

# Mature B Cell Acute Lymphoblastic Leukemia with MLL-AF9 Transcripts: Three Case Reports and Literature Reviews

**Yuxia Guo**

Chongqing Medical University Affiliated Children's Hospital

**Min Zhou**

chengdu women and children hospital

**Pinli Zou**

Chongqing Medical University Affiliated Children's Hospital

**Xin Liao**

Chongqing Medical University Affiliated Children's Hospital

**Jianwen XIAO** (✉ [tomahawk6502@sohu.com](mailto:tomahawk6502@sohu.com))

Chongqing Medical University Affiliated Children's Hospital <https://orcid.org/0000-0002-8322-9286>

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## Case Report

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# Abstract

**Background:** Mature B cell acute lymphoblastic leukaemia (BAL) is characterized by ALL-L3 morphology and the presence of surface immunoglobulin (sIgM) light chain restriction.  $t(8;14)(q24;q32)$  or its variants related to the MYC rearrangement (MYCr) are usually present in BAL, and BAL is considered the leukaemic phase of Burkitt lymphoma (BL). BAL with MLL rearrangement (MLLr) is rare.

**Case presentation:** Three children with BAL and MLLr are presented. We also reviewed the context of 24 previously reported cases, and the features, treatment and prognosis were analysed. Three BAL patients with MLLr were reported, accounting for 1.37% of the B-ALL population; 24 patients were found in the literature. Thirteen males and 14 females were included, and the average age at diagnosis was  $19.5 \pm 4.95$  m. Renal, CNS and skin involvement were present in 6, 4 and 3 patients, respectively. Twenty-six (96.30%) patients showed non-ALL-L3 morphology; negative or suspicious expression of CD20 was found in 64% of patients. MLLr was reported, but MYCr was not observed. Twenty-five (92.59%) patients achieved complete remission. Prospective event-free survival (pEFS) in patients who received allogeneic haematopoietic stem cell transplantation (allo-HSCT) was higher than that in patients who received chemotherapy (83.33% vs 41.91%).

**Conclusion:** BAL patients with MLLr had unique manifestations, including a younger age at diagnosis and overexpression of CD19; expression of CD20 was rare, and MYCr was undetectable. The patients were sensitive to chemotherapy, but the pEFS was higher in patients undergoing allo-HSCT than in patients undergoing chemotherapy.

## Background

Acute lymphoblastic leukaemia (ALL) is the most common neoplasm in children, and B cell acute lymphoblastic leukaemia (B-ALL) accounts for 75–80% of all ALL cases [1]. B-ALL cases were classified into several subtypes according to the 2016 World Health Organization (WHO-2016) classification according to morphology, immunophenotype, and cytogenetic and molecular genetic characteristics [1, 2]. For instance, the immunophenotypes of B-ALL populations were classified as precursor B-ALL (pB-ALL) and mature B-ALL (BAL) by flow cytometry (FCM) [2].

pB-ALL comprises 90% of B-ALL cases and is characterized by the morphologic type (FAB) of ALL-L1 or ALL-L2; CD19, CyCD22, CyCD79a, TdT, HLA-DR and/or CD22, CD10, CD20, CylgM, CD34 appear in pB-ALL [2–4]. Mixed lineage leukaemia (MLL) gene rearrangements (MLLr) are generally associated with ALL-L1/ALL-L2 pB-ALL and are present in 6% of paediatric ALL cases [2, 3]. Several MLLr genes, including MLL-AF4 and the MLL-AF9 fusion gene, have been reported in ALL cases, and the MLL-AF9 fusion gene is regarded as a reverse factor for ALL cases [4, 5].

BAL is uncommon in ALL patients and is characterized by ALL-L3 morphology; the presence of surface IgM (sIgM) with light chain restriction and the absence of immature B cell antigens are typical of BAL cases [2, 6]. BAL is often associated with the translocation  $t(8;14)(q24;q32)$  or its variants; the molecular

genetics of BAL is characterized by MYC gene rearrangements (MYCr), and it is considered the leukaemic phase of Burkitt lymphoma (BL) [6, 7]. MYCr is overexpressed in more than 95% of BL/BAL patients [2].

