KMT2A-ARHGEF12, a Therapy Related Fusion with Dismal Prognosis.

Nada Assaf (assaf.nada@outlook.com)  CH Versailles: Centre Hospitalier de Versailles  https://orcid.org/0000-0002-3352-2660

Raphael Liévin  CH Versailles: Centre Hospitalier de Versailles

Fatiha Merabet  CH Versailles: Centre Hospitalier de Versailles

Victoria Ragueneau  CH Versailles: Centre Hospitalier de Versailles

Jenifer Osman  CH Versailles: Centre Hospitalier de Versailles

Marc Spentchian  CH Versailles: Centre Hospitalier de Versailles

Rathana Kim  Hôpital Saint-Louis: Hopital Saint Louis

Florian Renosi  Université de Bourgogne: Universite de Bourgogne

Francine Gamache  Université de Bourgogne: Universite de Bourgogne

Virginie Eclache  Hôpital Avicenne: Hopital Avicenne

Isabelle Luquet  IUCT Oncopole: Institut Universitaire du Cancer Toulouse Oncopole

Lilia Corral Abascal  Centro ricerca tettamenti monza

Mariella D'Angió  University of Milan–Bicocca: Universita degli Studi di Milano-Bicocca

Patrizia Larghero  University of Frankfurt: Goethe-Universitat Frankfurt am Main

Claus Meyer  University of Frankfurt: Goethe-Universitat Frankfurt am Main

Rolf Marschalek  University of Frankfurt: Goethe-Universitat Frankfurt am Main

Philippe Rousselot  CH Versailles: Centre Hospitalier de Versailles

Christine Terré  CH Versailles: Centre Hospitalier de Versailles

Short Report

Keywords: KMT2A, ARHGEF12, acute myeloid leukemia, acute lymphoid leukemia, plasmacytoid dendritic cell

DOI: https://doi.org/10.21203/rs.3.rs-484660/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.  Read Full License
Abstract

Background: The detection of KMT2A gene rearrangements have an important impact on the prognosis and management of acute leukemias. These alterations most commonly involve reciprocal translocations at specific breakpoint regions within KMT2A. To date, more than 100 translocation partner genes of KMT2A have been identified, with different effects on risk stratification.

Methods and Results: We report the case of a mature plasmacytoid dendritic cells proliferation associated with B lymphoblasts harboring a KMT2A-ARHGEF12 fusion. This rare rearrangement, resulting from a cryptic deletion on the long arm of chromosome 11, is located outside the known major and minor breakpoint regions of KMT2A, not reported to date. The review of the few cases of KMT2A-ARHGEF12 reveals the tendency of this deletion to occur in therapy related hematologic neoplasms and confer unfavorable prognosis.

Conclusion: This review sheds light into the rare KMT2A-ARHGEF12 fusion in leukemia. Reporting rare chimeras is essential to improve knowledge about the biological mechanism and associated clinical consequences.

Introduction

The KMT2A gene, previously known as MLL, is located at chromosome 11q23.3. KMT2A acts as a catalytic, binding and recognizing part of the COMPASS complex, a promoter of embryonic development and early hematopoiesis. KMT2A rearrangements can occur in a wide variety of gene transformations including single nucleotide insertions or deletions, translocation of the KMT2A region into partner chromosomes or vice versa. Additional rearrangements, including gene duplication or reciprocal translocations, have also been shown to be involved in the modification of the KMT2A protein product. With the exception of internal partial tandem duplications, rearrangements at the KMT2A locus lead to the formation of hybrid genes composed of portions of KMT2A and portions of many partner genes. To date, more than one hundred different KMT2A fusion alleles have been described. The KMT2A gene exhibits two different breakpoint cluster regions (BCR): major and minor. Breakpoints in the major BCR localize mainly at introns 9–11, while breakpoints in the minor BCR occur within KMT2A introns 21–23. In many cases, chromosomal rearrangements of KMT2A result in the overexpression of HOX genes leading to increased hematopoietic stem cell proliferation and decreased regulated apoptosis.

The clinical consequences of the frequent KMT2A fusion are well known, however little is known about the effect of rare translocation partner genes (TPG). We hereby present the diagnostically challenging case of an adult therapy related B lymphoblastic leukemia (B-ALL) harboring the rare KMT2A-ARHGEF12 fusion at an unusual breakpoint region.

