

Progress and prognosis in chronic lymphocytic leukaemia



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A B S T R A C T

Chronic lymphocytic leukaemia (CLL) follows an extremely variable clinical course with survival ranging from months to decades. In recent years, although effective therapeutic approaches for CLL have been developed, some patients remain primarily refractory or develop resistance to such treatment during the course of the disease. This situation is associated with a particularly poor prognosis and short survival time. Molecular and cellular markers that may predict for refractory disease and disease progression in patients with CLL have recently been identified. Genomic aberrations, mutational profile of immunoglobulin heavy chain genes and ZAP-70 expression provide prognostic information for individual patients independently of clinical disease characteristics. These molecular markers have now entered the stage of risk stratification for individual patients in clinical trials.

Introduction

Chronic lymphocytic leukaemia (CLL) is a lymphoproliferative disorder characterised by the progressive accumulation of mature, but immunologically incompetent lymphocytes in the blood, bone marrow, and lymphatic tissues.¹ For CLL, lymphocyte counts in the blood are typically $\geq 5,000/\text{mm}^3$ with a characteristic immunophenotype (CD5^+ and CD23^+ B cells).² As the most common adult leukaemia in Western society representing 22–30% of all leukaemias,³ CLL occurs primarily in middle-aged and elderly adults and its frequency appears to increase in line with increasing age.⁴ However, up to 20% of CLL cases are reported in patients aged <55 years and CLL reported in this age group appears to be increasing. Thus, the optimal management of CLL remains a high priority.

Natural history and disease staging in chronic lymphocytic leukemia

The clinical course and prognosis of CLL are extremely variable with the disease progressing from an indolent lymphocytosis with no other evidence of disease to one of generalised lymphatic enlargement with concomitant pancytopenia (National Cancer Institute 2006).⁵ Some patients may survive for prolonged periods without treatment, while others require intensive treatment early after diagnosis.^{6,7} However, advanced stage CLL is associated with haemorrhage and infection, both of which represent a major cause of death in patients with CLL.⁸

Morphological examination is the routine first step in the diagnosis of haematological malignancies, including CLL, and disease staging in CLL enables a prediction of prognosis and

allows patient stratification to achieve comparisons of specific treatment data. The standard staging systems for CLL, the Rai system and the Binet classification, both of which are primarily based on tumour load, can be used to estimate the prognosis in patients with CLL (Table 1).⁹⁻¹¹

The Binet classification integrates the number of nodal groups involved in CLL with bone marrow failure. The major advantages associated with this staging approach are derived from the recognition of a predominantly splenic form of CLL, which may provide a more accurate prognosis compared with Rai staging, and from the recognition that the presence of anaemia or thrombocytopenia has a similar prognosis and does not merit a separate stage; anaemia and thrombocytopenia have

been identified as major adverse prognostic variables in CLL. Patients with thrombocytopenia or anaemia, or both, due to extensive marrow infiltration and impaired hematopoiesis (Rai III/IV, Binet C) appear to have a poorer prognosis than patients with immune cytopenias.¹² Neither the Rai staging system nor the Binet classification separate immune from non-immune causes of cytopenia.

Although staging systems for CLL allow the definition of distinct prognostic subgroups, their ability to predict the outcome for individual patients at the time of diagnosis remains limited, particularly in early-stage disease. Consequently, other factors related to the biology of CLL, such as molecular and/or genetic markers, are currently being evaluated for their prognostic value.

Table 1. Summary of the Rai and Binet staging systems for CLL.^{5,9-11}

RAI STAGING SYSTEM

Stage 0	Low risk
Stage 0 CLL is characterized by absolute lymphocytosis (blood: >15,000/mm ³ , bone marrow >40%) without adenopathy, hepatosplenomegaly, anaemia, or thrombocytopenia.	
Stage I	Intermediate risk
Stage I CLL is characterized by absolute lymphocytosis with lymphadenopathy without hepatosplenomegaly, anaemia, or thrombocytopenia.	
Stage II	Intermediate risk
Stage II CLL is characterized by absolute lymphocytosis with either hepatomegaly or splenomegaly, with or without lymphadenopathy.	
Stage III	High risk
Stage III CLL is characterized by absolute lymphocytosis and anaemia (haemoglobin <11 g/dL) with or without lymphadenopathy, hepatomegaly, or splenomegaly.	
Stage IV	High risk
Stage IV CLL is characterized by absolute lymphocytosis and thrombocytopenia (<100,000/mm ³) with or without lymphadenopathy, hepatomegaly, splenomegaly, or anaemia.	