However, rare cases of BAL with MLLr expression have been reported in children and adults [8, 9]. In this study, we describe the clinical features, lab findings, treatment and prognosis of three children with MAL with MLL-AF9 transcripts, and we also reviewed 24 case reports in the literature.

## Case Presentation

### Patients

Three BAL patients with MLLr were included in the study. Clinical data, including age, gender, laboratory findings, treatment and prognosis, were obtained from the patient records and retrospectively analysed. This study was approved by the Ethics Committee of the Children's Hospital of Chongqing Medical University (CHCMU) and Chengdu Women's & Children's Central Hospital (CWCCH), and written informed consent was obtained from all parents.

### Bone Marrow Analysis

Bone marrow (BM) samples were obtained at diagnosis and at different time points (TP) after chemotherapy. Classification according to morphology, immunophenotype, and cytogenetic and molecular genetics were performed at diagnosis according to the WHO-2016 classification of tumours of the haematopoietic and lymphoid tissues [2].

The morphologic type was classified as ALL-L1, L2 or L3 by FAB subtyping. The immunophenotype was determined by FCM with monoclonal antibody markers consisting of B cell, T cell, myeloid and stem/progenitor cell markers, and minimal residual disease (MRD) markers were also screened by FCM at diagnosis and monitored at different TP [1]. Chromosomal karyotyping and fluorescence in situ hybridization (FISH) of ETV6-RUNX1, BCR-ABL, MLLr, PDGFRB and MYCr and other rearrangements were performed as reported in the literature; 29 common fusion genes were assayed by multiplex nested reverse transcription-polymerase chain reaction (multiplex RT-PCR) and confirmed by split RT-PCR as reported in the literature [10].

### Treatment And Literature Review

Patients (Pts) 1 and 2 were treated according to the B non-Hodgkin lymphoma 2009 (B-NHL-2009) protocol for risk group 3, which was modified according to the MCP-84 protocol (S1 and S2). The details of the risk group and drug dosage are given in the Supplementary Materials (S3, S4 and S5). Pt 3 received chemotherapy according to the protocol of the Chinese Children's Cancer Group study ALL-2015 (CCCG-ALL-2015) [11]. Prophylactic intrathecal injections were administered for central nervous system (CNS)

involvement (S6); BM samples were obtained and evaluated at different TP as protocols required. BM smears and MRD were monitored by FCM, and RT-PCR was used to verify the results.

Reports on MAL patients with MLLr in the literature were retrieved from databases including PubMed, the Web of Science and CNKI. Data on clinical characteristics, laboratory findings, treatment and prognosis were collected and analysed.

## Results

### Clinical and lab findings

A total of 198 newly diagnosed B-ALL patients, including 21 BAL patients, were admitted to CHCMU and CWCCCH between January 2017 and November 2019, and 3 BAL patients with MLL transcripts were identified, accounting for 1.37% of the B-ALL population. The clinical and laboratory findings for the 3 reported patients are listed in Table 1. BM samples were obtained at diagnosis, and the results of the BM examinations are listed in Table 2, Fig. 1 and Fig. 2.

### Treatment And Prognosis

Pt 1 and Pt 2 were treated with the B-NHL-2009 protocol, and Pt 3 was treated with the CCG-ALL-2015 protocol. They achieved complete remission (CR) according to morphological, FCM and molecular examination after one course of chemotherapy. Allogenic haematopoietic stem cell transplantation (allo-HSCT) was considered by clinicians and was refused by the parents of the patients. A total of six courses of chemotherapy were completed for Pt 1 and Pt 2; as of the last follow-up in January 2020, these two patients had a CR status, and their event-free survival (EFS) times were 32 and 29 months, respectively. Chemotherapy was continued for Pt 3, and the EFS time was 3 months.

#### Literature review

The literature was searched in the abovementioned databases, and 12 articles involving 24 patients suffering from BAL with MLLr were found. Clinical and laboratory findings, BM examination results, and the treatment and prognosis of these cases in the literature reports are listed in Tables 3, 4 and 5 [8, 9, 17–26].