Case Presentation

A 56-year-old male patient was admitted in September 2016 with hypercalcemia (serum calcium = 2.69 mmol/L), normocytic anemia (hemoglobin = 11.7 g/dL; mean corpuscular volume = 95fL) and an IgA Kappa peak of 48.36 g/L. Bone marrow aspiration (BMA) revealed a 31% infiltration by dysplastic plasma cells. In the presence of specific bone lesions, a diagnosis of Multiple Myeloma (MM), International Staging System 3 was made. He was treated according to the CASSIOPEIA study, standard arm, with 4 cycles of bortezomib, thalidomide and dexamethasone. After complete remission, autologous hematopoietic stem cell transplant (HSCT) was performed following a conditioning regimen with melphalan 200 mg/m². Thalidomide was omitted during consolidation due to the development of stage 2 neuropathy. Until September 2019, our patient was placed on maintenance daratumumab with serial follow up by flow cytometry.

Upon presentation for the last cycle of daratumumab, the patient was found to have petechia. He was asymptomatic and denied fever, night sweats or recent weight loss. His vital signs were normal. There was no lymphadenopathy, splenomegaly, spontaneous/provoked bone pain nor evidence of external plasmacytoma. The neurologic exam was negative, except for residual peripheral stage 1 neuropathy. Complete blood count showed macrocytic anemia (hemoglobin = 7.4 g/dL; mean corpuscular volume = 105fL) and thrombocytopenia (platelets = 23 x 10⁹/L) and leukocytosis (white blood cells = 21.8 x 10⁹/L). The peripheral blood was also marked with recent (< 2 months) monocytosis (absolute number = 2.02 x 10⁹/L; percentage = 16%) and eosinophilia (absolute number = 0.63 x 10⁹/L; percentage = 5%). Circulating blasts were found, accounting for 1% of the total white blood cell population. Serum protein electrophoresis/immunofixation was negative. Proteinuria and MRD for MM were undetectable.

BMA morphologic examination revealed a hyperplastic marrow with dysgranulopoiesis, monocytesis (10%), eosinophilia (10%) and 13% agranular blasts. Flow cytometry indicated a pro-B lymphocytic origin (EGIL classification) of the observed blasts due to their expression of the B lymphoid markers CD19 (strong intensity), CD20, CD22, and the immature markers CD34, CD45 (low), Tdt and HLA-DR. Blasts were also positive for CD123 and aberrantly expressed the myeloid markers CD13 and CD33 (partial). They were negative for CD10, surface and intracytoplasmic IgM and Myeloperoxidase (MPO) by direct immunofluorescence.

In addition, plasmacytoid dendritic cells (pDCs) were observed accounting for 5% of the total marrow cell population by morphology. This population consisted of mature large cells characterized by an abundant, clear cytoplasm with extending projections and a small nucleus with coarse chromatin and absent nucleoli (Fig. 1, panels A-D). The pDCs, accounting for 8% of cells by flow cytometry, were positive for CD123 (strong), CD4 and the specific markers CD303, CD304, FcER1 and Lamp-5 (BAD-LAMP). CD56 and cTCL1 were negative. These observations were confirmed by a bone marrow biopsy and referral to a specialized center (Etablissement Français du Sang Bourgogne-Franche-Comté, Besançon, France).

Next Generation Sequencing (NGS) performed on the bone marrow sample revealed the presence of NRASG13D, RUNXT1F136C, DNMT3N757D and R792 mutations.
B-ALL molecular analysis revealed the absence of fusion transcripts using a targeted approach by reverse transcriptase multiplex ligation-dependent probe amplification (RT-MLPA)[6]. No clonal Ig-TCR rearrangements were identified using EuroMRD guidelines. [7]

No abnormalities were observed on conventional karyotyping (46,XY[20]). As recommended by the GFCH (Groupe Francophone de Cytogénétique HématoLOGique) for normal karyotype B-ALL, FISH for the detection of KMT2A rearrangements (break apart double color 11q23, Metasystems Probes, Heidelberg, Germany) was performed. In contrast with RT-MLPA results, a rearrangement was detected in 96/100 examined cells with loss of the signal (Fig. 1, panel E). Referral to the German Diagnostic Center of Acute Leukemia (DCAL) Goethe-University Frankfurt allowed the characterization of the fusion partner using capture probes on the entire KMT2A locus.[8] A novel KMT2A (intron 35) fusion with ARHGEF12 (intron 1) was identified. Specific PCR probes matching our patient’s KMT2A rearrangement were then designed for serial follow up (Table 1).

| Primer | Sequence |
|--------|----------|
| MLL_intron35-Fprimer | ATCCTGCACCTCCTTGTGTTG |
| ARHGEF12_intron1-Rprimer | AAGCTTGCTAGGTGTTAAGTTTGG |
| MLL-ARHGEF12_junction-Probe | ACTGCTTCCAGCGGGTCACAGATACC |

Treatment with Tagraxofusp, an anti-CD123 cytotoxin-targeting B lymphoblasts and pDCs, was initiated in preparation for allogenic HSCT which could not be performed due to disease complication by bacterial septicemia resulting in patient death.

