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Original contribution

Mucin 4 protein is expressed in B-acute lymphoblastic leukemia and is restricted to BCR::ABL1-positive and BCR::ABL-like subtypes☆

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Summary Mucin 4 (MUC4) is a transmembrane mucin that, like most mucins, is not expressed in normal hematopoietic cells, but little is known about its expression in malignant hematopoiesis. B-acute lymphoblastic leukemia (B-ALL) consists of genetically distinct disease subtypes with similarities and differences in gene expression most frequently studied at the mRNA level, which is less amenable to widespread routine clinical use. Here, we demonstrate using immunohistochemistry (IHC) that MUC4 protein is expressed in less than 10% of B-ALL, with expression restricted to *BCR::ABL1*+ and *BCR::ABL1*-like (*CRLF2* rearranged) subtypes of B-ALL (4/13, 31%). None (0/36, 0%) of the remaining B-ALL subtypes expressed MUC4. We compare clinical and pathologic features of MUC4+ and MUC4− *BCR::ABL1*+/like cases and most significantly report a possible shorter time to relapse for MUC4+ *BCR::ABL1* B-ALL that would need to be validated in larger studies. In conclusion, MUC4 is a specific, albeit insensitive, marker for these high-risk subtypes of B-ALL. We propose that MUC4 IHC may be used diagnostically to rapidly identify these B-ALL subtypes, particularly in resource-limited settings or when an aspirate sample is not available for ancillary genetic studies.
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1. Introduction

Mucin 4 (MUC4) is a transmembrane glycoprotein and a member of the large family of secreted or transmembrane mucins, characterized structurally by tandem repeat extracellular PTS (proline, threonine, serine) domains, which undergo extensive O-glycosylation [1,2]. Like other members in this protein family, MUC4 is normally expressed on the luminal surface of epithelial cells, such as respiratory, colonic, gastric, and cervical epithelium, where the protein provides a barrier function, protecting against cellular injury. However, transmembrane mucins like MUC4 can also be involved in downstream signaling, particularly in pathologic states. With the exception of MUC1 (recognized by the epithelial membrane antigen/EMA and other antibodies) [3], physiologic protein expression of mucins, including MUC4, has not been reported in hematopoietic cells or hematopoietic organs.

Despite their generally protective function, upregulation or aberrant expression of certain mucins has been reported in some human malignancies and neoplastic proliferations. MUC4 is known to be overexpressed in a subset of pancreatic, breast, colonic, and lung carcinomas, and MUC4 immunohistochemistry (IHC) has recently emerged as a sensitive and specific marker for low-grade fibromyxoid sarcoma [4,5]. IHC for other mucins—MUC1, MUC2, MUC5AC, and MUC6—is routinely used in diagnostic pathology to clarify the cell of origin or differentiation of neoplasms such as intraductal papillary mucinous neoplasm or gastric carcinoma. MUC1 (EMA) detection is also relevant in hematopathology as this protein is expressed in neoplastic cells in a subset of Hodgkin lymphoma (more likely nodular lymphocyte predominant type) as well as in some non-Hodgkin lymphoma (such as anaplastic large cell lymphoma). Little is known about MUC4 protein expression in hematologic malignancies.

B-acute lymphoblastic leukemia (B-ALL) is the most prevalent malignancy in the pediatric population, although about 40% of total cases occur in adults. It is characterized by the proliferation of immature B lymphoid blasts, most frequently replacing the marrow and circulating in peripheral blood ("leukemia"); less frequently, this disease may manifest as a tissue-based mass lesion without appreciable bone marrow involvement ("lymphoblastic lymphoma"). Until recently, the classification of B-ALL was based on the identification of recurrent genetic abnormalities: *BCR::ABL1* (Philadelphia chromosome; Ph+), *ETV6::RUNX1*, *TCF3::PBX1*, *IGH::IL3*, *MLL* rearrangement, internal amplification of chromosome 21 (iAMP21), hyperdiploidy and hypodiploidy. The 2017 World Health Organization (WHO) classification introduced a new subtype *BCR::ABL1*-like (Ph-like B-ALL), previously included in the B-ALL not otherwise specified (NOS) category, that was identified based on the presence of a gene expression profile similar to *BCR::ABL1*+ B-ALL but lacking the *BCR::ABL1* translocation [6,7]. Instead, about 90% of *BCR::ABL1*-like B-ALL cases harbor other genetic alterations resulting in the activation of kinase and cytokine receptor signaling, most commonly rearrangements involving *CRLF2* [8]. *BCR::ABL1*+ and *BCR::ABL1*-like B-ALL are considered high-risk subsets of B-ALL and together comprise about 10–20% of pediatric and about 50% of adult B-ALL [6,9,10].

