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Double-hit pancreatic B-lymphoblastic lymphoma with a variant translocation t(2;18)(p11;q21)

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Abstract

Double-hit lymphoma is typically categorized as “high-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements”, but in infrequent cases in which terminal deoxynucleotidyl transferase (TdT) expression is positive, it is categorized as B-lymphoblastic lymphoma (B-LBL). *BCL2* rearrangements are usually caused by t(14;18)(q32;q21); variant translocations are very rare. Here, we describe an unusual case of double-hit pancreatic B-LBL with a variant translocation t(2;18)(p11;q21). A 69-year-old man was admitted because of an abdominal mass. Computed tomography scans demonstrated a diffusely enlarged pancreas and massive ascites. Cell block preparations of ascites cells revealed marked proliferation of blastic lymphoid cells positive for CD19, CD10, CD79a, PAX5, and TdT, indicating a diagnosis of B-LBL. G-banding and spectral karyotyping showed 45,XY,+X,t(2;18)(p11;q21),-4,der(5)t(1;5)(q12;p15),der(6)t(6;21)(q21;q?),t(8;14)(q24;q32),-15. Fluorescence *in situ* hybridization detected split *BCL2* and *IGH/MYC* fusion signals. Almost all ascites cells were diffusely and strongly positive for MYC and BCL2. The patient died of progressive disease 20 days after admission. To our knowledge, this is the first reported case of *MYC* and *BCL2* double-hit B-LBL with t(2;18)(p11;q21). High coexpression of MYC by t(8;14) and BCL2 by t(2;18) may be implicated in the development of B-LBL. Furthermore, double-hit B-LBL may be associated with a less favorable outcome compared with typical B-LBL.

Key words: double-hit lymphoma, variant translocation, t(2;18)(p11;q21), B-lymphoblastic lymphoma, primary pancreatic lymphoma

Introduction

Double-hit lymphoma (DHL) that has a *MYC* rearrangement in combination with a *BCL2* and/or *BCL6* rearrangement is recognized as a distinct entity within B-cell lymphomas and is associated with poor survival outcomes when treated with standard chemotherapy [1]. Under the 2016 revision of the World Health Organization classification of lymphoid neoplasms, DHL is included in a single category as a "high-grade B-cell lymphoma (HGBL), with *MYC* and *BCL2* and/or *BCL6* rearrangements", except for cases that fulfill the criteria for a follicular lymphoma (FL) or lymphoblastic lymphoma (LBL) [2]. In DHL, rearrangement partners of *MYC* at 8q24 involve non-immunoglobulin (non-*IG*) genes as well as the immunoglobulin (*IG*) genes, *IGH*, *IGK*, and *IGL*, by t(8;14)(q24;q32), t(2;8)(p11;q24), and t(8;22)(q24;q11), respectively [3,4]. In comparison, rearrangements of *BCL2* at 18q21 are usually caused by t(14;18)(q32;q21), while the variant translocations t(2;18)(p11;q21) and t(18;22)(q21;q11) involving *IGK* and *IGL*, respectively, are very rare [3,5-10].

LBL is a highly aggressive neoplasm of T-/B-lymphoblasts resembling acute lymphoblastic leukemia (ALL), with no or limited bone marrow involvement [11]. The diagnostic hallmark is the expression of a T-/B-precursor immunophenotype. B-LBL is always positive for the B-cell markers CD19, CD79a, and CD22. Furthermore, CD10, CD24, PAX5, and terminal deoxynucleotidyl transferase (TdT) are expressed in most cases. Of these, TdT is usually used to determine the immaturity of B-LBL. Namely, DHL is infrequently categorized into B-LBL when TdT expression is positive [2]; a small number of cases of double-hit B-LBL have been reported [12-15]. Here, we describe an unusual case of double-hit B-LBL with t(2;18)(p11;q21), which arose mainly from the pancreas.

Case report

A 69-year-old man was admitted to our hospital because of an abdominal mass, ascites, and

icterus. He did not have any significant medical history, including lymphadenopathy. Peripheral blood analysis yielded hemoglobin 11.2 g/dL, platelets 389×10^9/L, and leukocytes 6.7×10^9/L with 81% segmented neutrophils, 1% eosinophils, 9% monocytes, and 9% lymphocytes. Blood chemistry revealed hepatic and pancreatic dysfunction: aspartate aminotransferase 214 U/L (normal range, 13–31 U/L), alanine aminotransferase 187 U/L (8–34), γ-glutamyl transpeptidase 666 U/L (9–57), alkaline phosphatase 3418 U/L (109–321), lactate dehydrogenase 783 U/L (115–217), total bilirubin 16.2 mg/dL (0.3–1), direct bilirubin 13.3 mg/dL (0–0.2), amylase 250 U/L (37–125), lipase 620 IU/L (16–60), and elastase 1 4605 ng/dL (0–300). Computed tomography scans of the abdomen demonstrated a diffusely enlarged pancreas, and a mass lesion in the portal hepatis with intrahepatic bile duct dilatation (Fig. 1a,b). Endoscopic ultrasound–guided fine needle aspiration biopsy (EUS–FNAB) detected the infiltration of atypical blastic cells positive for CD10, CD79a and PAX5 among pancreatic acinar cells (Fig. 2a,b,c,d). These findings suggested a diagnosis of B-LBL, although the positive rate of TdT expression was low (Fig. 2f).

