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A unusual three way complex rearrangement t(6;8,21)(p23;q22;q22) with RUNX1/RUNX1T1 fusion resulting in partial trisomy for region 8q22 to 8qter : a new variant of t(8;21) in acute myeloid leukemia(M2)

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Abstract

Background: Acute myeloid leukemia (AML) with t(8;21)(q22;q22) : RUNX1-RUNX1T1 fusion is a distinct type of acute myeloid leukemia (AML), especially in FAB M2. AML with t(8;21)(q22;q22) is associated with a favorable prognosis with good response to chemotherapy with cytosine arabinoside.

Material and methods: Morphology, Immunphenotyping, Cytogenetic, FISH and Molecular analysis were performed on bone marrow sample.

Results: Morphological and Flow cytometry evaluation classified the case as Acute Myeloid Leukemia with M2 subtype. Chromosomal analysis revealed novel variant with complex three break rearrangement involving chromosome 6,8 and 21 leading to RUNX1/RUNX1T1 fusion by Fluorescence in situ hybridization (FISH) along with probable deletion of 6p23-pter and partial trisomy of 8q22-qter. Molecular markers were negative for FLT3, NPM1 and CEBPA mutations and c-Kit mutations for exons 8 and 17 by DNA.

Conclusion: Based on the cytogenetic and FISH findings we report a new variant t(8;21) translocation case in complete remission with a follow-up of 26 months. In our present case a different region of 6p23 is identified resulting from a complex translocation leading to homozygous deletion of chromosome 6 at band 6p23-pter and partial trisomy of 8q22-qter region reinforces the utility of combination of Karyotyping and FISH to characterization of chromosome abnormalities.

Complete remission status after 26 months of treatment supports the favorable prognostic behavior of the variant translocations as reported for other variant t(8;21) translocations. Absence of mutations of/within molecular markers such as FLT3, NPM1, c-Kit and CEBPA may lead to a less burden of additional mutational events and help in treatment decisions.

Keywords: Karyotyping, FISH, Molecular, Hematological malignancies, three-way translocation.

Introduction

Acute Myeloid Leukemia (AML) with recurrent genetic abnormalities involved in rearrangements of genes that encode transcription factors are associated with distinct clinical and morphologic features and assist in classification.[1] Acute myeloid leukemia (AML) with t(8;21)(q22;q22) resulting in RUNX1-RUNX1T1 (formerly known as AML1/ETO) fusion is a distinct type of acute myeloid leukemia (AML), especially in FAB M2.[2-5] The translocation leads to the fusion of the RUNX1 and RUNX1T1 genes on the derivative chromosome 8, and patients positive for the chimeric gene are known to have a favorable prognosis. The t(8;21) abnormality is found in approximately 5%–10% of all AML cases and 10%–22% of AML cases with maturation corresponding to FAB class M2.[4-12] RUNX1/RUNX1T1 fusion alter sub nuclear routing and fidelity of transcriptional control that result in a differentiation block and increase proliferation of myeloid progenitors.[13] AML with t(8;21)(q22;q22) generally is associated with a favorable prognosis and, in particular, with good response to chemotherapy with cytosine arabinoside.[14] Although it is generally associated with a favorable prognosis, many additional genetic lesions may impact outcome.[15,16]

Additional abnormalities are also seen in cases showing t(8;21) and most frequently associated abnormalities are the loss of either X or Y-chromosome (together 46.8%), followed by del(9q) (15.1%), and trisomy 8 (5.8%), *FLT3*-ITD, *FLT3*-TKD,c-KIT mutations at molecular level.[17,18] Although rearrangements of genes that encode transcription factors may lead to impaired maturation of one or more myeloid lineages, mutations of genes such as FLT3, JAK2, RAS, or c-KIT that encode proteins involved in signal transduction pathways may result in differential prognosis.[18] In the case of t(8;21)(q22;q22); RUNX1-RUNX1T1, most published reports indicate a higher relapse rate and lower overall survival when mutation for KIT gene is present.[18,19] Variant/complex translocations of t(8;21)(q22;q22) involving chromosomes 8, 21, and other chromosomes account for approximately 3% of all t(8;21)(q22;q22) in acute myeloid leukemia (AML) patients and multiple gene lesions along with chromosomal translocations may cooperate and influence the clinical characteristics in AML including response to therapy and overall survival.[5] Literature studies have shown that the variant t (8; 21) translocations are formed with involvement of almost every chromosome except for the 22 and Y chromosome with chromosome 1 as the most commonly involved chromosome. The involvement of chromosome 1 in t(8;21) has been previously reported in approximately more than 10 reports.[14,20-25] On chromosome 1 the band regions reported are variable and are on both the p as well as the q arm. This illustrates that chromosome 1 is more accessible to the complex formation during chromatin reorganization phase. Studies on the potential genes involved in such translocations would lead to understanding whether or not the different chromosome regions play an important role in pathogenesis of acute myeloid leukemia. More cases are needed to elucidate its biologic mechanisms. Chromatin reorganization study for RUNX1 gene and the different chromosomes involved will throw light on the variant formation mechanisms.