Gene expression profiling has previously identified MUC4 overexpression at the RNA level in *BCR::ABL1*+ and *BCR::ABL1*-like B-ALL compared with other subtypes of B-ALL [7,11,12]. MUC4 mRNA overexpression is included in the 8-gene low-density array (LDA) predictor used in the identification of *BCR::ABL1*-like B-ALL cases in Children's Oncology Group clinical trials [13]. We sought to evaluate whether MUC4 mucin is indeed expressed at the protein level in B-ALL, to determine its differential expression in subtypes of B-ALL and the potential significance of such differential expression.

2. Materials and methods

2.1. Patient selection

Patients diagnosed with B-ALL between 2015 and 2019 at University Hospitals (UH) Cleveland Medical Center (UHCMC) and UH Rainbow Children's Hospital were identified from the UH pathology information system and a clinical database maintained by the Department of Pediatric Hematology/Oncology at Rainbow Children's Hospital. Initial diagnostic bone marrow samples were used for staining in the majority of cases. For 2 cases, the initial bone marrow biopsy was not available, and relapse bone marrow biopsy specimens from these patients were stained instead. Patients with inadequate or unavailable B-ALL positive bone marrow archival blocks for immunohistochemical evaluation were excluded. Only de novo B-ALL cases were included; cases with lymphoid blast phase of a preceding chronic myeloid leukemia were excluded.

In total, there were 49 patients, who ranged from 1 to 82 years old at the time of initial diagnosis. A total of 11 normal bone marrow samples were used as controls. These consisted of negative staging bone marrow biopsy specimens from patients with Hodgkin lymphoma (n = 4), neuroblastoma (n = 3), and diffuse large B cell lymphoma (n = 4). The ages of the patients ranged from 4 months to 64 years, with a mean age of 27 years and a median of 17 years. The study was approved by the UH Institutional Review Board.

2.2. B-ALL diagnosis and classification

All B-ALL diagnosis and classification was made by board-certified hematopathologists based on morphologic evaluation, flow cytometry, and genetic studies and

conformed to WHO 2017 criteria. Standard karyotype and fluorescence in situ hybridization (FISH) testing to identify B-ALL with *BCR::ABL1*, hyperdiploidy (FISH probes for +4, +10, +17), *ETV6::RUNX1*, *TCF3::PBX1*, hypodiploidy, and *MLL* rearrangement (*KMT2A* break apart probe) were performed at UHCMC. *BCR::ABL1*-like cases were identified by *BCR::ABL1*-like FISH panel testing at Cincinnati Children's Hospital (https://www.testmenu.com/cincinnatichildrens/Tests/540531) for the commonest *BCR::ABL1*-like associated rearrangements (breakapart probes for *CRLF2*, *ABL2*, *PDGFRB*, *CSF1R*, JAK2, *ABL1*, *EPOR*) or by *BCR::ABL1*-like LDA testing followed by confirmatory genetic testing for positive cases performed at UNM/Tricore Laboratories [13] for patients enrolled in COG trials. All *BCR::ABL1*-like cases in our cohort had a *CRLF2* rearrangement and overexpressed CRLF2 by flow cytometry performed at UHCMC (phycoerythrin conjugated anti-CRLF2/TSLP-R antibody, clone 1B4, Biolegend). Intrachromosomal amplification of chromosome 21 (iAMP21) is detectable using the RUNX1 probe used for *ETV6::RUNX1* testing. B-ALL with t(5;14) is detectable by standard karyotyping. However, no cases fulfilling this criterion were identified in our cohort. Patients for whom any defining cytogenetic abnormalities above were not identified were designated NOS. In 8 of 11 NOS cases, *BCR::ABL1*-like testing is unknown because testing was either not performed or was unsatisfactory. Karyotype on 2 of these NOS cases yielded a culture failure, but FISH testing was negative for recurrent abnormalities. Nevertheless, the possibility of hypodiploidy in these cases cannot be completely excluded. B-ALL with t(5;14) is unlikely based on the lack of characteristic morphologic findings (increased eosinophils) in these patients' samples.