BINET CLASSIFICATION

Clinical stage A^a	
Clinical stage A CLL is characterized by no anaemia (Hb ≥10 g/dL) or thrombocytopenia (platelets ≥100,000/mm ³) and <3 areas of lymphoid involvement.	
Clinical stage B^a	
Clinical stage CLL is characterized by no anaemia (Hb ≥10 g/dL) or thrombocytopenia (platelets ≥100,000/mm ³) with ≥3 areas of lymphoid involvement.	
Clinical stage C	
Clinical stage C CLL is characterized by anaemia (Hb <10 g/dL) and/or thrombocytopenia (platelets <100,000/mm ³) regardless of the number of areas of lymphoid enlargement.	

^aLymphoid areas include cervical, axillary, inguinal and spleen.

Prognostic factors in patients with chronic lymphocytic leukemia

There has been dramatic progress in our understanding of molecular pathogenesis and outcome predictors in CLL. Genetic parameters such as genomic aberrations (e.g. 11q deletion [11q-], 17p deletion [17p-]), the mutation status of the variable segment of immunoglobulin heavy chain genes (V_H) and surrogate markers (e.g. CD38, zeta-associated protein [ZAP-70], lipoprotein lipase [LPL]) provide prognostic information for individual patients independently of clinical disease characteristics (e.g. stage)¹³⁻²⁰ and can identify attenuated versus progressive types of CLL, offering the potential to facilitate risk-adapted treatment strategies. The development of highly sensitive assays for genetic analyses has greatly improved our understanding of these prognostic markers. While conventional cytogenetic analyses have previously only detected chromosome aberrations in 40–50% of CLL cases, new molecular cytogenetic methods, such as fluorescence in situ hybridization (FISH) has greatly enhanced our ability to detect chromosomal abnormalities in over 80% of CLL cases.^{15,21,22}

The use of highly sensitive assays has also

led to the identification of a monoclonal B lymphocytosis (subgroup of CD5⁻) of undetermined significance (MLUS), a benign condition of clonal lymphocytosis identified in 3% and 6% of adults aged >40 years and >60 years, respectively.^{23,24} MLUS resembles and can be indistinguishable from early stage CLL²⁵ and many published series of CLL include a subgroup of CD5⁻ cases. However, CD5⁻ B cell CLL remains a controversial topic with its nature and true incidence remaining unclear.²⁵

A number of studies have identified that the prognosis of patients with CLL can now be assessed by using factors such as cytogenetic subgroup, immunoglobulin mutational status, ZAP-70 and CD38 expression.^{1,13-15,17,26-28} Prognostic factors in CLL can essentially be considered from the perspective of the mutation status of genomic aberrations or the immunoglobulin heavy chain (V_H) genes.

Independent prognostic factors associated with rapid disease progression and short survival times include 17p- and 11q- (approximately 67% to 75% 2-year survival), whereas 13q deletion (13q-) as the sole abnormality is associated with favourable outcome (approximately 91% 2-year survival).^{15,29} The probability of survival among patients with these genetic deletions has been

