

Loss of CD20 Expression after Rituximab Therapy for B-Cell Lymphomas: A Review of the Literature

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Received: April 1, 2012 Accepted: May 6, 2012 Online Published: May 16, 2012

doi:10.5539/cco.v1n2p1 URL: <http://dx.doi.org/10.5539/cco.v1n2p1>

Supported in part by grants (20014012, 20590364, 20014010) from the Ministry of Education, Science, Culture, Sports and Technology, Japan

Abstract

Rituximab (Rx), a chimeric anti-human CD20 antibody, is used widely for the treatment of B-cell non-Hodgkin's lymphomas (NHL) worldwide. Loss of CD20 expression in relapsed B-cell lymphomas after Rx treatment, however, is observed in some cases, which might be a cause of B-cell NHL unresponsiveness to Rx retreatment. The frequency of loss of CD20 expression after Rx treatment and radiotherapy, its correlation with histological changes, and its clinical implication together with possible molecular mechanisms are discussed in this review of pertinent literature. In high-grade B-cell NHL, loss of CD20 expression after Rx treatment was observed less frequently in Japan than in Australia. Evaluation of CD20 expression by immunohistochemical and flow cytometric methods is a reliable guide for employment of Rx treatment for B-cell lymphomas.

Keywords: B-cell lymphomas, high-grade, low-grade, rituximab

1. Introduction

CD20, a hydrophobic transmembrane protein with a molecular weight of approximately 35 kD, is expressed in pre B and mature B lymphocytes (Nadler et al., 1981). Rituximab (Rx) is a chimeric anti-human CD20 antibody that is used widely for the treatment of B-cell lymphomas (Reff et al., 1994) and immune-related diseases, such as rheumatoid arthritis (Edwards et al., 2006). The mechanisms of action of Rx for elimination of non-neoplastic and neoplastic B-cells include antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity, and stimulation of the apoptotic pathway (Reff et al., 1994). Rx was employed originally for the treatment of low-grade B-cell lymphomas or follicular lymphoma (FL). Later, the combined use of Rx with conventional chemotherapy (cyclophosphamide, doxorubicin, vincristine, and prednisone) (Rx-CHOP) was found to be effective for more aggressive diffuse large B-cell lymphoma (DLBCL) (Feugier et al., 2005).

DLBCL, the most common type of malignant lymphoma worldwide, is a diffuse proliferation of large neoplastic B-lymphoid cells. On the basis of gene expression profiles, DLBCL can be categorized into two distinct subtypes: germinal center B-cell (GCB) and activated B-cell (ABC), or non-GCB (Alizadeh et al., 2000). Generally, the non-GCB type is associated with an unfavorable prognosis compared to the GCB type before the employment of Rx therapy.

Positive regulatory domain 1 (PRDM1), a master regulator of the differentiation of mature B lymphocytes into plasma cells, has two isoforms, PRDM1-alpha and -beta, the expressions of which are regulated by the transcriptional regulator NF-kappaB. Using microdissected DLBCL cells, Liu et al. reported that both PRDM1-alpha and -beta were expressed in the non-GCB, but not the GCB, type of DLBCL (Liu et al., 2007). Expression of the PRDM1-beta gene was shown to be correlated with an unfavorable prognosis of non-GCB patients when treated with CHOP, but this was not observed in those undergoing Rx-CHOP treatment (Liu et al., 2007), suggesting a favorable effect of Rx. This same study also reported that B-lymphoma cells resistant to chemotherapy expressed PRDM1-beta, and this expression was suppressed by Rx, possibly through NF-kappaB inactivation. Thus, expression of PRDM1-beta could be a prognostic marker for the non-GCB type of DLBCL.

Rx-CHOP is now employed as a standard therapy for DLBCL, but recurrence of disease is encountered not infrequently. In such cases, histologic examination of relapsed tumors is not usually performed because DLBCL does not transform to more become more, or less, aggressive. Therefore, reports on the loss of CD20 expression after Rx therapy for B-cell non-Hodgkin's lymphomas (NHL) have been relatively limited.