2.3. MUC4 IHC

The IHC was performed by the Tissue Resources division of the Human Tissue Procurement Facility (HTPF) at Case Western Reserve University. Unstained 5 µm sections of bone marrow cores (after B-plus fixation, decalcification, and paraffin embedding) were baked for 75 min at 60 °C. Deparaffinization (using xylene), antigen retrieval (using pH6.0 citrate buffer, 125 °C), and blocking of endogenous peroxidase and nonspecific antibody binding were performed prior to antibody staining. Prepared slides were stained with MUC4 mouse monoclonal antibody (8G7 clone from Cell Marque, 1:50 dilution, 1 hr at room temperature) and subsequently with horseradish peroxidase-based reagents (BioCare Medical M4U534). Pancreatic ductal adenocarcinoma (PDAC) specimens were used for antibody validation, titration, and optimization of staining conditions, and also as a positive control when B-ALL samples were evaluated. MUC4 stained B-ALL slides were reviewed in a blinded manner by a board-certified hematopathologist and a pathologist-in-training. Staining was cellular, and nonspecific stromal staining was not seen. The intensity of staining was scored as no staining (0), weak (1+), or strong (2+). Cases with both weakly staining and strongly staining blasts were scored as 2+. The specificity of MUC4 staining was confirmed by negative staining when the primary antibody was omitted from the staining protocol.

3. Results

3.1. MUC4 expression in B-ALL

To determine MUC4 protein expression in B-ALL, we selected a total of 49 B-ALL cases (24 pediatric and 25 adult) diagnosed from a bone marrow sample at our institution. This included 5 cases of *BCR::ABL1*-like B-ALL, all with a *CRLF2* rearrangement and overexpression by flow cytometry, 8 de novo *BCR::ABL1*+ B-ALL, and 33 cases representing the other most commonly recognized subtypes of B-ALL, determined based on cytogenetic studies performed at the time of diagnosis: hyperdiploidy (n = 10), *ETV6::RUNX1* (n = 6), *TCF3::PBX1* (n = 4), hypodiploidy (n = 2), *MLL* rearranged (n = 3), NOS (n = 11). No cases of B-ALL with t(5;14) and B-ALL with iAMP21 were represented.

Only 4 of these 50 B-ALL cases (8%) demonstrated positive staining for MUC4 in lymphoblasts. The MUC4 staining was diffusely positive in over 50% of blasts in all 4 cases. No MUC4 staining was seen in 11 normal pediatric and adult bone marrow cases, consistent with prior reports that MUC4 mucin is not normally expressed in the hematopoietic system. All 4 MUC4+ B-ALL cases were either *BCR::ABL1*+ (3/8, 38%) or *BCR::ABL*-like, *CRLF2* rearranged (1/5, 20% of cases). All 3 *BCR::ABL1*+ cases had strong (2+) staining while the positive *BCR::ABL1*-like case demonstrated weak (1+) staining (Fig. 1).

3.2. Pathologic features of MUC4+ and MUC4- *BCR::ABL1+/BCR::ABL1*-like B-ALL

We subsequently compared the clinical and pathologic features of MUC4+ *BCR::ABL1+/BCR::ABL1*-like B-ALL with MUC4− B-ALL in our cohort, which are summarized in Tables 1 and 2. All the *BCR::ABL1+/BCR::ABL1*-like cases showed extensive marrow involvement by lymphoblasts independent of MUC4 expression. Marrow blast frequency determined by aspirate or touch prep enumeration ranged from 70 to 92% (mean 82%) in the MUC4+ group and from 65 to 95% (mean 83%) in the MUC4− group. In about half of the cases (7/13), the blasts demonstrated typical B-ALL lymphoblast cytology of small cells with very scant cytoplasm, round nuclei, and dense to open chromatin. Variant morphology occurred similarly in both MUC4+ (2/4) and MUC4− (4/9) and included increased cell size, nuclear clefting/irregularities, abundant cytoplasm, and/or presence of cytoplasmic vacuolation or granules (Table 1; Fig. 2).