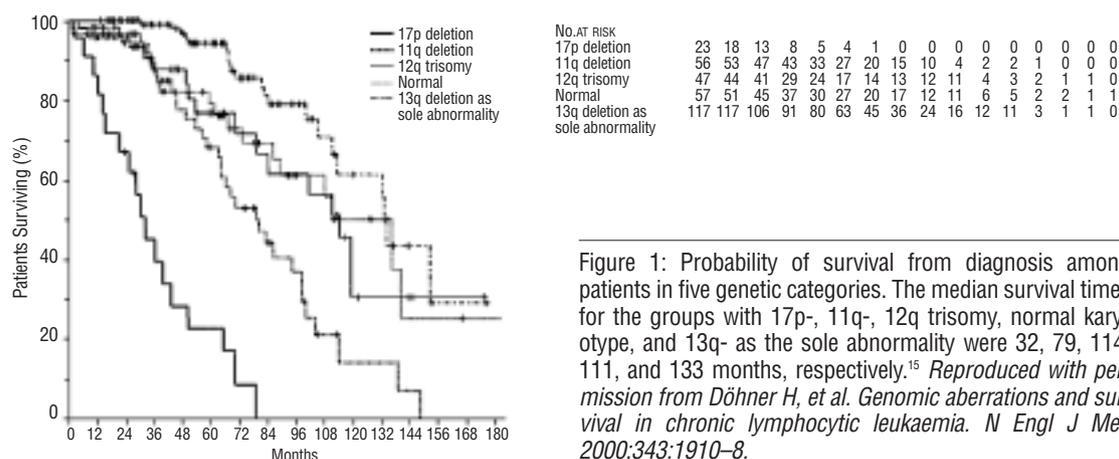
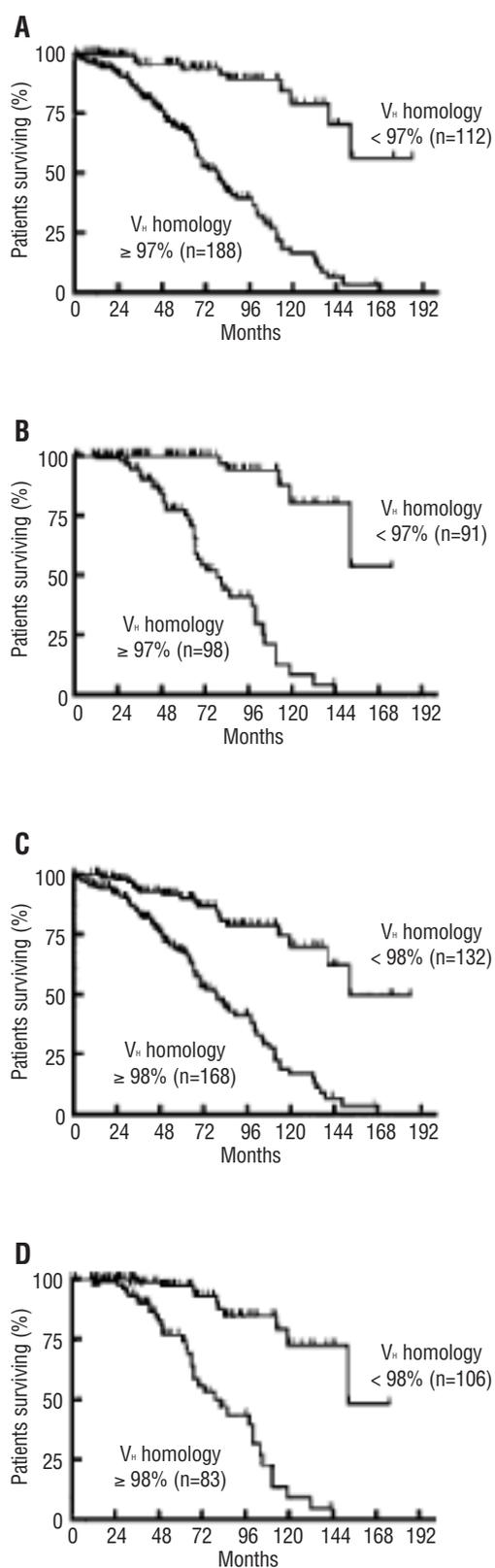


Figure 1: Probability of survival from diagnosis among patients in five genetic categories. The median survival times for the groups with 17p-, 11q-, 12q trisomy, normal karyotype, and 13q- as the sole abnormality were 32, 79, 114, 111, and 133 months, respectively.¹⁵ Reproduced with permission from Döhner H, et al. *Genomic aberrations and survival in chronic lymphocytic leukaemia. N Engl J Med 2000;343:1910-8.*



compared (Figure 1). Genetic subgroups with distinct clinical features have been identified. For example, 11q- is associated with marked lymphadenopathy and rapid disease progression while 17p- predicts for treatment failure with alkylating agents, as well as fludarabine and short survival times.^{21,30-34}

