AL-KUFA JOURNAL FOR BIOLOGY
ISSN (Print): 2073-8854 **ISSN (online):** 2311-6544

Original Research Paper

Screening JAK2 V617F Mutation in Acute Myeloid Leukemia (AML) and Acute Lymphoblastic Leukemia (ALL) Patients

Maytham A. Muftin Alkaby [1], Maytham. A. Dragh[2]

[1,2] *Department of Biology, Faculty of Science, University of Misan, Maysan, Iraq*

Article history
Received: 05/6/2024
Revised: 13/08/2024
Accepted: 22/9/2024

*Corresponding Author:
Maytham A. Muftin Alkaby , Department of biology, Faculty of science, University of *Misan, Maysan* , Iraq;
Email: maithamdragh@uomisan.edu.iq

DOI: 10.36320/ajb/v16.i3.17071

Abstract: The *JAK2* gene codes for the production of the Janus kinase 2 protein, which is essential for signaling from cell surface receptors to the nucleus of cells. The regulation of the generation of hematopoietic stem cells depends on these signals. A single nucleotide alteration called the JAK2 V617F mutation causes the protein's position 617 to shift from valine (V) to phenylalanine (F). Due to the overactivation of the JAK-STAT signaling system brought on by this mutation, hematopoietic stem cells proliferate uncontrollably in the bone marrow. Only infrequent reports of acute myeloid leukemia (AML) patients have included the JAK2 V617F mutation, which is recognized for its relationship with myeloproliferative neoplasms (MPN) and myelodysplastic syndromes (MDS). The purpose of this study is to determine if Iraqi patients with acute lymphoblastic leukemia (ALL) and AML have the JAK2 V617F mutation.

Keywords: JAK2 V617F mutation, MPN, MDS, AML, ALL

1.Introduction

The JAK2-STAT signal-transduction pathway plays a crucial role in hematopoiesis and is frequently dysregulated in various hematologic malignancies. The JAK2 V617F mutation, resulting in the substitution of valine for phenylalanine, has been identified as a driver mutation in myeloproliferative neoplasms (MPN) and myelodysplastic syndromes (MDS). However, its presence in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) remains unclear. Understanding the prevalence and clinical implications of this mutation in AML and ALL is essential for improved disease management and targeted therapies. Gene mutations are the most frequent genetic variation for most genetic variants. Genetic diseases are frequently, but not always, brought on by gene polymorphisms. The most frequent base pair variation in humans is a single nucleotide polymorphism (SNP), which is the most prevalent type of nucleotide variation [1]. Numerous SNPs have been linked to leukemias in previous research, and Janus kinase 2 (*JAK2*) was one of the SNPS associated with AML [2]. JAK2 signaling serves significant functions in embryonic development, hemopoiesis, cancer, and inflammation, making it relevant in both physiology and pathology[3,4].JAK2, a member of the JAK family of protein tyrosine kinases (PTKs), is widely expressed in almost all cell types and serves as an essential intracellular mediator of cytokine or hormone signaling [5].

Interest in *JAK2* was greatly sparked by the finding that a single point mutation in the nonreceptor tyrosine kinase JAK2, which results in the substitution of a valine residue by phenylalanine at amino acid 617 (JAK2V617F), is responsible for triggering a subset of MPN [6,7]. Philadelphia – negative (Ph-neg) MPNs, particularly polycythemia vera (PV), have been linked to a somatic point mutation (c.1849G>T) in exon 14 of the *JAK2* gene, a component of the JAK2-STAT signal-transduction system [8,9].The *JAK2* gene, which is important in the pathophysiology of PV, has a few more mutations in

DOI: 10.36320/ajb/v16.i3.17071

exon 12. These mutations are present in 3% of (PV) cases[10,11].

The genetic foundation for the Breakpoint cluster region-Abelson1(BCR-ABL1)-negative myeloprol- iferative neoplasm is frequently the JAK2 V617F mutation (MPN). The majority (~95%) of polycythemia vera and approximately half of the essential thrombocythemia and primary myelofibrosis patients carry this mutation [12]. However, it is rarely observed in de novo (AML) and (MDS) [13,2].