**Discussion**

**Diagnostic challenge:**

Myelomonocytic hyperplasia, B lymphoblasts and mature pDCs along with preservation of some normal hematopoiesis were present in our patient’s bone marrow. Dysplasia and monocytosis along with the absence of a BCR-ABL1 fusion transcript, PDGFRα, PDGFRβ and JAK2 rearrangements suggest a diagnosis of Chronic Myelomonocytic Leukemia (CMML)[4], confirmed by findings persistence for >3 months. The presence of B lymphoblasts suggest disease transformation.

Expansion of plasmacytoid dendritic cells presents in 2 distinct pathologic forms: Blastic pDC Neoplasm (BPDCN) and Mature pDC Proliferation (MPDCP). These entities are distinguished by the mature morphology of expended cells in MPDCP and the absence of CD56 expression.[14] MPDCP is associated with the presence of a myeloid neoplasm (frequently CMML), but also with MDS or acute myeloid leukemia (AML) with monocytic differentiation. In CMML, nodules of mature pDCs can be seen in around 20% of bone marrow biopsies.[15] MPDCP constitutes a rare entity, with around 80 cases described in the medical literature.[9] Patients are predominantly males above 60 years of age. [10] Treatment and prognosis depend on the underlying myeloid condition rather than the associated MPDCP.[9]

**KMT2A rearrangement:**

A well-recognized characteristic of KMT2A rearrangements is their ability to associate with multiple TPGs. Using long-distance inverse PCR analysis, Meyer et al report that 9 different fusion genes account for 90% of KMT2A rearrangements while the remaining 10% are spread among not less than 100 TPGs.[2] Thus, diagnosis using predesigned PCR probes complementary to the most common variants results in around 10% false negative rate. In addition, around 15% of KMT2A rearrangements are cryptic resulting in a visually normal chromosome 11[11], reflected in our case. As such, reflex testing using break apart dual color FISH is recommended in all suspected B-ALL and mixed lineage acute leukemia cases to rule out a KMT2A rearrangement.[11] Once the partner gene is identified, specific PCR probes can be designed and used for MRD monitoring. This approach was verified in several prospective studies.[12-14]

The KMT2A-ARHGEF12 fusion was first described by Kourlas et al. in a case of complex karyotype AML.[15] (Table 2; case 1). To date, only three additional cases were reported in the literature.[16-18] (Table 2; cases 2–4). Two other patients with this rare fusion were diagnosed at the DCAL (Table 2; cases 5–6). The majority are treatment related malignancies or occurring after long term herbicides exposure. This suggests a molecular trigger for this unusual fusion. ARHGEF12 is located on chromosome 11, telomeric to KMT2A. The rearrangement mechanism thus involves a 2MB interstitial deletion resulting in KMT2A-ARHGEF12 fusion, usually occurring within the major BCR of KMT2A. In our case (Table 2; case 7), the rearrangement resulted from the in-frame fusion of intron 35 of KMT2A and intron 1 of ARHGEF12 where nearly the entire KMT2A protein is fused to the entire ARHGEF12 protein. This KMT2A breakage, occurring outside the major and minor BCR,[8], has never yet been described, to the best of our knowledge. In addition to the identity of the TPG, the location of the KMT2A breakpoint was found to affect the clinical behavior and prognosis of the hematological malignancy.[2, 19] Failure of complete remission and short overall survival was observed for all reported adult patients with KMT2A-ARHGEF12 fusion (Table 2). The clinical effect of the rare breakpoint found in our patient remains to be elucidated.
Table 2
Characterization of 4 cases with KMT2A-ARHGEF12 gene rearrangement.