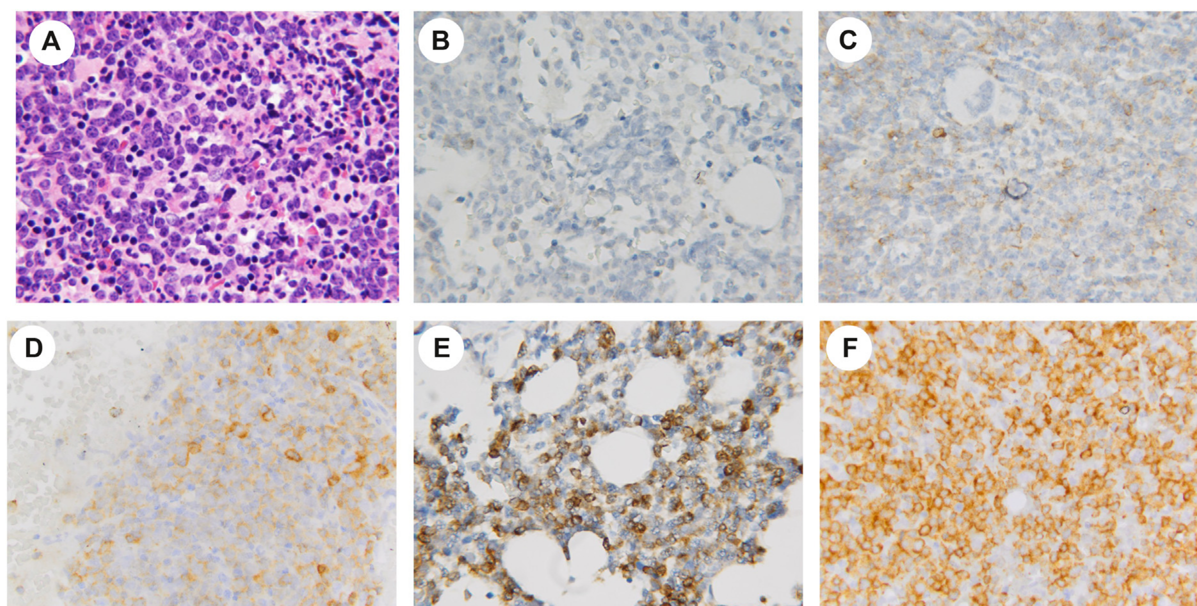

Fig. 1 MUC4 immunohistochemistry in B-ALL. A, Representative hematoxylin-eosin image of bone marrow core biopsy showing extensive marrow involvement by lymphoblasts. B, Representative negative staining of case #4 (score 0). C, Weak staining blasts of case #1 (score 1+). D, Strong staining blasts in a background of weaker staining blasts of case #13 (score 2+). E, Strong staining blasts of case #6 (score 2+). F, Strong staining blasts of case #9 (score 2+). All images are at ×400x magnification (×40x objective).

Flow cytometric analysis was possible in 12 cases (for 1 case, case #2, biopsy was performed at an outside institution). The lymphoblasts were all positive for CD19, CD10, and Tdt in 12 of 12 cases. In all cases, all or a majority of blasts were also positive for CD22 and CD34; 1 case each (cases #12 and 8, both MUC4− cases) had a CD22 or CD34 subset comprising 20% and 40% of blasts, respectively. Most cases in both groups showed aberrant expression of CD13/33 and CD304 (3/4 MUC4+, 6/8 MUC4− for both analyses). Notably, CD20+ B-ALL, defined based on the clinically relevant threshold of >20% of blasts positive for this antigen [14], were all MUC4− (0/4 MUC4+ and 4/8 MUC4− B-ALL were CD20+). Also, all the MUC4+ cases (4/4) showed aberrantly decreased or absent CD38 expression in blasts, whereas this was noted in only 4 of 8 MUC4− cases. There was aberrant T-cell marker expression, mostly aberrant CD7 expression, in 3 of 4 MUC4+ cases but only 2 of 8 MUC4− cases.