Acute lymphoblastic leukemia, with other types of leukemia, have all been associated with *JAK* mutations. Most *JAK* mutations are discovered in (ALL) [14].ALL is the outcome of immature B or T cell transformation. Approximately 85% of ALL patients are B-ALL, which has a better prognosis than T-ALL, which is particularly fatal in adults[15].

This motivated us to test our patients with acute myeloid and acute lymphoblastic leukemias for the JAK2V617F mutation.

2.Methodology

Samples collection

This study will include a retrospective analysis of peripheral blood samples collected from Iraqi patients diagnosed with AML and ALL. DNA will be extracted, and polymerase chain reaction (PCR) amplification will be performed to detect the JAK2 V617F mutation. Sanger sequencing and allele-specific PCR, will be employed to confirm the presence of the mutation.

The investigated group comprised 40 individuals, including 20 ALL and 20 AML, who were diagnosed and registered by the Iraqi Center for Hematology. These patients had acute leukemia, specifically acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). The age range of the participants was 14 to 80 years. The control group consisted of 20 voluntary blood donors. These individuals were chosen to match the investigated group in terms of ethnicity and geographical location. The DNA used for the research was isolated from peripheral blood samples collected between July 23, 2022, and September 26, 2022. Informed consent was obtained from all individuals before their participation in the study, ensuring they were aware of the study's purpose and procedures. The samples were stored under the same conditions to maintain consistency. To extract DNA from the whole blood samples, the gSYNCTM DNA Extraction Kit Functional Test Data (Geneaid, Taiwan) was used. This kit is specifically designed for DNA extraction and purification.

Column purification

Genomic DNA was isolated from blood samples. The amount of isolated DNA was varied from 52.392 to 88.712 ng/µl and an absorbance ratio of A260/280 was obtained in the range of 1.71–1.98.

Tetra-ARMS-PCR

Tetra-primer, ARMS, which were allele-specific, was utilized to create a DNA fragment containing the JAK2 polymorphism's special allele [16]. The t-ARMS-PCR method is a technique used for the detection and genotyping of specific mutations, such as the JAK2V617F mutation in leukemia patients. This method utilizes four primers: a forward outer primer (FO), a reverse outer primer (RO), a forward inner wild type-specific primer (Fwt), and a reverse inner mutant-specific primer (Rmt). These primers are designed to specifically target the JAK2 V617F mutation.

The PCR reaction is performed in a total volume of 25 µL. The reaction mixture includes 5 µL of DNA template (ranging from 25-1250 ng), 12.5 µL of GoTaq® Green Master Mix (2x concentration), 1 µL of each FO, RO, and Fwt primers, 1 µL of Rmt primer, and 3.5 µL of nuclease-free water to achieve the final volume.

The PCR amplification steps are carried out on a thermal cycler with the following program. Initial denaturation: 94ºC for 15 minutes, denaturation: 94ºC for 30 seconds, annealing: 58ºC for 30 seconds, extension: 72ºC for 30 seconds, repeat steps 2-4 for 32 cycles, final extension: 72ºC for 10 minutes, pause: 15ºC. After the PCR amplification, the products are analyzed using agarose gel electrophoresis. A 2% agarose gel containing ethidium bromide is prepared, Photographs are taken using a digital camera to document the results.

In the t-ARMS-PCR method, the presence or absence of specific PCR products indicates the presence or absence of the JAK2V617F mutation. The control 463-bp band is expected in all cases, while the 267-bp mutant fragment indicates the presence of the mutant allele. On the other hand, the 229-bp wild-type fragment suggests the absence of the mutation and the presence of the wild-type allele. By analyzing the sizes and patterns of the PCR products, the genotype of the *JAK2* gene can be determined in leukemia patients and healthy control samples. Primers were designed according Badrawy and Ibrahim [17]. These primers were provided from (ScientificReseracher. Co. Ltd. Iraq) as following tables:

Table 1: The Tetra-ARMS-PCR Primers for JAK2V617F mutation with their sequence and amplicon size:

Primer	Sequence (5'-3')	Product size
FO:	TTG GAT TTT TCC	463bp

Forward outer	TTT TTG CTT	
RO: Reverse outer	GGC CTG GAA TCT CCT CTA TCA	
Fwt: Forward wild-type specific	TCC TCA GAA CGT TGA TGG CAG	229bp
RMT: Reverse mutant-type	GTTTTACTTACTCT CGTCTCCACAAAA	267bp

To determine the sequence of nitrogen bases following confirmation of sample amplification, samples of 20 microliters of the PCR product were sent to Macrogen Company in South Korea to acquire the real sequences of the nitrogenous bases for the required pieces of genes.