Data from 27 patients suffering from BAL with MLLr, including the 3 patients described in our articles, were collected and analysed. Thirteen males and 14 females were included, and the average and median age at diagnosis was  $19.5 \pm 4.95$  m and 12 m, respectively (ranging from 6 wk to 9 year); 14 (51.85%) and 24 (88.89%) patients were  $\leq 1$  and  $\leq 2$  years of age, respectively. Renal, testicular, CNS and skin involvement at diagnosis were present in 6, 1, 4 and 3 patients, respectively. The average white blood cell (WBC) and platelet (PLT) counts and haemoglobin (Hb) levels were  $87 \pm 35.24 \times 10^9/L$ ,  $69.96 \pm 5.38 \times 10^9/L$  and  $65.12 \pm 12.34$  g/L, respectively.

Twenty-six (96.30%) of the 27 patients showed non-ALL-L3 morphology, and 1 patient presented with ALL-L3 morphology. The mature B-ALL phenotype was confirmed by FCM in all 27 patients. Expression of CD19, CD22, and sIgM with light-chain restriction was detected, and TdT and CD34 were not present in most cases. Expression of CD20 was found in 25 patients. Interestingly, negative or suspicious expression of CD20 was found in 16 (64%) patients, and positive expression of CD20 was detected with a monoclonal antibody in 9 (36%) patients. Although 2 of 3 patients in our report presented with positive CD20 expression, the expression level of CD20 was less than 30%.

Chromosomal karyotype results were reported for 26 patients, while 2 patients had no metaphase chromosomes to be analyzed; 11q23-related abnormal karyotypes were found in 11 patients. Results of FISH of MLLr were reported in 23 patients, and 22 (95.65%) cases were positive. The results of the detection of MLLr transcripts were presented for 16 patients. Fourteen patients were positive and were found to have MLL-AF9 (6 cases), MLL-AF1 (2 cases), MLL-AF10 (2 cases), and MLL-ENL (2 cases), while 1 patient did not have a clear result. FISH may be the most accurate tool for the detection of MLLr. t(8;14) and its variant were not detected by karyotyping; MYCr was detected by FISH in 17 patients, and the results were negative.

The 27 patients who received chemotherapy included patients treated with ALL-like (12 cases), BL (8 cases) or Interfant-99 [28] (7 cases) protocols. One patient died of sepsis, 1 patient presented with refractory status, and 25 patients achieved CR, and the CR rate was 92.59%. In addition, 6 patients received allo-HSCT, 1 (16.67%) patient relapsed 6 months later, and the prospective 2-yr EFS (pEFS) was 83.33%, as reported in the literature. Nineteen patients subsequently received chemotherapy according to the Interfant-99 (6 cases), BL (6 cases) or ALL-like (7 cases) protocols, and 9 (47.37%) of them relapsed. The 2-yr pEFS was 41.91% (Fig. 3). Four patients in the Interfant-99 and BL groups relapsed, and the 2-yr pEFS in these groups were 40% and 33.33%, respectively. One patient in the ALL-like group relapsed, but the median value of follow-up was only 5 m.

## Discussion And Conclusion

BAL has been described as an uncommon subtype of B-ALL; it presents with a unique immunotype characterized by the expression of pan-B-cell markers (CD10, CD19, CD20, cCD79a, etc.) and sIgM with light-chain restriction, whereas pB-ALL with surface light-chain immunoglobulin restriction has also been reported [15]. The clinical features, biological characteristics, treatment and prognosis of BAL are similar to those of BL [2, 7]. BAL patients often show an ALL-L3 FAB morphology; BAL, as well as BL, is characterized by translocations of MYC (chromosome 8q24) to an immunoglobulin gene locus, and overexpression of the MYC gene was detected in most cases[2, 14]. In patients treated with an intensive short course of chemotherapy, the EFS of BAL and BL has exceeded 90% [14].

MLLr genes, which are also called lysine-specific methyltransferase 2A (KMT2A)-related genes, occur in 2.5-5% of paediatric ALL patients and 70% of infant ALL patients [2]. The presence of MLLr genes is often

correlated with the phenotype of pB-ALL and leads to a worse prognosis [1, 2], but the presence of MLLr in BAL is an uncommon molecular biological feature, and the prognosis of such patients is unclear.