Ascites progressively increased after admission (Fig. 1c,d). We performed an abdominal paracentesis and analyzed ascites cells. May–Grünwald–Giemsa–stained cytospin preparations showed medium to large-sized blastic lymphoid cells with fine nuclear chromatin, nucleoli, and basophilic cytoplasm (Fig. 3a). Hematoxylin and eosin–stained cell block preparations showed the marked proliferation of blastic atypical lymphoid cells with densely stained, round nuclei (Fig. 3b). Immunohistochemistry on cell blocks revealed that these cells were diffusely and strongly positive for CD19, CD10, CD79a and PAX5 (Fig. 3c,d,e,f). Only 20% of cells were sporadically positive for CD20 (Fig. 3g), whereas about 60% of cells were positive for TdT (Fig. 3h). Double staining for TdT and CD20 demonstrated that cells were divided into large-sized TdT(-)CD20(+), medium-sized TdT(+)CD20(-), and TdT(-)CD20(-) populations, and without any apparent TdT(+)CD20(+) cells (Fig. 3i). The

positive rate of Ki-67 was about 40% (Fig. 3j). Immunophenotyping with flow cytometry (FCM) showed that gated lymphoid cells were positive (>20%) for CD10 (87.2%), CD19 (96.8%) and HLA-DR (50.4%) but negative for CD20, CD34, κ-chain, λ-chain, and other T-lymphoid and myeloid markers (Fig. 4). Unfortunately, we could not examine TdT expression by FCM.

Chromosome analysis of ascites cells showed 45,XY,+X,t(2;18)(p11;q21),-4,der(5)t(1;5)(q12;p15),add(6)(q13),t(8;14)(q24;q32),-15[16] (Fig. 5a). Spectral karyotyping (SKY) confirmed both der(2)t(2;18)(p11;q21) and der(18)t(2;18)(p11;q21) (Fig. 5b). SKY also confirmed der(14)t(8;14)(q24;q32), whereas the small segment 14q32→14qter on der(8)t(8;14)(q24;q32) could not be visualized. Finally, the karyotype was revised as follows: 45,XY,+X,t(2;18)(p11;q21),-4,der(5)t(1;5)(q12;p15),der(6)t(6;21)(q21;q?),t(8;14)(q24;q32),-15. Fluorescence *in situ* hybridization (FISH) detected split 5' *BCL2* and 3' *BCL* signals on der(2)t(2;18) and der(18)t(2;18) in all 18 metaphase spreads, respectively (Fig. 5c). FISH also detected *IGH/MYC* and *MYC/IGH* fusion signals on der(14)t(8;14) and der(8)t(8;14) in 19 of 20 metaphase spreads, respectively (Fig. 5d). Immunohistochemistry revealed that almost all ascites cells were diffusely and strongly positive for MYC and BCL2 (Fig. 3k,l). Furthermore, blastic cells infiltrating into the pancreas were also strongly positive for MYC and BCL2 (Fig. 2h,i).

Bone marrow biopsy showed normocellular marrow with no evidence of lymphoma cell infiltration. Considering the characterization of an immature B-cell immunophenotype (CD19+, CD10+, TdT+, surface IG-), and rearrangements of *MYC* and *BCL2* leading to high expression of MYC and BCL2, a diagnosis of *de novo* double-hit, double-expressor B-LBL was made. Before chemotherapy, we placed an endoscopic retrograde biliary drainage tube to reduce obstructive jaundice. The levels of total bilirubin, direct bilirubin, and alkaline

phosphatase decreased from 25.3 to 6.9 mg/dL, from 21.7 to 2.8 mg/dL, and from 4813 to 2555 U/L, respectively, within 4 days. After initial therapy with prednisolone and rasburicase, reduced CHOP therapy (cyclophosphamide, doxorubicin, vincristine and prednisolone) was started because of a poor performance status (PS4), and liver and renal dysfunction. However, the response to chemotherapy was poor and the patient subsequently died of progressive disease 20 days after admission. Autopsy was not performed.

Discussion

We have detected a rare variant translocation t(2;18)(p11;q21) and confirmed *MYC* and *BCL2* rearrangements that led to MYC and BCL2 dual expression in ascites cells of *de novo* pancreatic B-LBL. The patient presented with an aggressive clinical course, resistance to chemotherapy, and a poor outcome. As shown in Table 1, six cases of hematological malignancies with t(2;18)(p11;q21) and 8q24 translocations have been reported: four presented with t(8;14)(q24;q32) and two presented with t(8;22)(q24;q11) [3,6-10]. Among these possible DHL cases, rearrangements of both *MYC* and *BCL2* were confirmed only in the present case. The diagnoses were heterogeneous: diffuse large B-cell lymphoma (DLBCL), FL, B-cell lymphoma, unclassifiable (BCLU), and B-LBL. Thus, this is the first case of *MYC* and *BCL2* double-hit B-LBL with t(2;18)(p11;q21). High coexpression of MYC by t(8;14) and BCL2 by t(2;18) may be implicated in the development of B-LBL, although, compared with a standard translocation, the influence of this variant translocation on BCL2 expression and disease phenotype remains to be fully elucidated.