3.3. Clinical features of MUC4+ and MUC4− *BCR::ABL1*+/*BCR::ABL1*-like B-ALL

All MUC4+ patients were adults (age at diagnosis = 22, 49, 53, and 67 years), and their age at diagnosis (mean = 48 years) was not statistically different from the MUC4− cases (mean = 37 years, P = .35). Actually, with the exception of 1 patient (*BCR::ABL1*-like patient, 4 years of age at diagnosis), all *BCR::ABL1*+ or *BCR::ABL1*-like cases in our cohort were over 18 years old at diagnosis (range = 22−67 years), which is consistent with the known predilection of these subtypes of B-ALL for older individuals. Notably, none of the 13 adult non-*BCR::ABL1*+/*BCR::ABL1*-like B-ALL patients in our cohort (age range = 19−88 years, mean = 51 years) were positive for MUC4 versus 4 of 12 adult *BCR::ABL1*+/*BCR::ABL1*-like B-ALL (age range = 22−67 years, mean = 43 years, P = .32), indicating that MUC4 expression is not simply a function of age (P = .039).

Pediatric and adolescents and young adults (AYA) patients (patients #2, #3, #8, #10, and #13 who were aged 31, 4, 24, 24, and 22 years, respectively, at diagnosis) were treated with COG AALL1131 protocols. All the remaining patients, except patient #12, were initially treated with hyperCVAD (Cyclophosphamide, Vincristine, Adriamycin, Dexamethasone), and *BCR::ABL1*+ patients in addition also received dasatinib (an ABL kinase inhibitor). Patient #12 had a modified treatment due to comorbidities that omitted anthracyclines (Adriamycin), and cyclophosphamide was renal dose adjusted. Vincristine and dasatinib administration was also inconsistent due to drug side effects. This patient rapidly relapsed and died from the disease 7 months after diagnosis.

All 5 *BCR::ABL1*-like patients were refractory to treatment or subsequently relapsed after achieving remission. The only MUC4+ case (patient #1, *BCR::ABL1*-like) was the only one of the 13 *BCR::ABL1*+/like patients with CSF+ disease. After treatment, the patient initially showed partial response, with leukemic lymphoblast frequency decreasing from 76% to 12% and then to 5%. However, overt disease returned with 54% blasts, and despite multiple attempts at salvage therapy, the patient died 14 months after initial diagnosis. Patient #5 (MUC4−) similarly

Table 1 Pathologic features of MUC4+ and MUC4- BCR::ABL1+/BCR::ABL1-like B-ALL.

Case #	Genetics	Age (y)	MUC4	Blast (%)	Blast morphology	CD19, CD10, Tdt	CD22	CD34	CD13 or CD33	CD304	CD20	CD38	Aberrant T-cell marker expression
1	*CRLF2-R*	49	1+	76	Small, round nuclei, very scant cytoplasm	+	+	+	−	−	−	↓	−
2	*CRLF2-R*	31	0	73	Small-medium, round nuclei, scant cytoplasm with vacuoles	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
3	*CRLF2-R*	4	0	90	Small, round nuclei, very scant cytoplasm	+	+	+	−	+	+	+	Partial dim CD7 (5%)
4	*CRLF2-R*	44	0	65	Small, round nuclei, very scant cytoplasm	+	+	+	+	+	+	+	−
5	*CRLF2-R*	37	0	89	Small, round nuclei, very scant cytoplasm	+	+	+	+	−	−	+	−
6	*BCR::ABL1*	67	2+	92	Small, round nuclei, very scant cytoplasm	+	+	+	+	+	−	−	Partial dim CD2 (50%)
7	*BCR::ABL1*	63	0	95	Small, round nuclei, very scant cytoplasm	+	+	+	+	+	−	↓	−
8	*BCR::ABL1*	24	0	80	Small, round nuclei, very scant cytoplasm	+	+	+/− (60%)	−	−	+	+	−
9	*BCR::ABL1*	53	2+	88	Small-medium, irregular/clefted nuclei, scant cytoplasm	+	+	+	+	+	−	↓	Partial dim CD7 (7%)
10	*BCR::ABL1*	24	0	95	Small-medium, irregular/clefted nuclei, scant cytoplasm	+	+	+ bi-modal	+	+	+	↓	Partial dim CD4 (20%)
11	*BCR::ABL1*	59	0	73	Medium, round nuclei, moderately abundant cytoplasm	+	+	+	+	+	−	↓	−

(*continued on next page*)

Table 1 (*continued*)