Tumors occasionally become resistant to therapies that are initially effective. The same phenomenon is observed in B-cell NHL treated with Rx-containing chemotherapy, followed by reduced responsiveness of lymphoma cells to the therapy; loss or reduction of CD20 in lymphoma cells is one cause. In this paper, changes in CD20 expression in B-cell NHL after Rx therapy are discussed with respect to clinical behavior and molecular mechanisms. Davis et al. reported that the response rate of relapsed B-cell NHL to Rx after Rx therapy was less than 50% (Davis et al., 2000). In such cases, loss of CD20 expression in relapsed B-cell lymphomas could be a robust indicator of resistance to Rx therapy.

2. Resistance to Rx Therapy in B-cell Lymphomas

Possible mechanisms of the resistance of B-cell NHL to Rx therapy include three patterns: protection of the tumor cells from Rx-triggered elimination by ADCC / complement-dependent cytotoxicity and apoptotic stimulation, inadequate binding of Rx to the CD20 molecule, and loss of CD20 expression.

2.1 Antibody-Dependent Cellular Cytotoxicity (ADCC)

ADCC by natural killer (NK)-cells plays a major role in elimination of B-lymphoma cells during Rx therapy. Rx-induced ADCC was attenuated upon ligation of killer immunoglobulin-like receptors, inhibitory receptors expressed on NK-cells, by human leukocyte antigen (HLA) molecules expressed on human B-lymphoma target cells (Borgerding et al., 2010). Therefore, protection of tumor cells from ADCC by inhibition of NK-cell function through increased HLA expression on tumor cells might explain the failure of Rx treatment for CD20 positive B-cell NHL.

2.2 Binding of Rx to CD20

Inadequate binding of Rx to the CD20 molecule might be caused by mutations or polymorphisms of the CD20 gene that affect its structure. However, Sar et al. reported that no mutations were detected in the coding region of the CD20 gene in any of 11 patients with DLBCL who showed a poor prognosis with Rx-CHOP therapy (Sar et al., 2009); one case showed a synonymous single nucleotide polymorphism in exon 2. Johnson et al. reported similar results, demonstrating that mutations of the Rx epitope in the CD20 gene, encompassing exon 5 of the *MS4A1* gene, were detected in only one of 264 (0.4%) or one of 15 (6%) biopsies taken at diagnosis or relapse, respectively (Johnson et al., 2009a). No polymorphic sequence variants were detected in this region. Taken together, CD20 mutations involving the Rx epitope are rare in both *de novo* and relapsed DLBCL.

2.3 Loss of CD20 Expression after Rx Therapy for B-cell NHL

Loss of CD20 expression after Rx therapy for B-cell lymphomas is observed as a consequence of purging CD20-expressing B-lymphoma cells. Putative mechanisms are described below.

2.3.1 Mutations of the CD20 Gene

Terui et al. reported that deletion mutations in the C-terminus of CD20 were found in 4/50 (8.0%) cases of B-cell lymphomas; 2/22 cases of DLBCL, 1/7 of FL, 1/1 of mantle cell lymphoma, and 0/20 of other B-cell lymphomas (Terui et al., 2009). This resulted in decreased mean fluorescence intensity of CD20 expression on fresh lymphoma cells compared to cells with non-mutated genes. Three of their 44 (6.8%) patients who had received Rx-CHOP therapy had C-terminal deletion mutations, and they showed progressive disease after Rx-CHOP therapy, suggesting a role for mutation in disease progression. Radiotherapy was employed in two of Terui et al.'s cases before administration of Rx, and deletion of the C-terminus of the CD20 gene and disease progression were found in these cases. Radiation before Rx administration might also cause mutation of the CD20 gene. On the contrary, effects of the CD20 gene sequence on the level of CD20 protein expression were not found in other studies (Tomita et al., 2007; Czuczman et al., 2008). Tomita and colleagues (Tomita et al., 2007) reported epigenetic regulation of CD20 expression in a CD20-negative mature B-cell line, RRBL1, established from a patient treated repeatedly with Rx-containing chemotherapy.

The Gene Scan analysis in our study revealed partial or complete persistence of the same-sized peaks in 11 DLBCL cases, indicating the same origin of tumor cells before and after Rx-containing therapy; however, changes in the peak pattern were also found in many cases, suggesting the presence of genetic instability (Figure 1) (Wada et al., 2009). These findings may explain partly the occurrence of CD20-negative DLBCL after Rx-containing therapy.