With regard to partner genes in DHL, Johnson et al. analyzed 54 cases and showed that *MYC* partner sites were heterogeneous: 16 cases with t(8;14)(q24;q32), 11 cases with t(8;22)(q24;q11), 3 cases with t(2;8)(p11;q24), 13 cases with t(8;9)(q24;p13), and 11 cases with others [3]. In comparison, almost all partner sites of *BCL2* translocations were *IGH*: 51

cases with t(14;18)(q43;q21), 1 case with t(2;18)(p11;q21), 1 case with t(18;22)(q21;q11), and 1 case was unknown. Tomita et al. also showed that *MYC* partners varied in 27 cases of DHL: 14 cases with t(8;14), 4 cases with t(2;8), and 9 cases with t(8;22). Similarly, there were 26 cases with t(14;18) and 1 case with t(2;18) [10]. Accordingly, compared to the heterogeneity of *MYC* partner genes, variant *BCL2* translocations seem to be extremely rare in DHL. In general, t(14;18)(q32;q21) is specifically associated with FL, whereas the variant translocations t(2;18)(p11;q21) and t(18;22)(q21;q11) are preferentially associated with chronic lymphocytic leukemia as well as FL [5]. In contrast to the breakpoint at the 3' untranslated exon 3 (mbr) of *BCL2* in FL with t(14;18), it has been shown that t(2;18) resulted in the juxtaposition of the 5' region of *BCL2* and the J segment of *IGK* [7]. Cloning of *BCL2* breakpoints will be helpful to elucidate the pathogenesis of DHL with t(2;18).

Double-hit B-LBL is uncommon and may arise *de novo* or through transformation from FL [12-15]. Especially, *de novo* double-hit B-LBL is very rare, and only 11 cases including ALL have been reported and shown to be associated with frequent central nervous system involvement and complex karyotypes [14,15]. All previously reported double-hit B-LBL cases showed t(14;18)(q32;q21) resulting in an *IGH/BCL2* fusion and the acquisition of *MYC* rearrangement. The prognosis has been very poor with a median overall survival of 1.5 months and 4 months in *de novo* and transformed cases, respectively [13]. It is possible that patients with *de novo* double-hit B-LBL may have a preexisting low-grade FL, which may be masked by the aggressive lymphoma [13,16]. In the present case, a history or clinicopathological finding indicating a preexisting FL was not present, so we subsequently diagnosed the disease as *de novo* B-LBL. The diagnosis of a double-hit B-LBL was based on positive TdT expression. However, HGBL with TdT expression may present a diagnostic challenge, particularly when additional features are not supportive of immaturity [16,17]. The features that suggested an immature neoplasm included TdT positivity, diminished

CD45, a lack of CD20 or surface IG light chain expression by FCM, and a blastoid morphology [16]. The present case fulfilled these criteria.

Recently, Ok et al. summarized 13 cases of HGBL with TdT expression and divided these into four groups: 1) *de novo* HGBL with double-hit or triple-hit genetics and TdT expression, 2) FL followed by TdT-positive aggressive B-cell lymphoma (BCL), 3) initial TdT-negative aggressive BCL in patients who previously had FL, followed by relapses in which the neoplasm acquired TdT expression, and 4) mature BCL that acquired TdT expression at relapse [17]. For group 1, they characterized two cases of *de novo* composite lymphoma in which two components of CD20(+)TdT(-) DLBCL and CD20(-)TdT(+) blastic BCL were present in the same lymph node. *MYC* and *BCL2* rearrangements were shown in both components in one case, suggesting that both components arose from a common clone. Alternatively, the TdT(-) DLBCL component may have an intermediate step in developing TdT(+) blastic BCL [17]. In the present case, double staining with TdT and CD20 on ascites cells revealed the coexistence of medium-sized TdT(+)CD20(-) cells and a small number of large-sized TdT(-)CD20(+) cells. A similar composite mechanism may occur in the present case, although lymphoma cells could not be histologically distinguished. Furthermore, it is likely that TdT(-)CD20(-) cells predominated in the pancreatic tissue (Fig.2e,f). Unfortunately, we could not confirm whether these three TdT(+)CD20(-), TdT(-)CD20(+), and TdT(-)CD20(-) populations were monoclonal. However, G-banding and FISH showed that 95% of ascites cells had *MYC* and *BCL2* rearrangements. Immunohistochemistry revealed that almost all lymphoma cells in ascites and pancreatic tissue strongly expressed MYC and BCL2. These findings suggest that *MYC* and *BCL2* rearrangements occurred in TdT(+)CD20(-), TdT(-)CD20(+), and TdT(-)CD20(-) cells. Namely, it is possible that these three populations may be monoclonal. Like the present case, patients with double-hit B-LBL have a dismal prognosis compared to those with typical B-LBL. Accordingly, Ok et al. proposed a descrip-

tive term such as “blastic B-cell lymphoma with rearrangements in *MYC*, *BCL2*, and/or *BCL6*” rather than a diagnosis of B-LBL [17].

Another noticeable finding is that B-LBL arose mainly from the pancreas with massive ascites. Primary pancreatic lymphoma (PPL) is a rare form of extranodal lymphoma that makes up fewer than 2% of extranodal lymphomas and 0.5% of all pancreatic masses [18]. The diffusely enlarged pancreas in the present case seemed to be PPL, although a localized extrahepatic mass lesion was also observed. The most predominant histological subtype of PPL is DLBCL, and, less frequently, lymphoplasmacytic lymphoma. Namely, primary pancreatic LBL is extremely rare, with only two cases of primary pancreatic LBL/ALL having been reported to date. One B-ALL case with t(4;11) showed a diffusely enlarged pancreas, similar to the present case, and the other B-LBL case, with a complex karyotype, showed a solid mass in the pancreatic body, which was diagnosed by EUS–FNAB [19, 20]. Both reported cases remained in complete remission after intensive chemotherapy, whereas the present case presented an early death. Thus, this is the first case of pancreatic B-LBL with t(2;18)(p11;q21) and double-hit rearrangements that led to an unfavorable outcome.

Conflict of interest: The authors declare that they have no conflict of interest.

