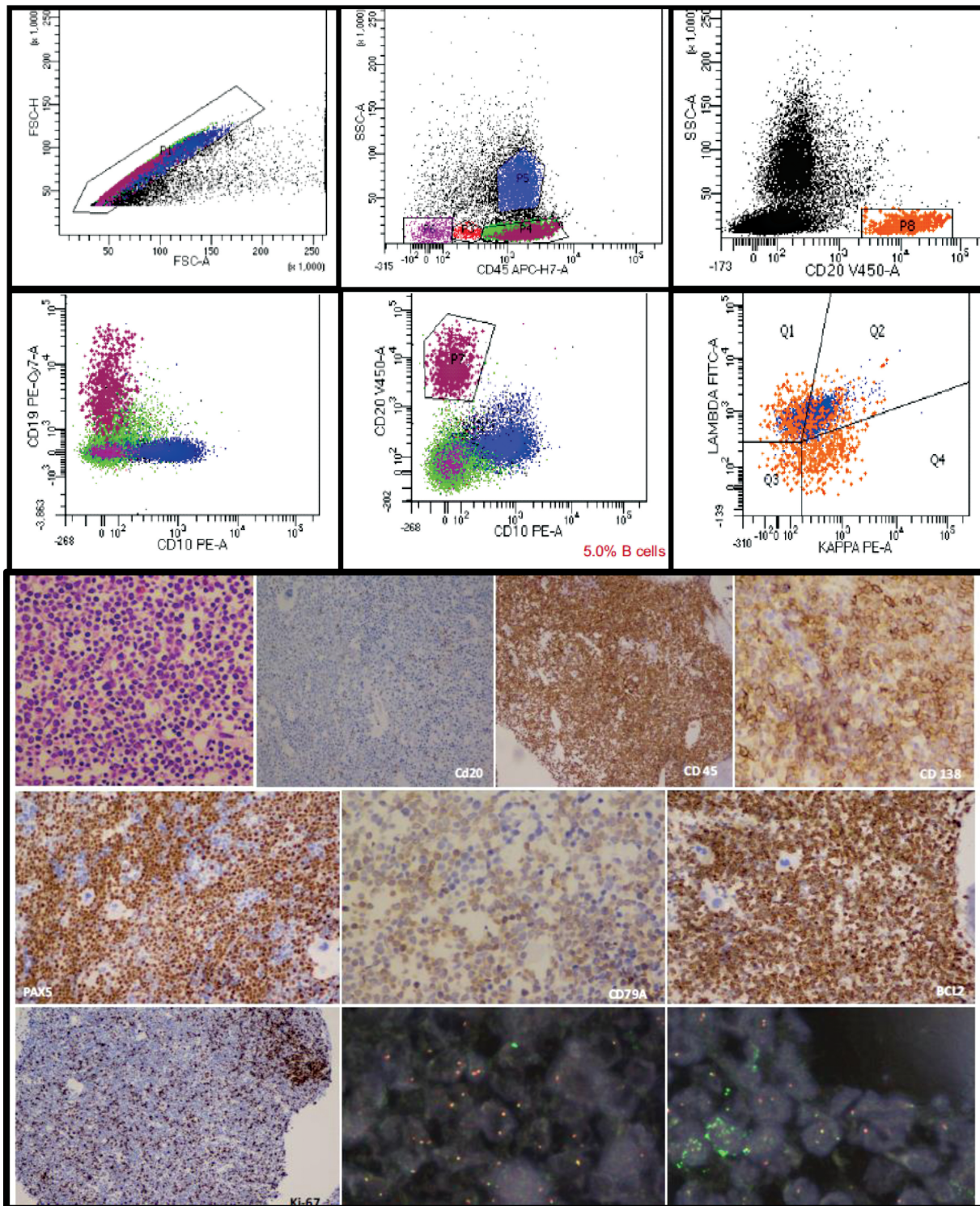


Fig. 1 Flow cytometric analysis of bone marrow aspiration of patient 1 revealing CD20 (orange) and CD19 (brown) gated population of mature lymphocytes with polyclonal expression of kappa and lambda. No atypical cells are seen (A–F). Hematoxylin and eosin image (40x) showing replacement of hematopoietic elements by atypical lymphoid cells; upper inset showing atypical cells on bone marrow imprint; lower inset showing myeloperoxidase negativity in atypical cells (G). On immunohistochemistry, these atypical lymphoid cells are negative for CD20, positive for CD45, CD138, PAX5, CD79A, BCL2, high Ki67 (H–N). Fluorescence in situ hybridization for BCL2, BCL6, and cMYC was negative with two red and green signals in each cell (O–Q).