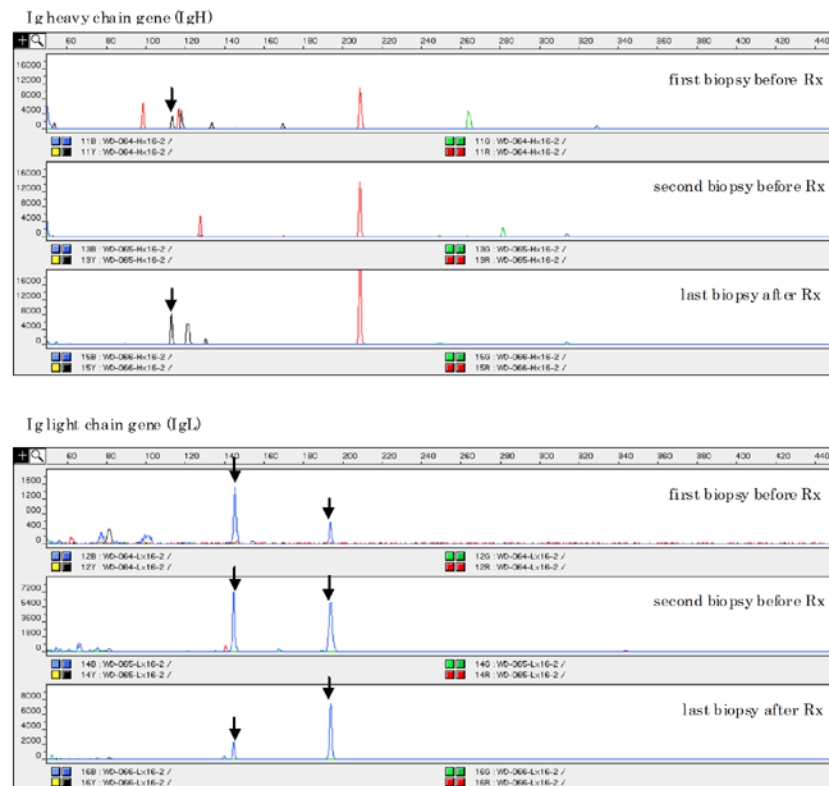


Figure 1. Polymerase chain reaction-based clonality analysis of immunoglobulin (Ig) gene rearrangement (Gene Scan analysis) in a case of diffuse large B-cell lymphoma revealed different peak patterns before and after rituximab (Rx) treatment, with partial persistence of the same-sized peaks (↓)

2.3.2 Immunohistochemical (IHC) and Flow Cytometric (FCM) Analysis

Johnson et al. reported that tumor cells in 43 of 272 (16%) DLBCL cases showed reduced CD20 expression, 35 of whom also exhibited bright CD19 expression (Johnson et al. 2009b). These 35 cases had a worse prognosis than the other cases with bright CD20 expression when treated with CHOP or Rx-CHOP, irrespective of the international prognostic index. Forty-one of the 43 cases with reduced CD20 expression by FCM showed strong staining for CD20 by IHC. Sequencing of exon 5 of the *MS4A1* gene, encoding the extracellular component of the CD20 antigen, did not reveal mutations that could explain the discrepant results between FCM and IHC. Cases showing loss of CD20 expression by FCM, but which were positive for CD20 by IHC, in recurrent tumors of DLBCL after Rx treatment were also reported (Wada et al., 2009; Kennedy et al., 2002); these cases showed progressive disease during Rx-containing therapy. These findings suggest that lack of detection of CD20 by FCM is a sign of resistance to Rx-containing therapy. FCM and IHC analyses detect different epitopes of CD20; extracellular surface epitopes are detected by FCM whereas intracellular ones are detected by IHC. Masking of the surface epitopes through binding of CD20 molecules, instead of gene mutations that affect surface epitopes but preserve intracellular ones, might occur.

2.3.3 Rx-resistant Cell Lines (RRCL) as a Model for Loss of CD20 Expression

Tsai et al. reported that rituximab-resistant cell lines (RRCL) exhibited a gradual loss of CD20 surface expression through repeated exposure to Rx (Tsai et al., 2012). They found that the promoter activity of the CD20 gene was decreased, due to reduced binding of several key positive regulatory proteins on the CD20 promoter. Forced CD20 expression restored cytoplasmic, but not surface, CD20, suggesting a defect in CD20 protein transport. Thus, addition of interleukin-4 (IL-4) might induce higher CD20 promoter activity and CD20 expression, improving the responsiveness of RRCL to Rx.