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Figure legends

Fig. 1. Radiological findings of the abdomen.

(a)(b) Contrast-enhanced computed tomography (CT) scans of the abdomen on admission. A diffuse enlargement infiltrating or replacing most of the pancreatic gland with mild dilatation of the main pancreatic duct is observed. Enhancement of the pancreas is poor but homogeneous. An extrahepatic soft tissue mass (arrow) with dilatation of the intrahepatic biliary duct was also found in the portal hepatis.

(c)(d) Unenhanced CT scans 10 days after admission. Massive ascites and pancreatic swelling are observed.

Fig. 2. Pathological findings of the pancreatic tissue collected by EUS-FNAB.

(a) Hematoxylin and eosin (H&E)–stained pancreatic tissue shows the infiltration of medium-sized atypical blastic cells with densely stained round nuclei among pancreatic acinar cells (×200).

(b)–(i) Immunohistochemistry of pancreatic tissue. Almost all lymphoma cells are diffusely and strongly positive for CD10 **(b)**, CD79a **(c)**, and PAX5 **(d)**. Only 10% of lymphoma cells are positive for CD20 **(e)** and TdT **(f)**. The positive rate of Ki-67 is about 80% **(g)**. Almost all lymphoma cells are diffusely and strongly positive for MYC **(h)** and BCL2 **(i)**.

Fig. 3. Pathological findings of ascites cells.

(b) May–Grünwald–Giemsa–stained cytospin preparations show medium- to large-sized blastic lymphoid cells with fine nuclear chromatin, nucleoli, and basophilic cytoplasm (×1000).

(c) H&E–stained cell block preparations show the proliferation of medium-sized blastic lymphoid cells with densely stained round nuclei (×400).

(c)–**(l)** Immunohistochemistry of cell block preparations (×400). Almost all lymphoma cells are diffusely and strongly positive for CD19 **(c)**, CD10 **(d)**, CD79a **(e)**, and PAX5 **(f)**. Only 20% of cells are sporadically positive for CD20 **(g)**, whereas about 60% of cells are positive for TdT **(h)**. Double staining for TdT and CD20 show that nuclei and surfaces of cells are positive for TdT (brown) and CD20 (red), respectively **(i)**. Cells are divided into large-sized TdT(-)CD20(+), medium-sized TdT(+)CD20(-), and TdT(-)CD20(-) populations, with no apparent TdT(+)CD20(+) double-positive cells. The positive rate of Ki-67 is about 40% **(j)**. Almost all cells are diffusely and strongly positive for MYC **(k)** and BCL2 **(l)**.

Fig. 4. Flow cytometric findings of ascites cells at diagnosis of B-LBL by CD45/side scatter (SSC) gating. The corresponding cell percentage demarcated by the gate is 95.3%. The results of two-color analyses with CD7 and CD10, CD19 and CD13, CD5 and CD23, CD25 and CD4, CD3 and CD20, CD34 and HLA-DR, and λ-chain and κ-chain for gated cells are shown. Corresponding cell percentages in each fraction are indicated. The gated cells are positive (>20%) for CD10, CD19 and HLA-DR.

Fig. 5. Cytogenetic findings of ascites cells.

(a) G-banded karyotype of ascites cells at diagnosis of B-LBL: 45,XY,+X,t(2;18)(p11;q21),-4,der(5)t(1;5)(q12;p15),add(6)(q13),t(8;14)(q24;q32),-15. Arrows indicate rearranged chromosomes.

(b) Spectral karyotyping (SKY) of metaphase spreads after spectrum-based classification (left side, reverse DAPI; right side, SKY). Only chromosomes 2, 8, 14 and 18 are shown. Two derivative chromosomes, der(2)t(2;18)(p11;q21) and der(18)t(2;18)(p11;q21), are confirmed. SKY also confirmed der(14)t(8;14)(q24;q32), whereas the small segment 14q32→14qter on der(8)t(8;14)(q24;q32) could not be visualized since this segment may

be smaller than the minimum genomic alteration detectable by SKY. Arrows indicate re-arranged chromosomes.

(c) Fluorescence *in situ* hybridization (FISH) with a Vysis LSI BCL2 Break Apart FISH Probe Kit (Abbott Molecular, Abbott Park, IL, USA) on metaphase spreads and interphase nuclei. Arrows indicate 1) a normal 5' *BCL2*/3' *BCL2* fusion signal (red/green, yellow) on a normal chromosome 18, 2) a 5' *BCL2* signal (red) on der(2)t(2;18), and 3) a 3' *BCL2* signal (green) on der(18)t(2;18). One each of yellow, red and green signals are also observed on an interphase nucleus (left).

(d) FISH with Vysis LSI IGH/MYC/CEP 8 Tri-Color Dual Fusion Probe Kit (Abbott Molecular) on metaphase spreads and interphase nuclei. Arrows indicate 1) *MYC* (red) and CEP 8 (blue) signals on a normal chromosome 8, 2) an *IGH* signal (green) on a normal chromosome 14, 3) *MYC/IGH* fusion (red/green, yellow) and CEP 8 signals on der(8)t(8;14), and 4) an *IGH/MYC* signal on der(14)t(8;14). Two yellow, one red, one green, and two blue signals are also observed on an interphase nucleus (inset).