Large cell lymphomas are identified by morphology and immunophenotyping via IHC and flow cytometry. However, CD20-negative lymphomas pose a diagnostic challenge. On flow cytometry, they can be confused with a B cell acute

lymphoblastic leukemia due to CD20, kappa and lambda negativity; however, this population is generally moderate-to-bright positive for CD45 apart from expressing other B cell markers- CD19, cytoplasmic CD79a, CD22. On IHC, positivity

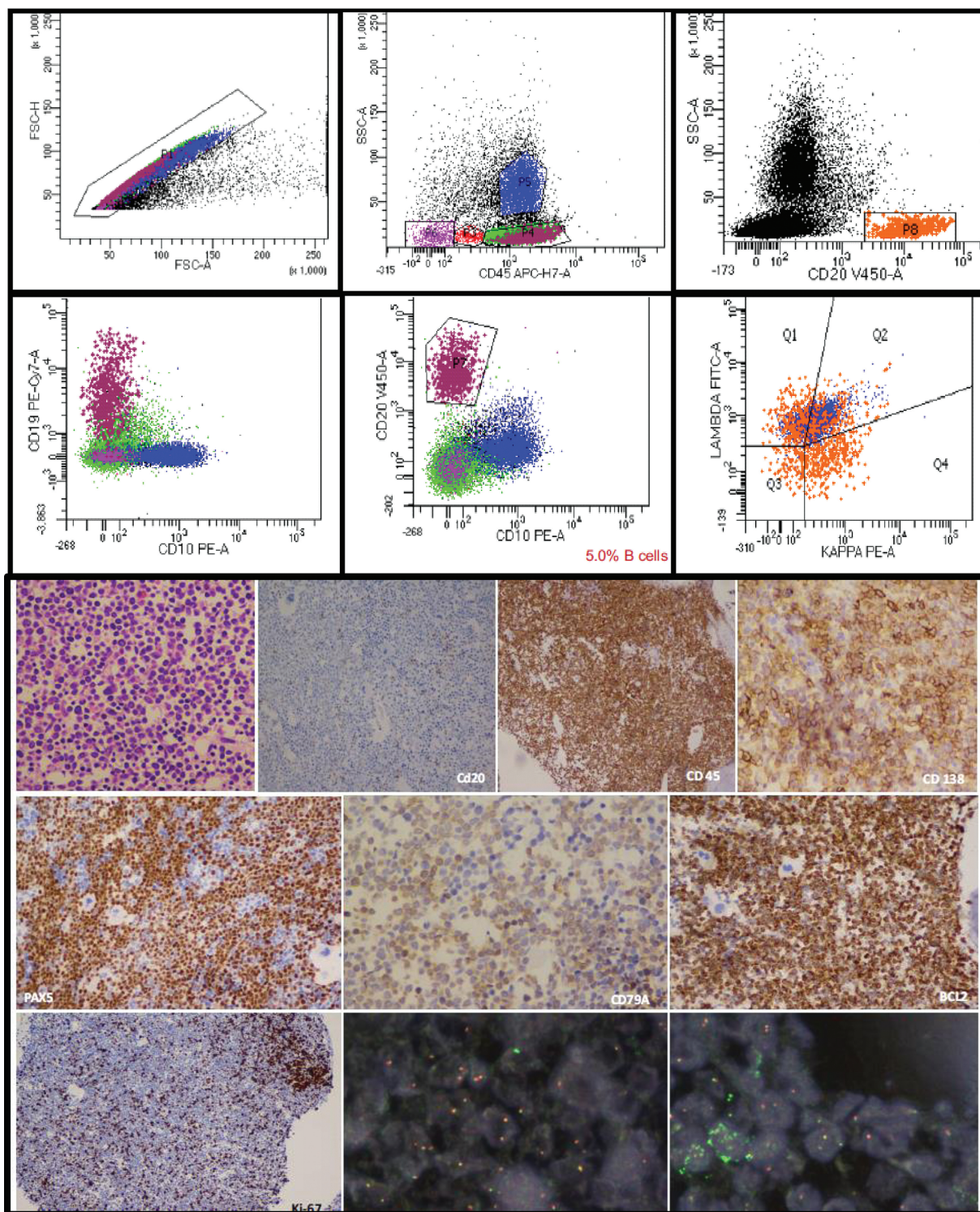


Fig. 2 Flow cytometric analysis of patient 2; Magenta pink colored abnormal population revealing moderate-to-bright CD45, positivity for CD19, CD10, CD43, kappa, CD38, negativity for CD20, CD34, CD117, myeloperoxidase, and positivity for CD79a (A–K). Hematoxylin and eosin (40x) revealed replacement by atypical lymphoid cells (L). On immunohistochemistry, these cells were positive for CD19, CD79A, PAX5, CD10, BCL2, BCL6, cMYC with high Ki67 and negative CD20 (400x; M–U).