3.Results and discussion

Preliminary results from a limited number of case reports have suggested the potential presence of the JAK2 V617F mutation in AML. However, comprehensive data on the frequency and significance of this mutation in AML and ALL among Iraqi patients are lacking.

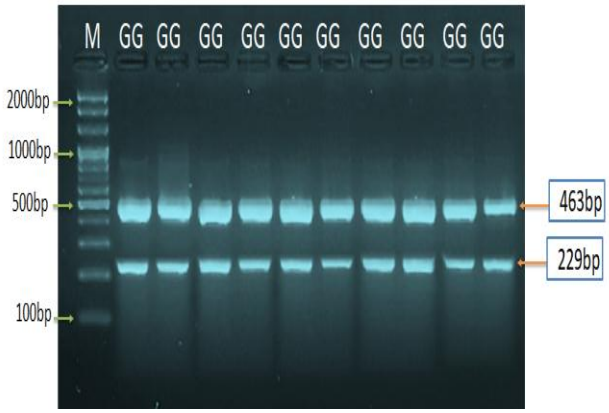

Fig.1. Agarose gel electrophoresis image that showed the T-ARMS-PCR product analysis of for JAK2V617F mutation in leukemia patients' samples. Where M: marker (2000-100bp). The lane (GG) normal wild-type JAK2 showed two bands at 229bp for the G allele and outer internal control was observed at 463bp T-ARMS-PCR product.

To in terpret these results, we note the following:
The presence of the band at 463 bp indicates that the sample contains a control band and does not have the JAK2V617F mutation. In other words, it is negative for the mutation. The presence of bands at both 463 bp and 267 bp suggests that the sample contains both a control band and a mutant band. This indicates a positive result for the JAK2V617F mutation, meaning the sample is of the mutant type.
The presence of bands at 463 bp and 229 bp indicates that the sample contains a control band and a wild-type band. This suggests that the sample is negative for the JAK2V617F mutation and is of the common wild type.
Based on these findings, it appears that the JAK2V617F mutation was not detected in the leukemia patients included in the study, regardless of whether they had acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML). Additionally, the mutation was not observed in the control group. The researchers also performed a multiple sequence alignment analysis of the JAK2 gene in the leukemia patients' samples and compared them to reference samples from the NCBI Gene Bank. The alignment analysis revealed a close similarity between the patients' samples and the NCBI-BLAST JAK2 Reference wild type (NG_009904.1) with minimal genetic changes ranging from 0.00150% to 0.00050%.

This suggests that the *JAK2* gene in the local Iraqi acute leukemia patients' samples is closely related to the wild type reference sequence, indicating a lack of significant mutations or alterations in this gene in the studied population figure 2.

Fig.2 The DNA Sequencing Chromatogram peaks of the JAK2 gene in local Homo sapiens acute leukemia Iraqi patients and control samples that showed only wild type allele (G allele) and there is no JAK2 mutant type's allele (T allele).

.