Fig. 1

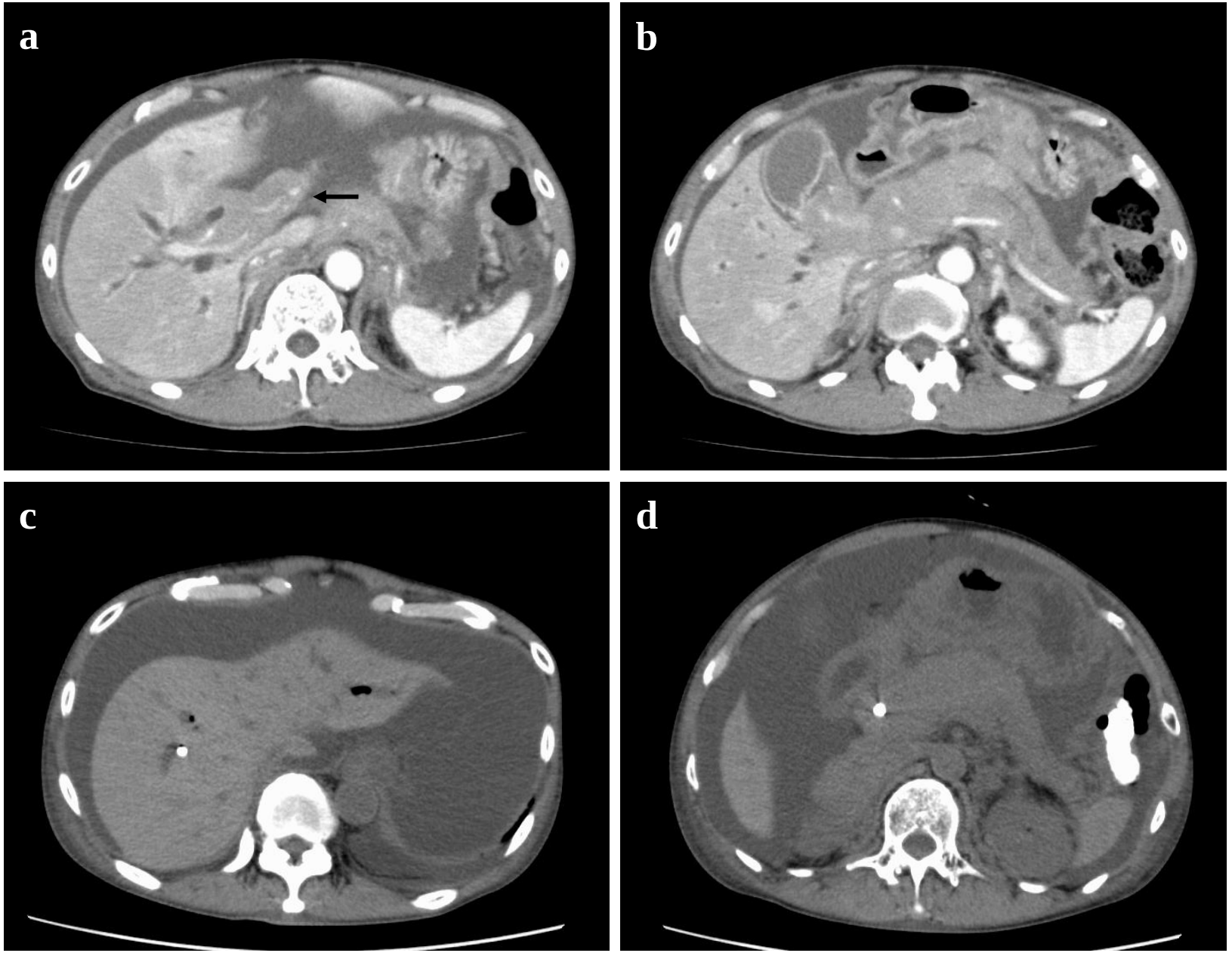

Fig. 2

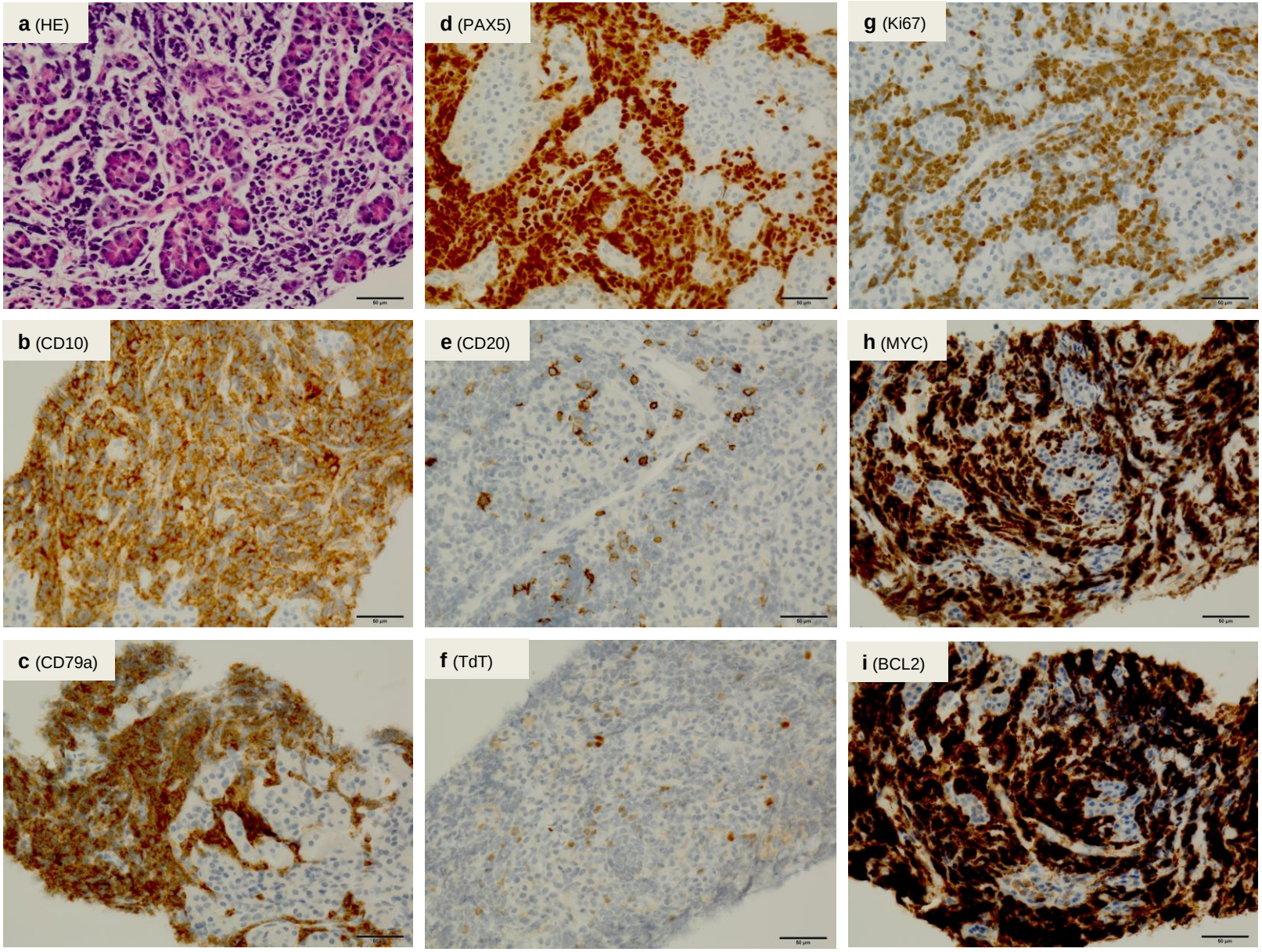

Fig. 3