Significant numbers of mutations in V_H genes have been associated with a median survival in excess of 10 to 20 years while the absence of mutations has been associated with a median survival of less than 5 to 10 years. However, V_H mutation status testing is a costly and time-consuming approach and is thus not currently available for general clinical use; surrogate prognostic markers for V_H mutation status are being evaluated.^{5,13,14,26} While the mutation status of V_H genes allows the separation of patients into long (mutated V_H) or short (unmutated V_H) survival times, V-gene usage and gene expression differences in the two subgroups allow insights into differential pathogenic mechanisms and provide further prognostic information. CD38, ZAP-70 and LPL as well as other novel factors, are prognostic markers in CLL related to V_H mutational status.^{13,16-20,26,35}

V_H mutation status and CD38 expression have

Figure 2. Probability of survival from the date of diagnosis among patients with a V_H homology <97% or ≥97% and <98% or ≥98%. (A) Estimated median survival time for the V_H homology ≥97% group was 79 months. The last observed death in the V_H homology <97% group was after 152 months of follow-up time (survival probability, 56%). (B) When only patients diagnosed at Binet stage A were evaluated, the estimated median survival times for the V_H homology ≥97% and the V_H homology <97% groups were 79 months versus not reached (last observed death after 152 months of follow-up time; survival probability, 53%). (C) Estimated median survival time for the V_H homology ≥98% and <98% groups were 79 months and 152 months, respectively. (D) When only patients diagnosed at Binet stage A were evaluated, the estimated median survival times for the V_H homology ≥98% and V_H homology <98% groups were 79 months versus 152 months.²⁹ Reproduced with permission from Kröber A, et al. V_H mutation status, CD38 expression level, genomic aberrations, and chronic lymphocytic leukaemia. *Blood* 2002;100:1410-6.

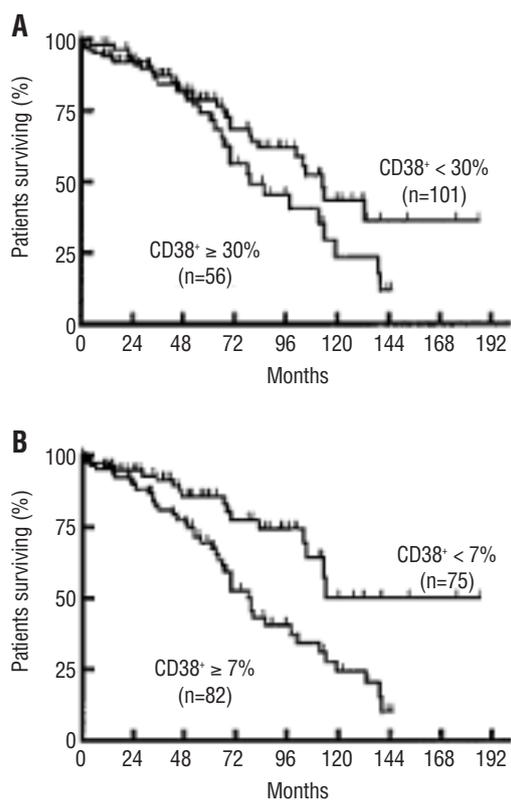


Figure 3: Probability of survival from the date of diagnosis compared among CLL subgroups with different CD38 expression levels. Estimated median survival times for the subgroups with (A) CD38⁺ <30% versus ≥30% were 113 months versus 79 months ($p=0.13$; log-rank test) and for the subgroups with (B) CD38⁺ <7% versus ≥7% were 114 months versus 79 months (Corrected $p=0.02$; derived from maximally selected log-rank statistics), respectively.²⁹ Reproduced with permission from Kröber A, et al. V_H mutation status, CD38 expression level, genomic aberrations, and chronic lymphocytic leukaemia. *Blood* 2002;100:1410–6.

an impact on prognosis in patients with CLL. In univariate analyses, unmutated V_H genes and high CD38 expression levels predicted for shorter survival times (Figures 2, 3) (n=300).²⁹ Although the overall incidence of genomic aberrations appeared similar in the V_H unmutated and V_H mutated subgroups, they varied in type. For example, high-risk genomic aberrations such as 17p- and 11q- occurred almost exclusively in the V_H unmutated subgroup, whereas favourable aberrations such

as 13q- and 13q+ as single abnormalities were overrepresented in the V_H mutated subgroup. Using a multivariate analysis, Kröber et al. (2002) confirmed that unmutated V_H, 17p- and 11q- were identified as independent prognostic factors, suggesting a complementary role of V_H mutation status and genomic aberrations to predict outcome in CLL.²⁹