We reviewed the literature in databases, and a total of 27 patients, including the 3 patients described in our manuscript, were found. These three BAL patients with MLLr have unique clinical manifestations and laboratory findings compared with pB-ALL and BL patients. Infant leukaemia patients comprised half of these patients, and most of these patients (24/27) were  $\leq 2$  years of age at onset, whereas the median ages of pB-ALL and BL patients were 9 and 2–5 years of age, respectively. Renal, CNS and skin involvement at diagnosis were not unusual and were present in 6, 4 and 3 cases, respectively, in patients with BAL with MLLr; however, renal involvement is not rare in BAL, and CNS or skin involvement is uncommon in both pB-ALL and MAL [1, 6]. Although the reported patients were classified as having a BAL phenotype by FCM, overexpression of CD19 was detected in most cases, and expression of CD20 was not detected; nevertheless, expression of both CD20 and CD19 is common in BL patients [2]. It has been revealed that rituximab [13], an anti-CD20 monoclonal antibody resulting in the selective depletion of B lymphocytes, was unsuitable to treat these patients; patients who appeared to be refractory and/or relapsed may benefit from chimeric antigen receptor T cell (CAR-T) immunotherapy targeting the overexpression of CD19 [28].

BL is often associated with t(8;14)(q24;q32) or its variants, and MYCr is detectable in more than 95% of the BL population [2]. Translocation is the essential driver of the overexpression of the MYC gene, and activation of the MYC gene leads to cell cycle progression, inhibition of differentiation, the promotion of cell proliferation and genomic instability and the activation of endogenous apoptotic programmes [13]. However, it is surprising that the MYC gene and its chromosomal translocation were undetectable in the MAL patients with MLLr.

Standard treatment of BAL patients with MLLr has not yet been established, and BAL patients seemed to be sensitive to chemotherapy, including chemotherapy administered according to the ALL, BL or Interfant-99 protocols. Most of these patients achieved CR after they received one course of chemotherapy, but the prognosis of patients subjected to different treatments was widely divergent. The pEFS was higher in the allo-HSCT group than in the chemotherapy group. Although 2 patients described in our report received chemotherapy with the BL protocol and survived more than 2 years, the prognosis of patients treated with chemotherapy has remained poor, and allo-HSCT should be recommended for patients with CR1 status.

## Abbreviations

Acute lymphoblastic leukaemia

ALL; allogeneic haematopoietic stem cell transplantation:allo-HSCT; B cell acute lymphoblastic leukaemia; B-ALL; bone marrow:BM; Burkitt lymphoma BL; central nervous system:CNS; chimeric antigen receptor T cell:CAR-T; complete remission:CR; flow cytometry FCM; haemoglobin:Hb; Mature B cell acute lymphoblastic leukaemia:BAL; Mixed lineage leukaemia; MLL; MLL gene rearrangement:MLLr; multiplex nested reverse transcription-polymerase chain reaction:multiplex RT-PCR; MYC rearrangement:MYCr;

platelet:PLT; precursor B-ALL pB-ALL; prospective event-free survival:pEFS; surface immunoglobulin:slgM; time points:TP; white blood cell:WBC; World Health Organization:WHO.

## Declarations

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### Authors' contribution

YXG, MZ, PLZ and XL collected the data, YXG wrote the draft of the manuscript, JWX reviewed and revised the manuscript. All authors read and approved the final manuscript.

### Funding

Not applicable.

### Availability of data and materials

The data are listed in the article and the Supplementary Materials.

### Ethics approval and consent to participate

This study was approved by the Ethics Committee of CHCMU and CWCCCH, and written informed consent was obtained from all parents.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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## Figures