Case #	Genetics	Age (y)	MUC4	Blast (%)	Blast morphology	CD19, CD10, Tdt	CD22	CD34	CD13 or CD33	CD304	CD20	CD38	Aberrant T-cell marker expression
12	*BCR::ABL1*	46	0	87	Medium-large, irregular nuclei, moderately abundant cytoplasm, prominent nucleoli	+	+/− (80%)	+	+	+	−	+	−
13	*BCR::ABL1*	22	2+	70	Medium-large, round nuclei, abundant cytoplasm, azurophilic granules	+	+	+	+	+	−	↓	Partial dim CD7 (22%)

NOTE. MUC4+ cases in bold font. N/A: flow cytometry at diagnosis is not available for case #2. CD22+/− & CD34+/− cases indicate partial staining with % of blasts positive in parenthesis. CD13/CD33: + indicates at least partial or dim expression of CD13 or CD33. CD304: + indicates at least partial or dim expression of CD304. CD20: + indicates 20% or more of blasts expressing CD20.

showed a limited response to treatment, with marrow blast frequency only decreasing from 89% to 27% after 1−2 months of treatment and subsequently fluctuating between 17% and 92% before the patient succumbed to the disease 11 months after diagnosis. The remaining 3 patients, MUC4− *BCR::ABL1*+/like patients (patients #2, #3, and #4), achieved remission but then subsequently relapsed several months later (46, 43, and 27, respectively). The adult patients (patients #2 and #4) have both died.

For *BCR::ABL1*+ patients (excluding patient #12 with compromised treatment), those with MUC4+ blasts had a shorter time to relapse than MUC4− patients. The 3 MUC4+ patients (#6, #9, and #13) relapsed at 11, 18, and 15 months after diagnosis, respectively, despite initially achieving morphologic remission. One of these patients (patient #9) died 19 months after diagnosis. In contrast, the remaining 4 MUC4− patients have either not relapsed (patients #7, #8, and #11) after more than 5 years of follow-up or took a longer time to relapse (43 months for patient #10), compared with the MUC4+ patients. None of the MUC4− patients have died.

Taken together, in *BCR::ABL1*+/like patients who achieved morphologic remission with optimal initial induction therapy, MUC4 protein expression by blasts was associated with a shorter time to relapse (mean of 15 months versus 40 months, $P < .01$) and a trend toward a higher rate of relapse (100% versus 57%, not significant).

4. Discussion

MUC4 overexpression has been increasingly identified in a variety of malignancies in the last several years. Here, by performing IHC on 49 de novo B-ALL bone marrows of different subtypes, we show that MUC4, which is not expressed in normal hematopoietic cells, is aberrantly expressed at the protein level in a small subset of B-ALL. We demonstrate that positive is specific to *BCR::ABL1*+/like case. By comparing morphologic and flow cytometric features of MUC4+ and MUC− *BCR::ABL1*+/like cases, we found the 2 groups to be largely similar, although there was a tendency toward some immunophenotypic differences (CD38 down-regulation and aberrant T-cell marker expression in the MUC4+ group, CD20 expression in the MUC4− group). Lastly, we found that MUC4+ *BCR::ABL1*+ cases tended to have a shorter time to relapse.

Our study and a recent study by Kaumeyer et al. [15] corroborate each other by showing the remarkable specificity but limited sensitivity of MUC4 IHC for *BCR::ABL1*+/like. In that study, the authors studied 54 B-ALL cases and found that 3/7 *BCR::ABL1*+, 9 of 25 *BCR::ABL1*-like and 2 of 22 non-*BCR::ABL*+/like cases were MUC4 IHC positive. For most of their positive cases, only a minority of the blasts stained positively for MUC4 and when only cases with ≥50% of blasts staining were considered, the positivity rate (2/7 *BCR::ABL1*+, 3/25

Table 2 Clinical characteristics of MUC4+ and MUC4- BCR::ABL1+/BCR::ABL1-like B-ALL.