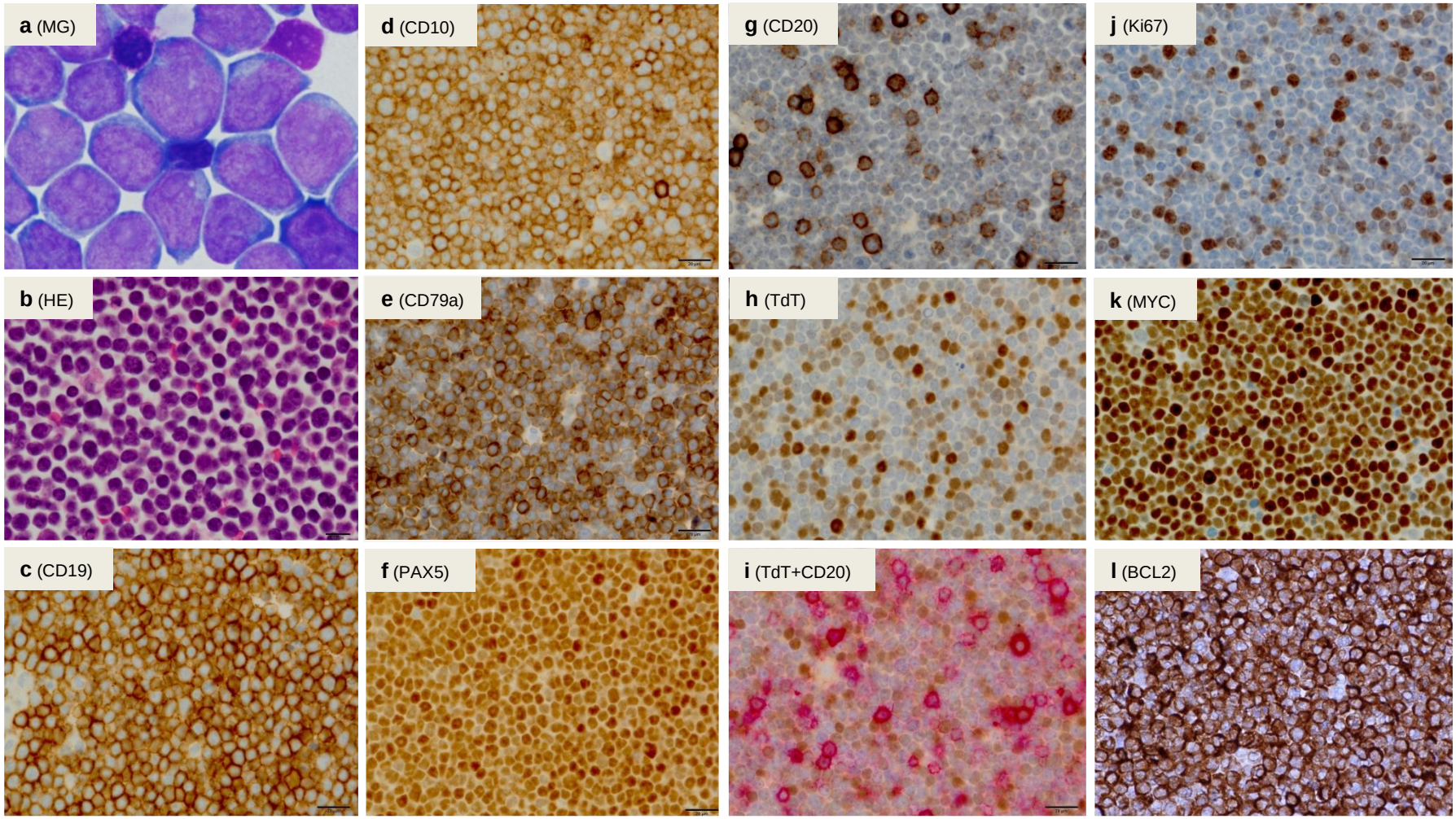
a (MG)
d (CD10)
g (CD20)
j (Ki67)
b (HE)
e (CD79a)
h (TdT)
k (MYC)
c (CD19)
f (PAX5)
i (TdT+CD20)
l (BCL2)