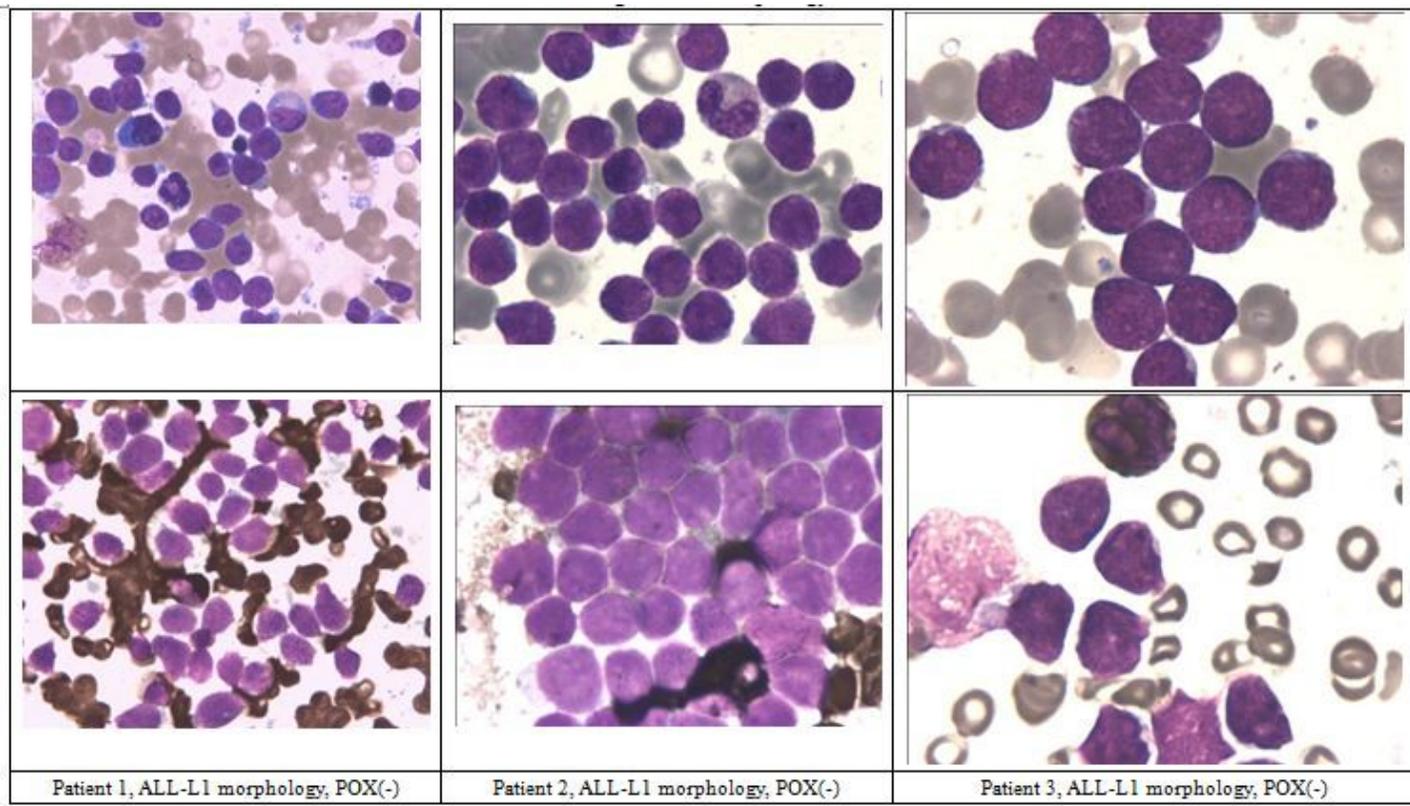
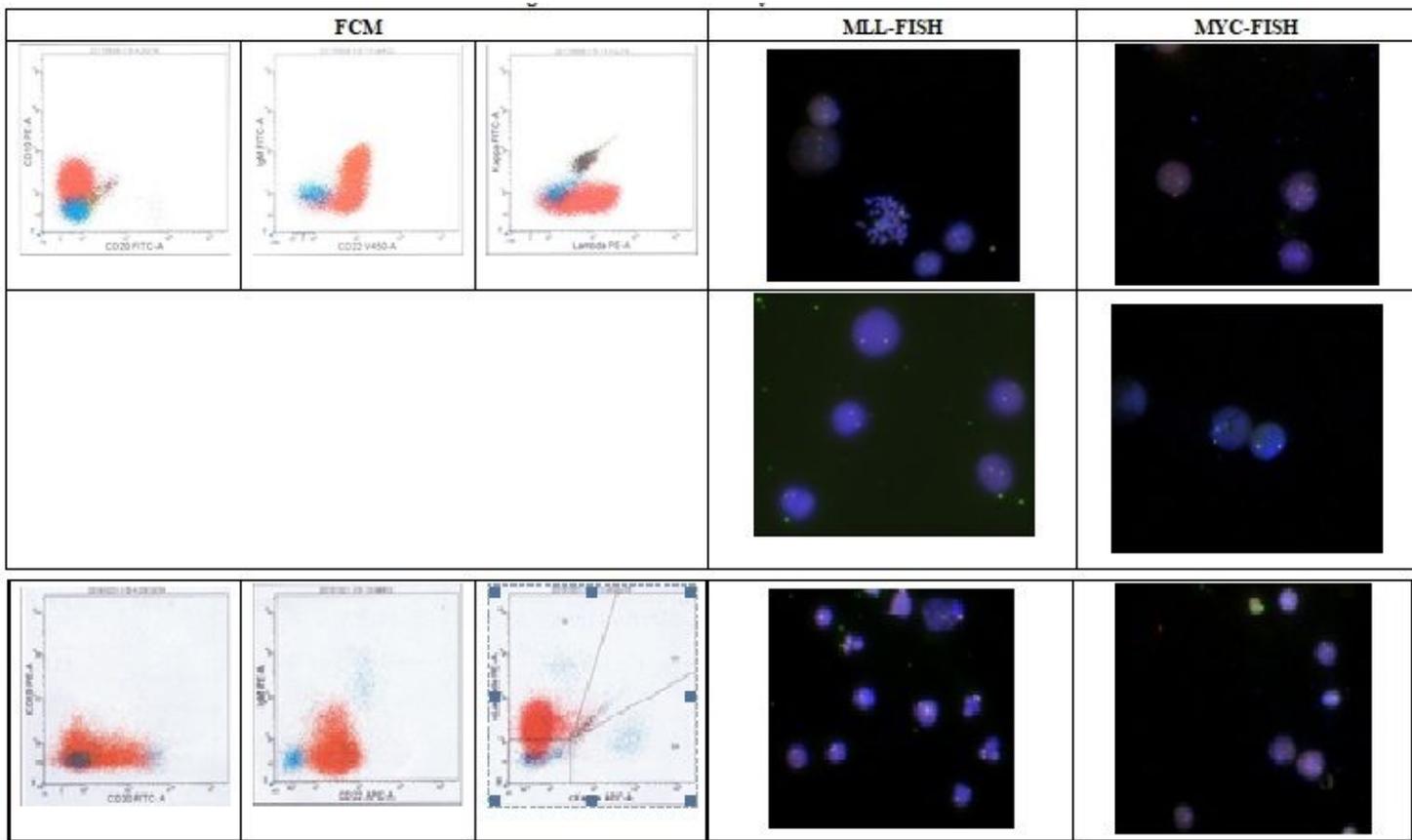