In this study, we present a novel variant with complex three break rearrangement involving chromosome6, 8 and 21 designated as 46, XY, -6, -6, der(6)t(6;8)(p23;q22)(8qter→8q22::6p23→6qter)x2,-8, der(8)t(8;21)(q22;q22)(8pter→8q22::21q22→21qter),-21, der(21)del(21)(q22:)(22pter→21q22:) leading to RUNX1/RUNX1T1 fusion along with probable deletion of 6p23-pter and partial trisomy of 8q22-qter. Follow-up of 26 months have shown that consolidation

chemotherapy with high dose cytarabine successfully has induced the patient into complete remission status.

Materials and methods

Morphology

For morphologic examination, all bone marrow (BM) aspirate/peripheral blood smears (PBS) were air dried and subsequently stained with Wright's Giemsa. Cytochemical stains included myeloperoxidase (MPO) and alpha naphthyl acetate esterase (ANAE).

Immunophenotyping

Immunophenotyping of leukemia blasts was done by flow cytometry using fluoresceine, iosthiocynate and phycoerythrin-conjugated monoclonal antibodies (Becton Dickson, Franklin Lakes, NJ). Antibodies against the following CD markers were used CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11c, Cd13, CD14, CD15, CD19, CD33, CD34, CD117, cMPO and HLADR.[26]

Cytogenetic analysis

Cytogenetic analysis of un-stimulated bone marrow cells was performed at the time of diagnosis and after one month of treatment using direct, overnight and 48 hours culture. The cells were grown in RPMI 1640 (Sigma, Schnelldorf, Germany) supplemented with 20% fetal bovine serum (GIBCO, Grand Island, NY). Harvesting and GTG banding were performed using a standard procedure.[27,28] Metaphase chromosomes were G-Banded and karyotype description was according to ISCN 2016 nomenclature.[29] At least 20 metaphase cells were analyzed, and 10 well-spread metaphases were photographed and karyotyped using ASI software. The fixed cell pellet stored in methanol: acetic acid fixative (3:1) at −20°C was used further for molecular cytogenetics – fluorescence in situ hybridization.

Fluorescence in situ hybridization (FISH) analysis

Metaphase-interphase fluorescence in situ hybridization (FISH) was performed using LSI Dual Color Dual Fusion probes for RUNX1/RUNX1T1 and PML/RARA, and Break Apart Rearrangement probes for CBFB and MLL (Abbott Molecular / Vysis, Des Plaines, IL, USA). FISH was performed on un-stimulated cultured bone marrow cells, on both interphase cells (500) and metaphase cells using optimized Vysis protocol. FISH analysis was done on an Olympus BX61 fluorescence microscope with appropriate filters using ASI (Applied Spectral Imaging) software.

Molecular Analysis

FLT3 ITD/TKD (D835), NPM1, CEBPA and c-KIT mutations were analyzed by direct sequencing. FLT3-ITD within the juxtamembrane domain was amplified by PCR using primers encoded in exons 14 and 15. The size of the ITD PCR product was determined by capillary electrophoresis. The activating mutation of TKD at codon 835 was determined by PCR/Sequencing.[30] NPM1 exon 12 mutations (small insertion - 4/5 bp) which alter the N-terminal domain nuclear localization signal leading to abnormal cytoplasmic accumulation of the NPM1 phosphoprotein are detected by PCR amplification with fluorescently labeled primers and small insertion mutations are identified by capillary electrophoresis-based fragment analysis. PCR-sequencing was performed for detection of N- and C-terminal mutations of CEBPA (CCAAT/enhancer-binding protein alpha) gene. The entire coding region was sequenced.[31] c-kit - Mutational analysis of exon 8 (the extracellular domain) and exon 17 (tyrosine kinase II domain) were studied by DNA – Sequencing.[17,18]