Fig. 4

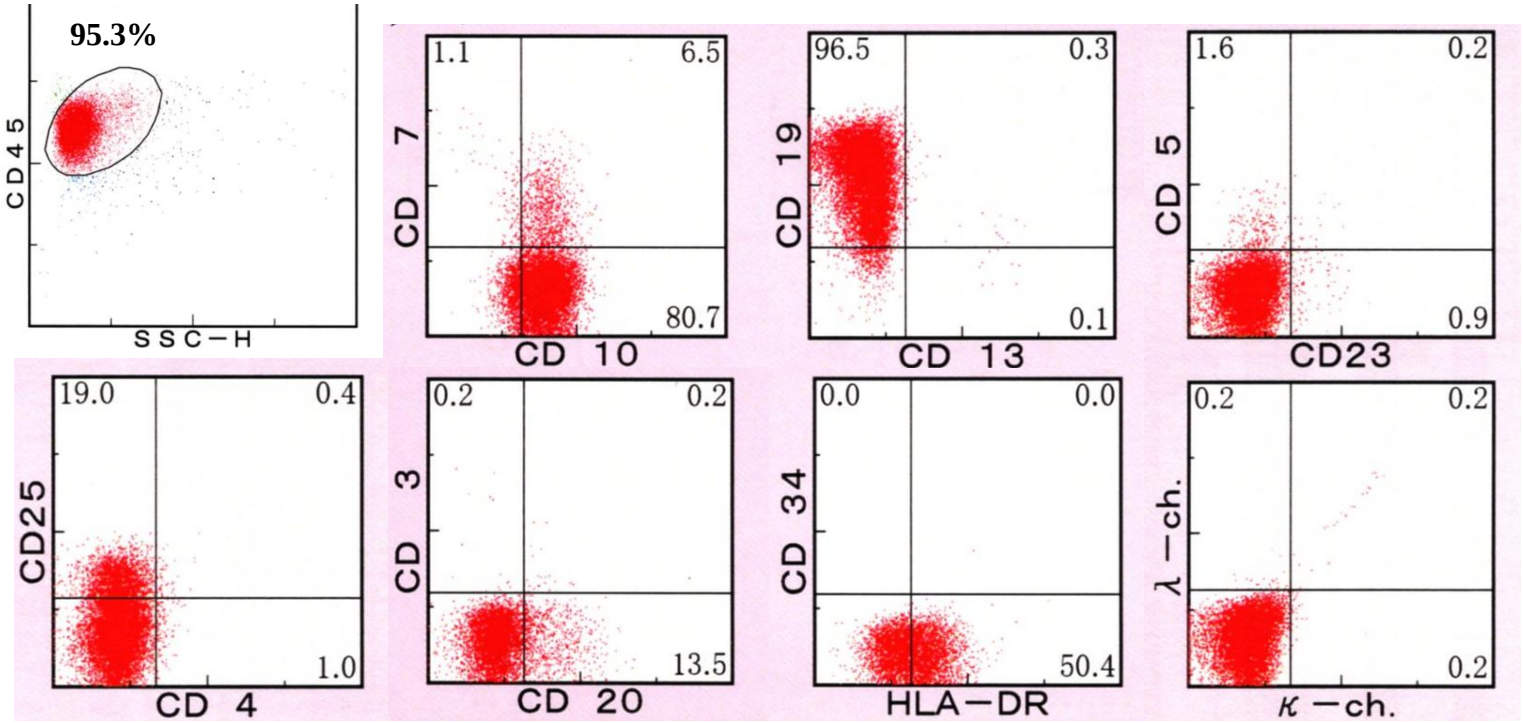
95.3%
CD45
SSC−H
1.1
6.5
80.7
CD 7
CD 10
96.5
0.3
0.1
CD 19
CD 13
1.6
0.2
0.9
CD 5
CD23
19.0
0.4
1.0
CD25
CD 4
0.2
0.2
13.5
CD 3
CD 20
0.0
0.0
50.4
CD 34
HLA−DR
0.2
0.2
0.2
λ−ch.
κ−ch.