for B cell markers (CD19, CD79a, PAX5) and negativity for immaturity markers (CD34, Tdt) are the major determinants in diagnosing a CD20-negative B-cell lymphoma and excluding B-cell acute lymphoblastic leukemia. Our both the cases had a similar picture and IHC confirmed them as lymphomas rather than leukemias. Cytogenetics and molecular studies

including FISH can be used for the detection of Bcl-2, Bcl-6, and cMYC rearrangements for diagnosis of double-hit/triple-hit lymphomas. We are reporting a rare “triple-expressor” primary bone marrow CD20-negative lymphoma.

Till date no consensus has been reached on treatment strategies for CD20-negative B-cell lymphomas. These

patients show poor response to R-CHOP regime. Studies have revealed use of CODOX-M/IVAC (cyclophosphamide, vincristine, doxorubicin, methotrexate alternating with ifosfamide, etoposide, cytarabine), dose-adjusted EPOCH (infusional etoposide, vincristine and doxorubicin along with bolus cyclophosphamide and prednisone),⁷ and hyper-CVAD (cyclophosphamide, vincristine, doxorubicin and dexamethasone alternating with high-dose methotrexate and cytarabine),⁸ as effective therapies. In addition, plasmablastic lymphomas respond to bortezomib in combination with infusional dose-adjusted EPOCH. Bcl-2 inhibitors are also being explored as a treatment option and requires further investigation.⁹

Conclusion

CD20-negative lymphoma is a novel rare entity with poor prognosis. It poses a diagnostic and therapeutic challenge. Further research needs to be done to establish the standard of care for this group of patients. We have reported these two cases to increase awareness among pathologists and clinicians and to highlight the implication of this rare entity on diagnosis and treatment, especially in laboratories using limited markers for immunophenotyping.

Consent

Consent was obtained from patients to use their case for publication.

Funding

None.

Conflicts of Interest

None Declared.

References

- 1 Castillo JJ, Chavez JC, Hernandez-Ilizaliturri FJ, Montes-Moreno S. CD20-negative diffuse large B-cell lymphomas: biology and emerging therapeutic options. *Expert Rev Hematol* 2015;8(03): 343-354
- 2 Garg M, Lee BE, McGarry K, Mangray S, Castillo JJ. CD20-negative diffuse large B-cell lymphoma presenting with lactic acidosis. *Am J Hematol* 2015;90(03):E49-E50
- 3 Tedder TF, Streuli M, Schlossman SF, Saito H. Isolation and structure of a cDNA encoding the B1 (CD20) cell-surface antigen of human B lymphocytes. *Proc Natl Acad Sci U S A* 1988;85(01): 208-212
- 4 Grillo-López AJ, White CA, Dallaire BK, et al. Rituximab: the first monoclonal antibody approved for the treatment of lymphoma. *Curr Pharm Biotechnol* 2000;1(01):1-9
- 5 Evans SS, Clemmons AB. Obinutuzumab: a novel anti-CD20 monoclonal antibody for chronic lymphocytic leukemia. *J Adv Pract Oncol* 2015;6(04):370-374
- 6 Gupta IV, Jewell RC. Ofatumumab, the first human anti-CD20 monoclonal antibody for the treatment of B cell hematologic malignancies. *Ann N Y Acad Sci* 2012;1263:43-56
- 7 Dunleavy K, Pittaluga S, Shovlin M, et al. Phase II trial of dose-adjusted EPOCH in untreated systemic anaplastic large cell lymphoma. *Haematologica* 2016;101(01):e27-e29
- 8 Linder K, Gandhiraj D, Hanmantgad M, Seiter K, Liu D. Complete remission after single agent blinatumomab in a patient with pre-B acute lymphoid leukemia relapsed and refractory to three prior regimens: hyperCVAD, high dose cytarabine mitoxantrone and CLAG. *Exp Hematol Oncol* 2016;5:20
- 9 Katchi T, Liu D. Diagnosis and treatment of CD20 negative B cell lymphomas. *Biomark Res* 2017;5:5. Doi: 10.1186/s40364-017-0088-5