3. Clinical Implications of Loss of CD20 Expression in B-Cell Lymphomas after Rx Therapy

3.1 Histological Changes and CD20 Expression (Table 1)

Low-grade B-cell NHL-expressing CD20 might recur as high-grade CD20-negative NHL, which could explain, at least in part, the resistance of recurrent tumors to Rx retreatment (Davis et al., 1999; Schmitz et al., 1999; Alvaro-Naranjo et al., 2003; Maeshima et al., 2009; Hiraga et al., 2009). However, Foran et al. reported that loss of CD20 expression after Rx treatment was not correlated with histological transformation from low-grade to high-grade NHL (Foran et al., 2001).

Table 1. Frequency of loss or significant decrease of CD20 expression after rituximab treatment for CD20-positive B-cell lymphomas

	Frequency of loss or significant decrease of CD20 expression (%)	Histological change (case no.)	Authors/Year
DLBCL	0/1 (0)	NA	Seliem et al/2006
	4/11 (36.4)	no remarkable change (3)	Maeshima et al/2009
		proliferation of plasmacytoid cells (1)	
	3/7 (42.9)	NA	Hiraga et al/2009
MZBCL	4/21 (19.0)	no remarkable change (4)	Wada et al 2009
	0/2 (0)	NA	Seliem et al/2006
	1/2 (50)	proliferation of plasmacytoid cells (1)	Maeshima et al/2009
	0/2 (0)	NA	Hiraga et al/2009
CLL/SLL	3/4 (75)	NA	Seliem et al/2006
	2/2 (100)	no remarkable change (2)	Maeshima et al/2009
FL		no remarkable change (5)	
		proliferation of plasmacytoid cells (2)	
	9/34 (26.5)	transformation to Hodgkin's lymphoma (1)	Maeshima et al/2009
		transformation to anaplastic large cell lymphoma-like undifferentiated lymphoma (1)	
MCL	2/7 (28.6)	transformation to DLBCL (2)	Hiraga et al/2009
	0/10 (0)	NA	Maeshima et al/2009
	0/1 (0)	NA	Hiraga et al/2009
LPL	1/1 (100)	NA	Seliem et al/2006
Burkitt or Burkitt-like	0/2 (0)	NA	Hiraga et al/2009
aggressive B-NHL*	6/10 (60)	NA	Kennedy et al/2002
B-NHL, NOS	1/1 (100)	NA	Seliem et al/2006

*DLBCL and MCL (mainly DLBCL)

DLBCL indicates diffuse large B-cell lymphoma; MZBCL, marginal zone B-cell lymphoma; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; LPL, lymphoplasmacytic lymphoma; B-NHL, B-cell non Hodgkin's lymphoma; NOS, not otherwise specified; NA, data not available

3.2 Transient Loss of CD20 Expression

Ferreri et al. reported transient loss of CD20 expression in a case of gastric DLBCL after Rx-containing treatment; the tumor cells were initially CD20-positive, turned negative at the first relapse, and restored CD20 expression at the second relapse (Ferreri et al., 2007). Because loss of CD20 expression could be a transient phenomenon, it is meaningful to evaluate CD20 expression at every relapse of tumors to inform decisions regarding Rx-containing regimens.

3.3 Frequency of Loss of CD20 Expression after Rx Therapy for Low- and High-Grade B-Cell NHL (Table 1)

Loss or a significant decrease in CD20 expression was found in various kinds of B-cell NHL and was relatively common in cases with CLL/SLL in previous reports (Seliem et al., 2006; Maeshima et al., 2009). There was no correlation between loss of CD20 expression and interval of biopsies, treatment modalities, clinical response, or frequency and dose of Rx therapy (Maeshima et al., 2009). Jilani et al. reported down-regulation of CD20 expression at the RNA level after exposure of CLL cells to Rx (Jilani et al., 2003). Thus, evaluation of CD20 expression might identify a subset of CLL/SLL patients who will not benefit from repeated therapy with Rx.

Kennedy et al. reported that loss of CD20 expression after Rx treatment was frequently observed in cases of DLBCL, which resulted in progressive disease (Kennedy et al., 2002). A lower frequency, 4 of 21 cases (19%), of loss of CD20 expression after Rx treatment for DLBCL was reported from Japan (Figure 2) (Wada et al. 2009). The mean Rx dose administered until CD20-negative relapse in the report of Kennedy et al. (5 doses) was lower than that in our cases (9 doses), but the difference was not significant (Wada et al., 2009). The mean time between the last administration of Rx and CD20-negative relapse in the Kennedy et al. cases (5.3 months) was similar to that of our cases (4.5 months).