A 30 year old male presented with generalized weakness and dyspnea on excursion since one month with complaints of high grade fever associated with chills and cough with expectoration. He did not have any history of bleeding from any site, no history of vomiting and did not have any history of loss of consciousness. Laboratory evaluation showed leucopenia as well as

thrombocytopenia with Hb-10.2 gm/dl,MCV-94.1,MCH-31.5,MCHC-33.4,TLC-400/cmm, platelet count-37000/cmm, HIV 1 and 2, Hepatitis B and C virus antigen was negative. His clinical examination showed O/E-,Pallor ++,P-82/min,BP-120/80mmHg,RR-18/min, absence of lymphadenopathy, Icterus, clubbing, cyanosis, S/E,RS- AEBE, Clear, CVS- S1S2 N,CNS- WNL, P/A-Soft, non-tender, and no organomegaly.

Bone marrow aspiration and biopsy was carried out which was suggestive of suppressed erythroid series with 84% blast cells. Biopsy showed patchy staining of marrow with MPO positivity and diagnosed as Acute Myeloid Leukemia.

Immunophenotyping investigation by flow cytometry showed blasts positive for HLA DR (99.2%), CD13 (97.2%), CD19% (97.3%), CD33 (98.4%), CD34 (98.9%) and cMPO (98.9%) classifying the patient as Acute Myeloid Leukemia with M2 subtype.

Chromosomal analysis revealed a complex karyotype pattern designated as 46, XY, -6, -6, der(6)t(6;8)(p23;q22)(8qter→8q22::6p23→6qter)x2, -8, der(8)t(8;21)(q22;q22)(8pter→8q22::21q22→21qter), -21, der(21)del(21)(q22:)(22pter→21q22:) in all the 20 metaphases analyzed. The analysis revealed translocation between one of the chromosomes 6, 8 and 21 at band region p23, q22 and q22 respectively.

Also the other homologous chromosome 6 designated as derivative chromosome 6 showed translocation in an uncommon form with part of 8q22 to qter on region 6p23, the same was confirmed by FISH.

Translocation between chromosome 8 and 21 at bands q22 and q22 respectively resulted in fusion of 5' RUNX1 present on chromosome 21 with 3' RUNX1T1 on chromosome 8. In an unusual form part of 5' RUNX1T1 instead of fusing with 3' RUNX1 on chromosome 21 has translocated on chromosome 6 at region p23 leading to loss of reciprocal fusion which is usually on derivative chromosome 21. Thus the karyotype and FISH studies indicate that there is partial homozygous deletion of chromosome 6 for region 6p23-pter and partial trisomy of chromosome 8 for the region 8q22 –qter[Figure 1].

FISH analysis revealed positive status for RUNX1/RUNXT1: (8;21) in a variant form and negative status for all the other markers i.e. PML/RARA, RARA gene rearrangement, MLL gene rearrangement and CBF beta gene rearrangement.

Using a dual-color dual fusion RUNX1 and RUNX1T1 probes for fluorescence insitu hybridization (FISH) analysis, we demonstrated fusion of RUNX1/RUNX1T1 with variant pattern in 100% of the interphase cells analyzed. On interphase cell the signal pattern was one fusion, three orange and two green signals indicating t(8;21) variant status [Figure 2A].

Metaphase FISH showed presence of RUNX1/RUNX1T1 fusion on the derivative chromosome 8. Two green RUNX1 signals on both chromosomes 21 indicated there is absence of reciprocal translocation between 8 and 21. Extra orange signals of RUNX1T1 seen on both homologous of chromosome 6 indicated partial trisomy of 8q22→qter on both the chromosome 6 [Figure 2B, Figure 2C] and the same was observed by karyotyping.