Fig. 5

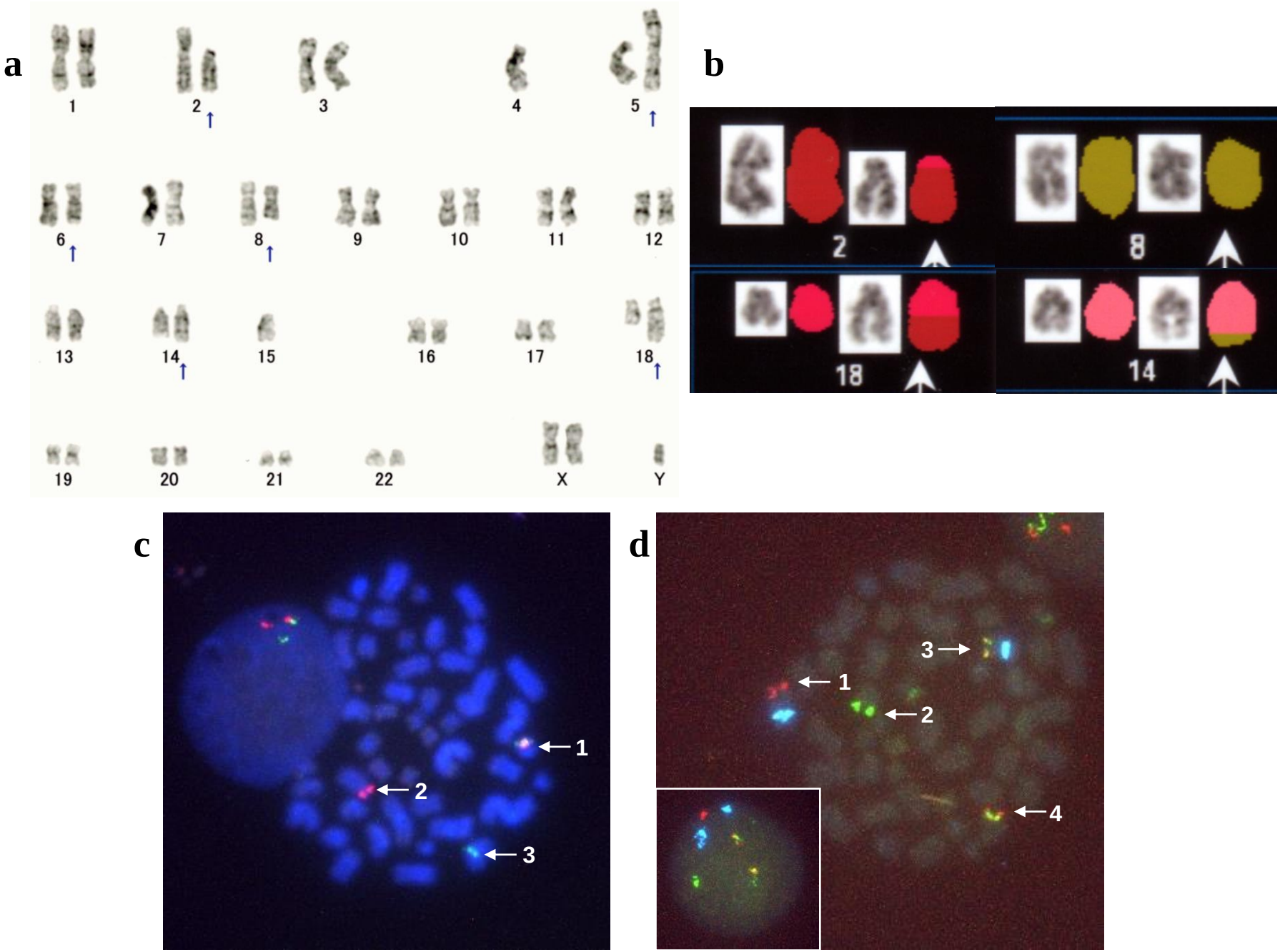
a
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
b
2
8
18
14
c
1
2
3
d
3
1
2
4

Table 1. Reported cases of hematological malignancies associated with t(2;18)(p11;q21) and 8q24 translocations

Case No.	*Age/ Sex*	*Diagnosis*	*Karyotypes*	*BCL2*	*MYC*	*References*
1	36/F	FL→ BLL	47,XX,-1,+der(1)t(1;?)(p36;?),**t(2;18)(p11;q21)**,**t(8;22)(q24;q11)**,-11,+der(11)t(7;11)(q11;q24), +der(11)t(7;11)(q11;q24),-13,+r	NA	NA	Aventin et al., 1990 [6]
2	61/F	DLBCL	50,XX,+X,+der(1;?)(q12;?),**t(2;18)(p11;q21)**,**t(8;14)(q24;q32)**,+12,+add(18)(q?)	R	NA	Hillion et al., 1991 [7]
3	NA/M	FL	47,XY,**t(2;18)(p11;q21)**,**t(8;14)(q24;q32)**,+der(18)t(2;18)(p11;q21)	NA	NA	Juneja et al., 1997 [8]
4	56/F	FL→ BCLU	46,X,-X,**t(2;18)(p11;q21)**,der(3)t(3;7)(q21;q11),der(4)t(3;4)(q12;p15),add(6)(?q21),+7,+8, **t(8;22)(q24;q11)**,t(12;15)(q24;q12),del(13)(q13q31),del(15)(q12q15)[6]/45,idem,-16[2]/ 47,idem,+del(7)(q?)[cp2]	R	NA	Henderson et al., 2004 [9]; Johnson et al., 2009 [3]
5	NA/M	BCLU	46,XY,i(1)(q10),**t(2;18)(p11;q21)**,t(3;22)(q27;q11),**t(8;14)(q24;q32)**,der(14)t(8;14)[20]	NA	NA	Tomita et al., 2009 [10]
6	69/M	B-LBL	45,XY,+X,**t(2;18)(p11;q21)**,-4,der(5)t(1;5)(q12;p15),der(6)t(6;21)(q21;?),**t(8;14)(q24;q32)**,-15[16]	R	R	present case

Abbreviations: F, female; M, male; NA, not available; FL, follicular lymphoma; BLL, Burkitt-like lymphoma; DLBCL, diffuse large B-cell lymphoma; BCLU, B-cell lymphoma, unclassifiable; B-LBL, B-lymphoblastic lymphoma; R, rearranged. Both t(2;18)(p11;q21) and 8q24 translocations are described in bold letters.