Case #	Genetics	Age (y)	MUC4	Treatment	Disease after induction?[a] (blast %)	Relapse?	Time to relapse (months)	Dead?	Survival/Follow-up time (months)	Additional comments
1	*CRLF2-R*	49	1+	HyperCVAD	Yes (12%)	Refractory	–	Y	14	CSF+ at diagnosis
2	*CRLF2-R*	31	0	COG AALL1131	MRD (0.01%)	Y	46	Y	76	Down syndrome
3	*CRLF2-R*	4	0	COG AALL1131	No	Y	43	N	71	
4	*CRLF2-R*	44	0	HyperCVAD	No	Y	27	Y	28	
5	*CRLF2-R*	37	0	HyperCVAD	Yes (27%)	Refractory	–	Y	11	
6	*BCR::ABL1*	67	2+	HyperCVAD, Dasatinib	No	Y	11	N	59	
7	*BCR::ABL1*	63	0	HyperCVAD, Dasatinib	No	N	–	N	64	
8	*BCR::ABL1*	24	0	COG AALL1131	No	N	–	N	78	
9	*BCR::ABL1*	53	2+	HyperCVAD, Dasatinib	No	Y	18	Y	19	
10	*BCR::ABL1*	24	0	COG AALL1131	No	Y	43	N	65	
11	*BCR::ABL1*	59	0	HyperCVAD, Dasatinib	No	N	–	N	75	
12	*BCR::ABL1*	46	0	Cyclophosphamide, Vincristine, Dexamethasone, Dasatinib	No	Y	4	Y	7	Anthracycline, methotrexate omitted due to comorbidities
13	*BCR::ABL1*	22	2+	COG AALL1131	MRD (0.1%)	Y	15	N	48	

NOTE. MUC4+ cases in dark bold font.

[a] Treatment response was evaluated 1–3 months after the start of induction chemotherapy.

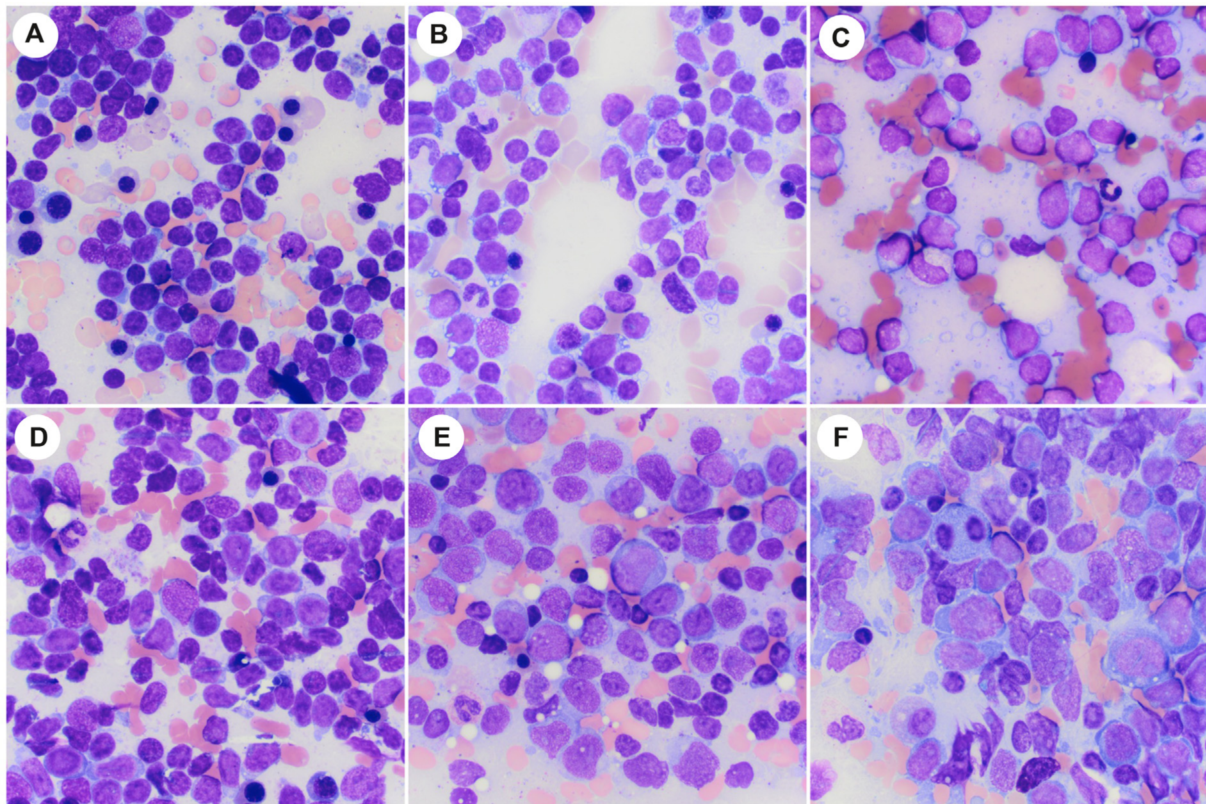

Fig. 2 Variable blast morphology in *BCR::ABL1* and *BCR::ABL1*-like cases. Giemsa-stained bone marrow aspirate smears. A, Case #1; B, case #2; C, case #9; D, case #11; E, case #12; and F, case #13. See Table 1 for a description of blast morphology. All images are at ×500x magnification (×50x objective, oil immersion).