Although gene-expression patterns of CLL cells with unmutated V_H genes appear similar to those of cells with mutated V_H genes, the patterns of both differ from those of other leukaemia and lymphoma cell types.^{26,27} Nevertheless, the two subtypes of CLL can be distinguished by the differential expression of the gene which encodes ZAP-70, an intracellular tyrosine kinase with a critical role in T-cell-receptor signaling.^{36,37} ZAP-70 has been found to be associated with enhanced signaling by the cell-surface immunoglobulin receptor of CLL cells, irrespective of their V_H mutation status,³⁸ and measurement of ZAP-70 can be used as a surrogate marker for the mutational status of V_H.^{16,28}

Sequencing of V_H genes and measurement of ZAP-70 expression in CLL cells (cohort of 307 patients followed by the Chronic Lymphocytic Leukemia Research Consortium [CRC]) has established that ZAP-70 is a stronger predictor of the need for earlier treatment compared with V_H mutation status (Figure 4).¹⁷ ZAP-70 positivity (>30%) correlates with an unfavourable median survival (6–10 years), compared with negative ZAP-70 which is associated with a median survival of >15 years.⁵ The test for ZAP-70 is now commercially available and has been proposed as a surrogate marker for the measurement of V_H mutational status.^{16,17,28}

As the concordance of V_H gene mutation status and ZAP-70 expression appears variable in CLL, additional factors which may characterise this discordance have been investigated.²⁰ In this study, V_H mutation status

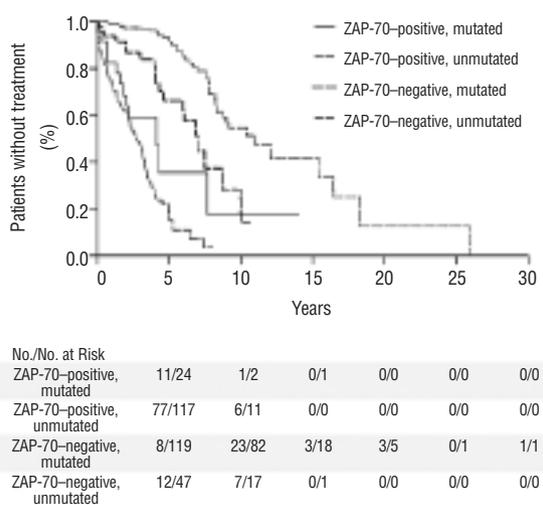


Figure 4: Relationship between ZAP-70 expression and V_H mutational status and the time from diagnosis to initial therapy. Kaplan-Meier curves depict the proportion of untreated patients with CLL according to the time since diagnosis. The patients are grouped with respect to their V_H mutational status and whether they did or did not express ZAP-70.¹⁷ Reproduced with permission from Rassenti LZ, et al. ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease progression in chronic lymphocytic leukaemia. *N Engl J Med* 2004;351:893-901. ©2004 Massachusetts Medical Society. All rights reserved.

and ZAP-70 expression were strongly related in the absence of additional genetic high-risk-features as defined by the lack of 11q- or 17p- and V3-21 usage (concordance 84%). However, the presence of such additional genetic high-risk features resulted in a significantly higher proportion of discordant cases (39%). In addition, discordant cases with V3-21 usage were almost exclusively V_H mutated and ZAP-70 positive (89%), whereas nearly all of the discordant cases with high-risk aberrations were V_H unmutated and ZAP-70 negative (92%). Thus, there appear to be characteristic modes of discordance between ZAP-70 and V_H mutation status depending on the presence or absence of additional genetic high-risk features such as 11q- and 17p- or V3-21 usage.