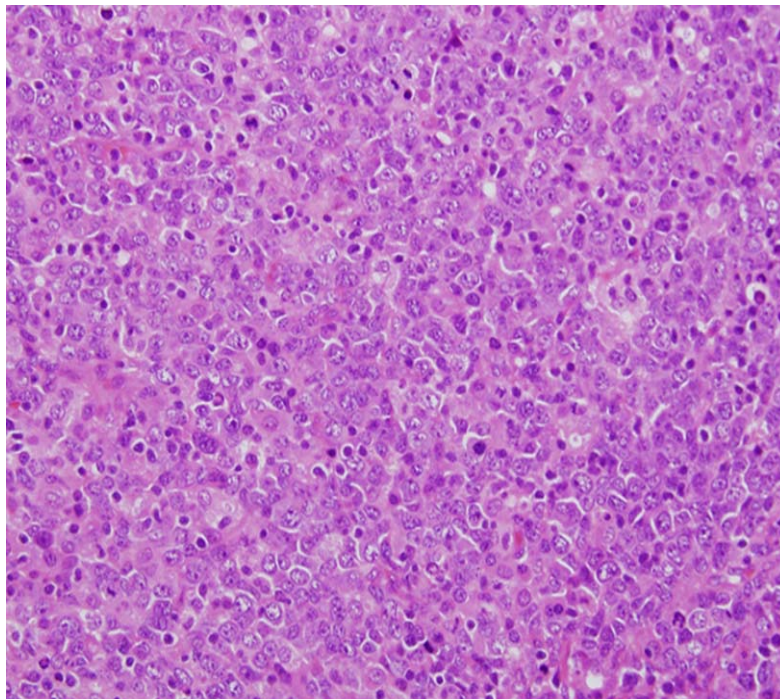


Figure 2. A. Initial diffuse large B-cell lymphoma (DLBCL) (one of our cases). H&E