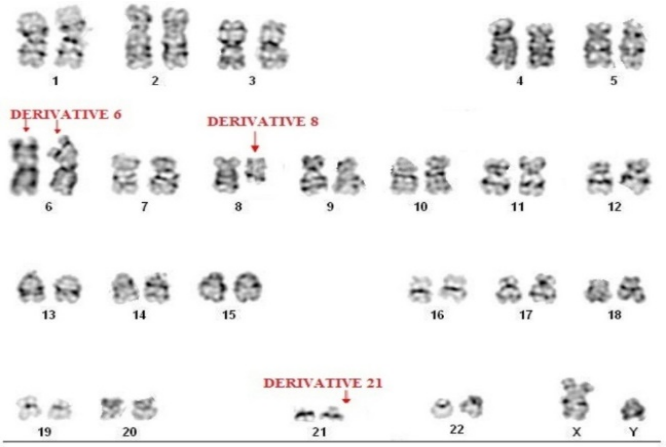

Figure 1: G-banded karyotype of the BM cells showing 46, XY,-6,-

6,der(6)t(6;8)(p23;q22)(8qter→8q22::6p23→6qter)x2, -8, der(8)t(8;21)(q22;q22)(8pter→8q22::21q22→21qter), -21, der(21)del(21)(q22:)(22pter→21q22:) Arrows indicate the aberrant chromosomes

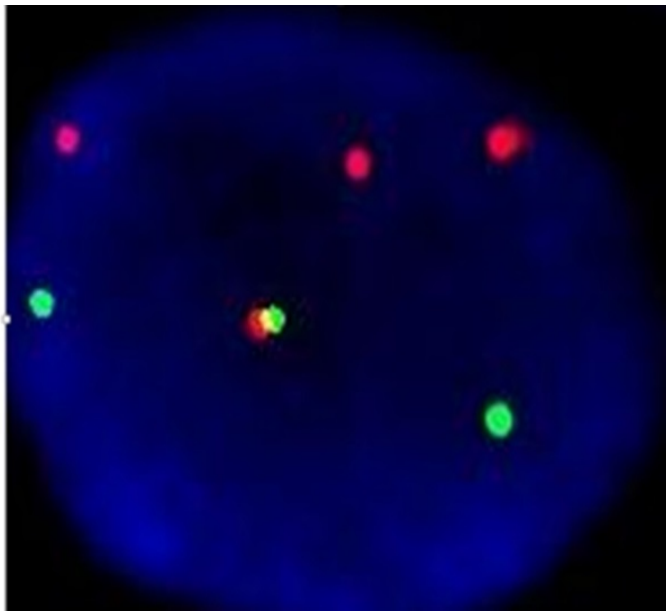

Figure 2A: FISH using dual-color probes for *ETO* (*RUNX*1*T*1) (Orange) and *AML*1 (*RUNX*1) (green) on an interphase cell showing one fusion, three orange, and two green signals.

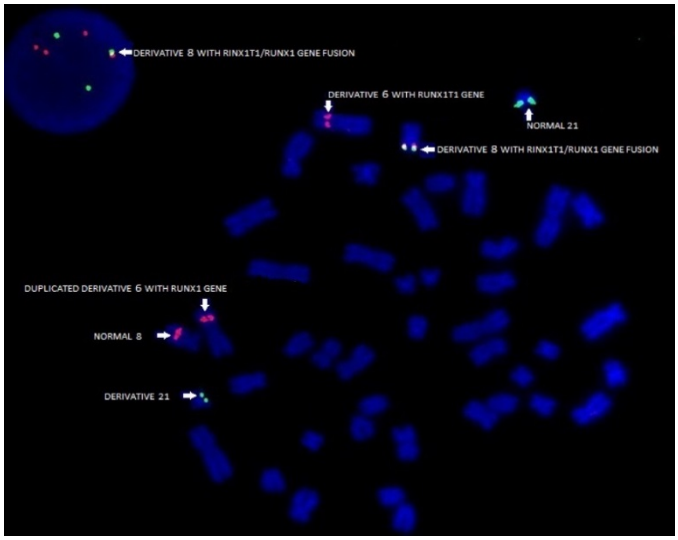

Figure 2B: FISH on metaphase cell using dual-color probes for *ETO* (*RUNX*1*T*1) (Orange) and *AML*1 (*RUNX*1) (green) on an interphase cell shows one fusion, three orange, and two green signals. The analysis showed complex translocation with three break rearrangement involving chromosome 6, 8 and 21 resulting in *RUNX1/RUNX1T1* fusion, loss of reciprocal *RUNX1/RUNX1T1* fusion and partial trisomy of chromosome for the region 8q22→qter.