| Case 1[^5] | Case 2[^6] | Case 3[^7] | Case 4[^8] | Case 5 | Case 6 |
|------------|------------|------------|------------|--------|--------|
| Gender/Age | M/36       | F/77       | M/69       | F/13   | F/52   | M/6    |
| Country    | USA        | China (Chang Gung Memorial Hospital) | China (Shanghai Institute of Hematology) | Norway (Norwegian Radium Hospital) | France (IUCT Oncopole, Toulouse) | Italy (Ce Tettaman) |
| Diagnosis  | AML-M4 (primary) | Pesticides exposure | AML-M5 (primary) | B-ALL (secondary) | AML-M4 (secondary) | AML (secondary) |
| Primary Diagnosis | AML-M4 with t(9;11) (p21;q23) | Endometrial adenocarcinoma | B-ALL | AML-M4 (primary) | AML | BL |
| Peripheral blood finding at diagnosis | N.A | N.A | N.A | pancytopenia | WBC 6.9x10^9/L; Hb 10.1 g/dL; PLT 39x10^9/L | WBC 34 | Hb 11.3 | PLT 131 |
| Blast counts in PB and BM | N.A | N.A | N.A | 90% in BM | 0.7% and 43% | 0.2% an |
| Immunophenotype | N.A | N.A | N.A | Pre-B phenotype | Positive: CD33, CD117, MPO, HLA-DR. | Negative: CD19, CD34 |
| Karyotype | 51,XY:+8,+19,+3mar[19]/46,XY[1] | 53,XX,+6,+8,+9,+11,+13,+22 | 46,XY | 45,XX,-9(14;19)(q32a13)[8]/46,XX[3] | 46,XY,t(1;8)(q31;p23)c | 46,XY |
| KMT2A FISH results | Nuc ish(5'MLLKMT2Ax2)[8/8] | N.A | N.A | nuc ish (5'KMT2Ax2,3'KMT2Ax1) (5'KMT2A con 3'KMT2Ax1) [179/200] | nuc ish(5'KMT2Ax2,3'KMT2Ax1) (5'KMT2A con 3'KMT2Ax1) [80/100] | nuc ish(5'KMT2) |
| Method for KMT2A-ARHGEF12 detection | RT-PCR | RT-PCR | RNA sequencing | RNA sequencing | LDI-PCR | NGS |
| Breakpoint KMT2A | Intron 9 | N.A | N.A | Intron 9 | Intron 11 | Intron 1’ |
| Breakpoint ARHGEF12 | Intron 11 | N.A | N.A | Intron 13 | Intron 11 | Exon 10 |
| Treatment | Standard induction chemotherapy followed by HSCT | Standard induction (2 courses) | vincristine, doxorubicin, methotrexate HSCT | N.A | First reg cytarabine doxorub Second and cyt |
| Clinical outcome | Death due to interstitial pneumonia | Failure of CR and death | CR | N.A | Failure |

[^5]: male; F: female; WBC: White Blood Cell; Hb: hemoglobin; PLT: platelets; PB: peripheral blood; BM: bone marrow; NGS: Next Generation Sequencing; LDI-PCR: Long Distance Isothermal PCR.

M: male; F: female; WBC: White Blood Cell; Hb: hemoglobin; PLT: platelets; PB: peripheral blood; BM: bone marrow; NGS: Next Generation Sequencing; LDI-PCR: Long Distance Isothermal PCR.

Conclusion
In conclusion, reporting unusual genetic aberrations is essential to improve our knowledge of the biology and clinical features of hematologic disorders. Additional information is needed for the determination of the prognosis and adequate treatment modalities of rare KMT2A rearrangements.

Declarations

Funding:
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Conflicts of interest/Competing interests:**

None.

**Availability of data and material (data transparency):**

Data presented in this study can be found in the Laboratory Information System of the centre hospitalier de Versailles and the Institute of Pharmaceutical Biology/DCAL, Goethe-University Frankfurt.

**Code availability (software application or custom code):**

Not applicable.

**Authors' contributions:**

N.A wrote the manuscript; R.L helped writing the manuscript; F.M, V.R, J.O, M.S, R.K, F.R, F.G, V.E, I.L, L.C.A, M.D-A, P.L, C.M, R.M, P.R reviewed the manuscript and participate in the diagnosis/clinical management and C.T had the manuscript idea and reviewed the manuscript.

**Ethics approval/Consent to participate (include appropriate statements):**

Patient's informed consent was obtained for anonymous publication of his case.

**Consent for publication:**

Not applicable.

**Acknowledgments:**

The authors would like to thank the Centre Hospitalier de Versailles for editorial assistance.