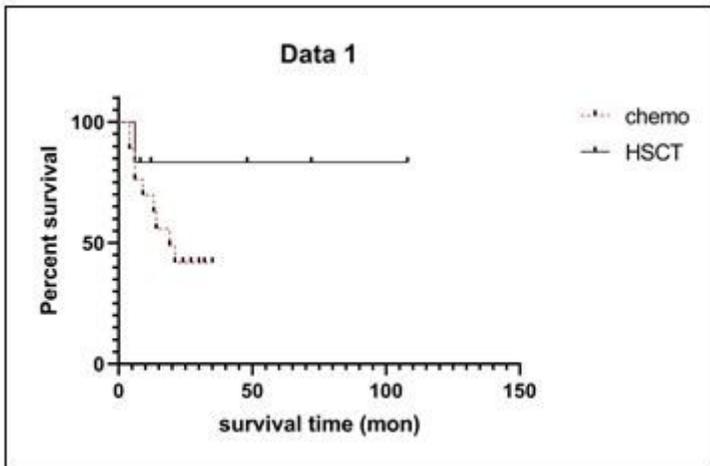
Figure 1

BM morphology



## Figure 2

BM Examination by FCM and FISH



Chemo: chemotherapy; HSCT: haematopoietic stem cell transplantation

## Figure 3

Survival curves of patients

## Supplementary Files

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