Other useful independent prognostic factors

in patients with CLL include age, white blood cell count and lactate dehydrogenase levels.²⁹ Lymphocyte doubling time (with a favourable prognosis associated with a doubling of the white blood cell count in >1 year)³⁹ and elevated levels of beta-2-microglobulin which can imply a worse prognosis⁴⁰ have also been identified. An important finding from multivariate analyses is that V_H mutation status and genomic abnormalities have independent prognostic values, appear to allow outcome prediction irrespective of the clinical stage, and may therefore allow a risk assessment of individual patients early in the course of the disease. The use of prognostic markers to aid treatment selection is continuing to evolve.

The influence of molecular/genetic prognostic factors on response to treatment in patients with chronic lymphocytic leukemia

In recent years, although innovative and highly effective therapeutic approaches for CLL have been developed, some patients remain primarily refractory or develop resistance to such treatment during the course of the disease. This situation is associated with a particularly poor prognosis and low median survival time. Prospective trials are currently underway to establish and verify the role of prognostic markers in CLL. However, no large multivariable analyses are yet available to test the relative power of these individual prognostic variables and their influence on response to treatment of CLL.⁵

Genes potentially involved in the pathogenesis of CLL have been identified in a subset of cases with 11q- (ATM) and in cases with 17p13- (p53). For example, p53-pathway based therapies, such as fludarabine, appear ineffective in patients with p53 genetic abnormalities associated with resistance to treatment. In addition, 17p- correlates with

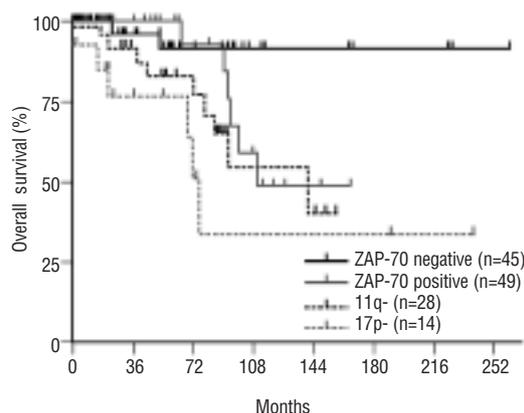


Figure 5: Kaplan-Meier curve of overall survival of the 17p-subgroup compared with other genetic aberrations.²⁰ Reproduced from Kröber A, et al. Additional genetic high-risk features such as 11q deletion, 17p deletion, and V3-21 usage characterize discordance of ZAP-70 and V_H mutation status in chronic lymphocytic leukaemia. *J Clin Oncol* 2006;24:969–75. Reprinted with permission from the American Society of Clinical Oncology.

mutated p53 along with poor response rates and short duration of response to the standard therapeutic options, including alkylating agents, rituximab and fludarabine (Figure 5) and with short survival duration in patients with CLL.^{21,30-33,41-43}

There is emerging evidence to suggest that the humanised anti-CD52 antibody alemtuzumab can be used to treat CLL in patients with genetic aberrations. For example, the efficacy of alemtuzumab has been evaluated in 36 patients with fludarabine-refractory CLL, 15 (42%) of whom had p53 mutations and/or deletions.⁴⁴ Clinical responses in patients with p53 mutations/deletions were achieved in 6/15 (40%) compared with 4/21 (19%) without genetic aberrations and the median duration of treatment response for this patient subset was 8 months (range: 3-17 months). Overall, these data suggest that alemtuzumab may be an effective therapy for patients with CLL with p53 mutations or deletions. Similarly, data from the GCLLSG CLL2H trial have confirmed alemtuzumab to be effective in

patients with CLL irrespective of genetic risk factors.⁴⁵

Conclusions

Beyond clinical trials, the standard procedures to estimate prognosis and evaluate the need for treatment in CLL are the staging systems developed by Rai, Binet and the NCI-sponsored working group criteria for active disease. However, molecular markers have now entered the stage of risk stratification for individual patients. CLL cases with unmutated V_H show more rapid disease progression and shorter survival times. Genomic aberrations and V_H mutation status, as well as surrogate markers such as ZAP-70, appear to give prognostic information irrespective of the clinical stage and may therefore allow a risk assessment for individual patients early in the course of their disease. These genetic aberrations (e.g. 17p-) can predict short survival times and treatment failure with alkylating agents and fludarabine, whereas p53 independent therapies as alemtuzumab are of importance. The identification of new treatments, such as alemtuzumab, that are effective in patients with genetic abnormalities represents a major therapeutic advance for the treatment of CLL.

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