BCR::ABL1-like, 0/22 non-*BCR::ABL1*+/like) was very similar to what we have observed in our cohort (3/8 *BCR::ABL1*+, 1/5 *BCR::ABL1*-like, 0/36 non-*BCR::ABL1*+/like). Differences between antibody vendor and staining protocols may in part explain the presence of minority partial staining in a subset of B-ALL cases (including non-*BCR::ABL1*+/like cases) in the prior study but not in ours. However, more significantly, the apparent difference between the overall *BCR::ABL1*-like positivity rate (9/25, 36%) in the prior study versus what we observed (1/5, 25%) may be attributable to the specific subtypes of *BCR::ABL1*-like used in both studies. Specifically, all cases in our study were *CRLF2* rearranged (*JAK-STAT* pathway activated group of *BCR::ABL*-like [16]), while Kaumeyer et al.'s study included *BCR::ABL1*-like cases with *JAK* class fusions as well as those with *ABL* class fusions and demonstrated a significant higher frequency of MUC4 expression in the latter versus the former (5/6 or 83% in the *ABL* group and 4/16 or 25% in the *JAK* group). In our study, the intensity of MUC4 staining was noticeably weaker in the *CRLF2*-rearranged positive case compared with the strong staining of all 3 *BCR::ABL1*+ cases. This raises the possibility of intensity differences in MUC4 staining between *ABL* class and *JAK* class *BCR::ABL1*-like B-ALL, which could be explored in future studies. Nevertheless, results of our current study and Kaumeyer et al.'s study indicate that: (1) MUC4 protein expression, especially when a majority of blasts are IHC+, occurs in less than 10% of B-ALL in about a third of *BCR::ABL1* B-ALL and 10−20% of *BCR::ABL1*-like cases, (2) MUC4 protein expression may be at least 3-fold more prevalent in *ABL* class versus *JAK2* class *BCR::ABL1*-like B-ALL, and (3) optimized staining protocols may be important in realizing the full potential of the specificity of this stain in clinical practice.

Our observations suggest that MUC4 expression may not simply be an epiphenomenon since MUC4+ *BCR::ABL1*+ patients appeared to have a worse outcome (shorter time to relapse) than MUC4− *BCR::ABL1*+ patients, although this conclusion is limited by the relatively small sample size. Nevertheless, this raises the question of what potential mechanistic role MUC4 may play in the pathogenesis of B-ALL. In solid tumors such as PDAC and cholangiocarcinoma [17], where MUC4 overexpression has been associated with a worse outcome, MUC4 is thought to promote tumorigenesis and cancer metastasis by modulating the interaction of tumor cells with their microenvironment, leading to downstream effects such as inhibition of tumor cell apoptosis [18]. Its expression can also contribute to the resistance of tumor cells to chemotherapy,

such as gemcitabine resistance in PDAC [19]. In some myeloid leukemias, myelomas, and cutaneous T cell lymphomas, another membrane-bound mucin, MUC1, has been shown to be expressed and promote malignancy by also inhibiting tumor cell death and decreasing sensitivity of tumor cells to chemotherapy [20−23]. In fact, MUC1 is capable of binding to and promoting the stability of BCR::ABL1 protein and promoting resistance of chronic myeloid leukemia cells to tyrosine kinase inhibition treatment [23]. Additional mechanistic studies are warranted to determine whether MUC4 has similar biochemical and cell biological effects in *BCR::ABL1*+ B-ALL and whether these effects are translatable to *BCR::ABL1*-like B-ALLs, especially those with *ABL* rearrangements without a *BCR* partner. Future studies using a larger sample, a prospective design and/or involve uniformly treated cohorts of patients would be important to confirm the prognostic significance of MUC4 expression in *BCR::ABL1*+/like B-ALL and whether MUC4 expression may be used to select patients who might benefit from MUC4-targeted therapy [24] or more aggressive treatment or monitoring protocols.

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