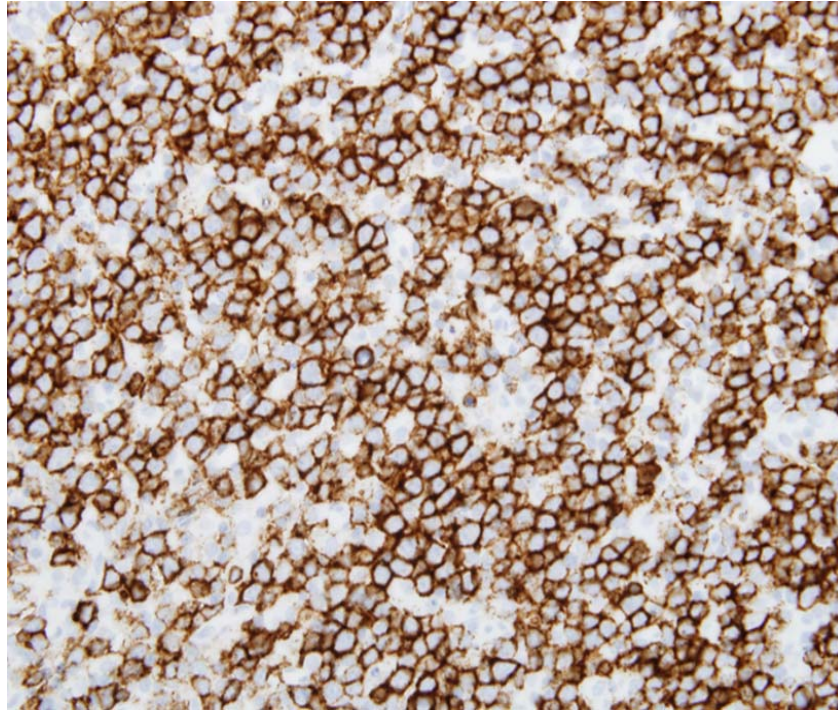


Figure 2. B. Tumor cells were CD20⁺ by immunohistochemistry

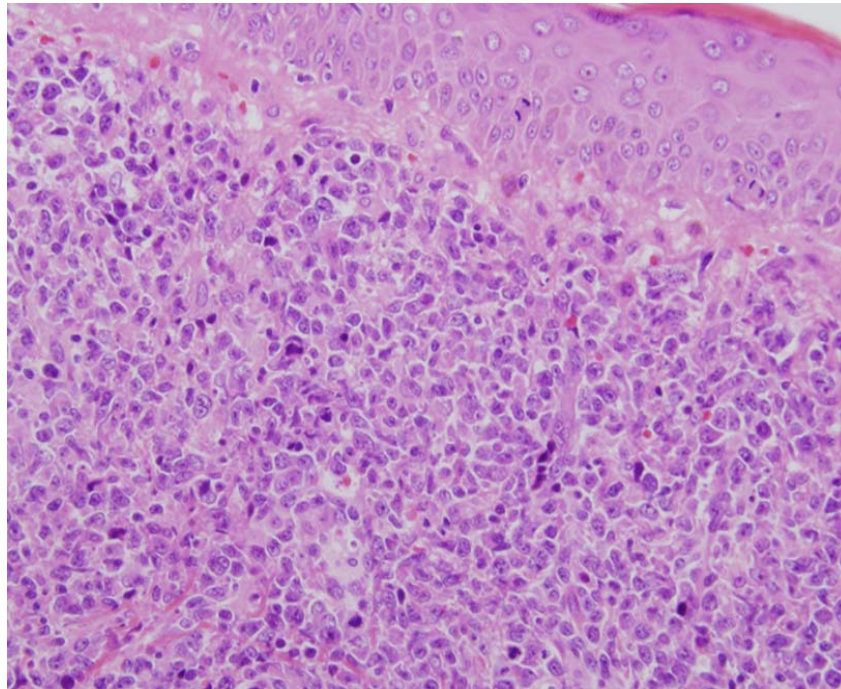


Figure 2. C. Recurrent DLBCL after rituximab treatment showed similar features to the initial DLBCL. H&E

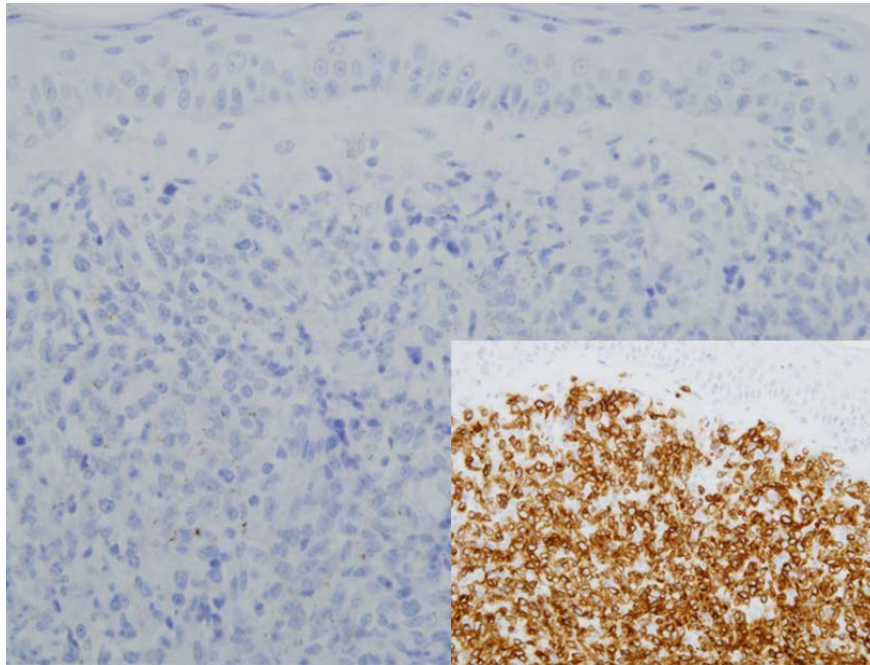


Figure 2. D. Recurrent tumor cells were CD20⁻. Inset: tumor cells were CD79a⁺. Magnification, x400

4. Conclusion

Loss of CD20 expression in neoplastic B-cells could be a cause of B-cell NHL unresponsiveness to Rx-containing chemotherapy, which might result in an unfavorable prognosis. Therefore, estimation of CD20 expression is a prerequisite for employment of Rx therapy for B-cell NHL.

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