**References**

1. Ntzichristos, P., et al., *Mechanisms of epigenetic regulation of leukemia onset and progression*. Adv Immunol, 2013. **117**: p. 1-38.
2. Meyer, C. and T. Burmeister, *The MLL recombinome of acute leukemias in 2017*. 2018. **32**(2): p. 273-284.
3. Tanja A. Gruber, J.E.R., *Chapter 62 - Acute Myeloid Leukemia in Children*, in *Hematology*, E.J.B. Ronald Hoffman, Leslie E. Silverstein, Helen E. Heslop, Jeffrey I. Weitz, John Anastasi, Mohamed E. Salama, Syed Ali Abutalib., Editor. 2018, Elsevier. p. 981-993.
4. Swerdlow SH, C.E., Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Vol. 2. 2017.
5. Moreau, P., et al., *Bortezomib, thalidomide, and dexamethasone with or without daratumumab before and after autologous stem-cell transplantation for newly diagnosed multiple myeloma (CASSIOPEIA): a randomised, open-label, phase 3 study*. Lancet, 2019. **394**(10192): p. 29-38.
6. van Dongen, J.J.M., et al., *Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: Report of the BIOMED-2 Concerted Action BMH4-CT98-3936*. Leukemia, 2003. **17**(12): p. 2257-2317.
7. van der Velden, V.H.J., et al., *Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data*. Leukemia, 2007. **21**(4): p. 604-611.
8. Meyer, C., et al., *Human MLL/KMT2A gene exhibits a second breakpoint cluster region for recurrent MLL-USP2 fusions*. 2019. **33**(9): p. 2306-2340.
9. Facchetti, F., et al., *Neoplasms derived from plasmacytoid dendritic cells*. Mod Pathol, 2016. **29**(2): p. 98-111.
10. Cook, J.R., *Chapter 11 - Fluorescence In Situ Hybridization*, in *Cell and Tissue Based Molecular Pathology*, M.H.S. Raymond R. Tubbs, Editor. 2009, Churchill Livingstone. p. 104-113.
11. de Haas, V., et al., *Initial Diagnostic Work-Up of Acute Leukaemia: ASCO Clinical Practice Guideline Endorsement of the College of American Pathologists and American Society of Hematology Guideline*. J Clin Oncol, 2019. **37**(3): p. 239-253.
12. Szczepanski, T., C.J. Harrison, and J.J. van Dongen, *Genetic aberrations in paediatric acute leukaemias and implications for management of patients*. Lancet Oncol, 2010. **11**(9): p. 880-9.
13. Burmeister, T., et al., *Monitoring minimal residual disease by quantification of genomic chromosomal breakpoint sequences in acute leukemias with MLL aberrations*. Leukemia, 2006. **20**(3): p. 451-7.
14. Van der Velden, V.H., et al., Prognostic significance of minimal residual disease in infants with acute lymphoblastic leukemia treated within the Interfant-99 protocol. Leukemia, 2009. 23(6): p. 1073-9.

15. Kourlas, P.J., et al., Identification of a gene at 11q23 encoding a guanine nucleotide exchange factor: evidence for its fusion with MLL in acute myeloid leukemia. Proc Natl Acad Sci U S A, 2000. 97(5): p. 2145-50.

16. Shih, L.y., et al., Characterization of fusion partner genes in 114 patients with de novo acute myeloid leukemia and MLL rearrangement. Leukemia, 2006. 20(2): p. 218-223.

17. Liu, Y.F., et al., Genomic Profiling of Adult and Pediatric B-cell Acute Lymphoblastic Leukemia. EBioMedicine, 2016. 8: p. 173-183.

18. Panagopoulos, I., et al., Therapy-induced Deletion in 11q23 Leading to Fusion of KMT2A With ARHGEF12 and Development of B Lineage Acute Lymphoblastic Leukemia in a Child Treated for Acute Myeloid Leukemia Caused by t(9;11)(p21;q23)/KMT2A-MLLT3. Cancer Genomics Proteomics, 2021. 18(1): p. 67-81.

19. Emerenciano, M., et al., The distribution of MLL breakpoints correlates with outcome in infant acute leukaemia. Br J Haematol, 2013. 161(2): p. 224-36.

Figures

Figure 1

Bone marrow aspirate and FISH. Bone marrow aspirate reveal myeloid hyperplasia and excess blasts. Plasma dendritic cells show typical 'hand mirror' morphology. Panel A: original magnification X500. Panels B, C and D: original magnification X1000. LB: Lymphoblasts, Mono: Monocyte, pDC: Plasma dendritic cell, EOS: Eosinophil, N: Neutrophil. Panel E: Break apart double color 11q23 for the detection of KMT2A rearrangements. The pattern of hybridization reveals the presence of two fluorescent signals on the normal chromosome 11 (Red: 5’KMT2A, Green: 3’KMT2A) and loss of the 3’ portion of KMT2A on the rearranged counterpart.