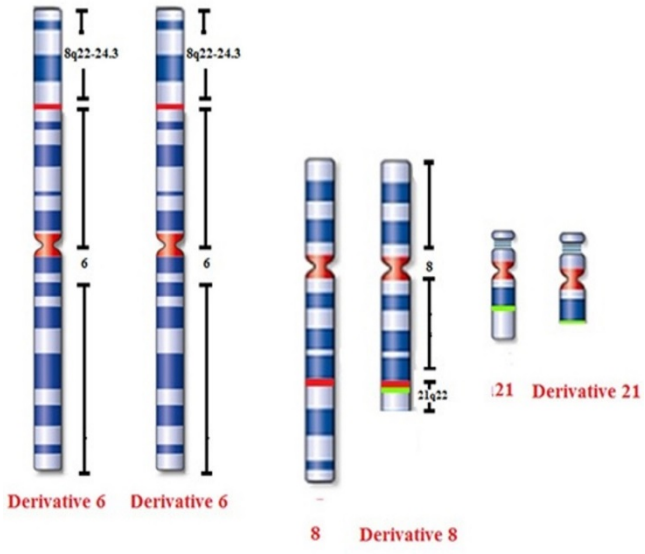

Figure 2C: Ideogram representation of complex rearrangement of chromosomes 6, 8 and 21 leading to fusion of *RUNX1/RUNX1T1* fusion

Mutations for several molecular markers considered as favorable or unfavorable prognostic factors in AMLpatients with t(8;21), such as FLT 3-ITD / TKD (D835), NPM 1, CEBPA, c-KIT mutations, were completely negative [Figure 3].

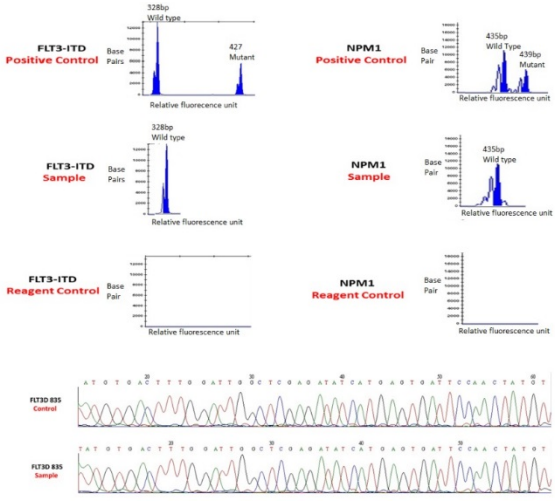

Figure 3: Mutation analysis for *FLT3*-ITD, *NPM1*, and *FLT3*-TKD(D835) genes by PCR, capillary electrophoresis-based fragment analysis and sequencing. The fluorescence of PCR products of *FLT3*-ITD (A) and *NPM1* (B) were analyzed on a 3500xL DNA Analyzer (Applied Biosystems). DNA sequencing data covering *FLT3*-TKD (D835) region are demonstrated (C). The position of a single base-pair substitution at codon 835 is indicated by asterisk. Positive controls are obtained from AML patients and samples are originated from the present case.

Patient received 3+7 standard Induction chemotherapy with Daunorubicin (60mg/m2) daily for 3 days and Cytosine arabinoside 100 mg/m2 daily for 7 days. Patient achieved complete remission after this induction chemotherapy proved by absence of RUNX1/RUNX1T1 fusion in 100% of the interphase cells of bone marrow aspirate. Following this, patient received consolidation chemotherapy with high dose cytosine arabinoside 3 gm/m2, 6 doses in each consolidation cycle for total of 3 cycles. Patient maintains his complete remission at end of 26 months follow up. Post induction day 28 marrow was in remission which was proved by no increase in CD34 and CD117 reactivity and absence of RUNX1/RUNX1T1 fusion by FISH.

Currently patient off treatment since 26 months and is in remission based on bone marrow with less than 5% blast and normal complete blood count at end of induction and consolidation chemotherapy also patient is maintaining a normal complete blood count on follow ups.

Discussion

Based on the cytogenetic and FISH findings we report a new variant t(8;21) translocation case in complete remission with a follow-up of 26 months. Although literature studies have reported 4 variant translocations involving chromosome 6 at bands 6p21 and 6p22, the region involved in our present case is a different region of 6p23 resulting from a complex translocation leading to homozygous deletion of chromosome 6 at band 6p23-pter and partial trisomy of 8q22-qter region.[32,33] Partial trisomy for region 8q22-qter has also been reported along with variant translocation t (8;12;21).[34]

Interphase FISH with a variant pattern of one fusion, three orange and two green signals along with metaphase FISH led to a better understanding of the complex mechanisms involving different chromosomes and for identification of RUNX/RUNX1T1 fusion on derivative 8. It also showed the absence of reciprocal fusion on chromosome 21. Our case reinforces the utility of combination of Karyotyping and FISH to determine accurate characterization of chromosome abnormalities.

Complete remission status after twenty six months of treatment supports the favorable prognostic behavior of the variant translocations as reported for other variant t(8;21) translocations.[23,35-37] Although literature studies have shown association of t (8;21) with favorable prognosis, poor outcomes in t(8;21) cases have been associated with several adverse prognostic indicators such as certain secondary cytogenetic aberrations, leukocytosis, CD56 expression and extra-medullary manifestations.[5,38,39] As there has been no clear prognostic significance established of variant cases with additional chromosomal abnormalities such as loss of sex chromosomes, trisomy 4, inversion in chromosome 16 and deletion 9q[40], additional abnormality of homozygous deletion of region 6p23-pter and partial trisomy of chromosome 8q22-qter in present case does not seems to abrogate the favorable prognosis and absence of unfavorable prognostic markers such as FLT3 and c-Kit mutations also may have influenced the prognostic behavior.

Interphase FISH pattern of one fusion, three orange and two green signals was indicative of a variant translocation and metaphase FISH along with Karyotyping elucidated the regions involved. Metaphase FISH with two green RUNX1 signals on both chromosomes 21 indicated absence of reciprocal translocation between 8 and 21. Extra red signals by FISH suggestive of extra copy of RUNX1T1 gene, on karyotype indicated partial trisomy of 8q22→qter on chromosome 6. The 5' region of RUNX1T1 fused with an unknown gene on both homologues of chromosome 6 at region 6p23 instead of forming a reciprocal fusion with RUNX1. Extra dosage of RUNX1T1 or its fusion to unknown gene at 6p23 and its clinical and prognostic significance can be understood with more number of case studies.

Absence of mutations of/within molecular markers such as FLT3, NPM1, c-Kit and CEBPA may lead to a less burden of additional mutational events and help in treatment decisions. The chromosomal region 8q22~qter could harbor genes responsible for leukemogenesis. Since t(8;21) is a necessary but not sufficient precondition for leukemic transformation (Miyamoto T, 2000), deregulation of oncogene suppressors or overexpression of an oncogene in 8q22~qter region could be the additional mutational event for leukemogenesis.[34] Additional events and disease status may have also play a role as in the prognosis and Gustafson et al., have reported that therapy related AML with t(8;21) shares many features with de novo AML-t(8;21)(q22;q22), but affected patients have a worse outcome.[41]

The identification of many potentially important relationships among recurrently mutated AML genes and pathways have provided a comprehensive foundation for an understanding of the genetic rules of pathogenesis and also suggest that mutational profiling could potentially be used for risk stratification and to inform prognostic and therapeutic decisions regarding patients with AML but still prognosis for AML with variant t(8;21) remains unclear.[42,43]

Based on the case presented and the literature data, it is apparent that cases with complex variants of t (8;21) comprise a distinct entity. The question calls for further investigation, in large series, both to understand the biology and to confirm the prognostic significance. A more precise genetic biomarker would be essential to further help in defining this class and newer technologies such as Microarray or Next Generation Sequencing would contribute to this study that would identify contributory molecular events which will lead to distinctly categorize and evolve as variant subclass. Large series study on these variants and their co-existing abnormalities would be needed during revisions of disease classification and possibly would be treated using a targeted therapy.

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