DOI: 10.36320/ajb/v16.i3.17071

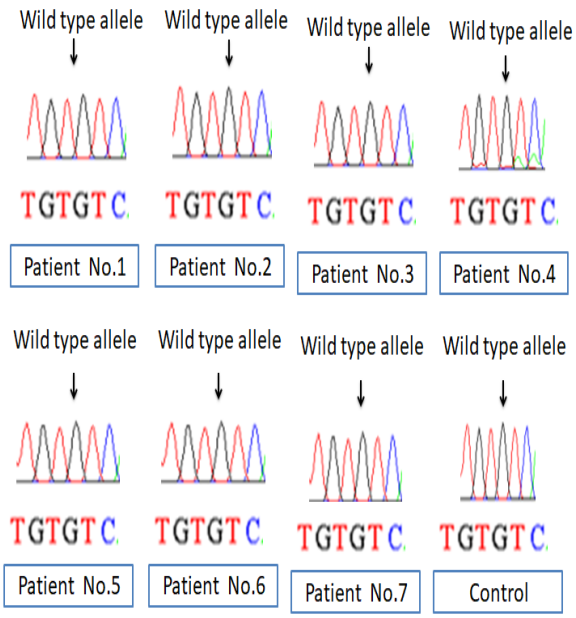

Fig.3 genetic tree analysis based on the JAK2 gene in Homo sapiens acute leukemia samples from Iraqi patients used to determine the JAK2V617F mutation. The tree was constructed using the unweight pairs group method with the arithmetic mean (UPGMA tree) in (MEGA version 6.0).

There are several studies that have investigated the presence of JAK2V617F mutation in acute myeloid leukemia (AML). Lee and his group[13] found that approximately 2.7% of AML cases contained a *JAK2* mutation. This study suggests that the incidence of the *JAK2* mutation in AML is relatively low. Similarly, Jekarl and his team [18] reported an incidence of 13.3% for the JAK2V617F mutation in AML samples studied.

However, Lee and his group[19] reported a higher incidence of the JAK2 V617F mutation in AML, conducted a Korean study and diagnosed the *JAK2* mutation in two cases of AML without previous infection with myeloproliferative neoplasms (MPN). Farasani [20] also found the presence of the *JAK2* mutation in 15% among 100 samples of the AML leukemia patients in Saudi Arabia.

Overall, these studies suggest that while the incidence of *JAK2* mutation in AML may vary, it appears to be relatively low overall. However, it is important to consider that the prevalence of *JAK2* mutation in AML may differ across different populations and geographical regions. Further research is necessary to fully understand the role of *JAK2* mutation in the pathogenesis of AML and its implications for disease progression and treatment.

We did not find the JAK2V617F mutation in any of the cases of acute myeloid leukemia (AML) that they studied. These findings are consistent with a study by Jaradat and his team [21], which also reported no mutation in any of the leukemia samples they examined, including cases of acute lymphoblastic leukemia (ALL), AML, chronic lymphocytic leukemia (CLL), and chronic myeloid leukemia (CML). Additionally, a previous study conducted by Badrawy and Ibrahim [17] that included 90 samples, 30 of which were from AML, also did not find the JAK2V617F mutation in their study samples. The mutation may not be present or significant in the cases of AML that were examined in the mentioned studies. It is essential to acknowledge that scientific studies often focus on specific samples and populations, which means that the findings might not be universally applicable to all cases of AML. Each case of AML can have unique genetic characteristics and mutations, and further research is necessary to gain a comprehensive understanding of these mutations and their implications in AML and other types of leukemia.

By conducting additional studies, researchers can investigate other genetic mutations that might play a role in AML development, progression, or treatment response. This ongoing research will contribute to improving our knowledge of the underlying mechanisms of AML and potentially lead to more effective targeted therapies or diagnostic approaches tailored to specific genetic profiles.

Itzykson and his group[22] found that approximately 10% of chronic myeloid leukemia(CML) cases carry the JAK2V617F mutation. This mutation is associated with the activation of JAK2 kinase, which plays a role in the development of myeloproliferative neoplasms (MPNs) such as CML.

JAK2V617F is more commonly observed in acute myeloid leukemia (AML) cases that arise from a pre-existing MPN (MPN turning into AML). However, noted that this mutation is very rare in de novo AML, which refers to cases of AML that develop without a preceding MPN,in the study of 222 AML patients mentioned, the researchers found that although phosphorylation (activation) of STAT3, a downstream target of *JAK2*, occurred relatively frequently in myeloblasts (immature cells in AML), the JAK2V617F mutation was only identified in four cases (2% of the total). Among these four cases, three had a pre-existing MPN and one had AML secondary to primary thrombocythemia, which is a type of MPN characterized by elevated platelet counts [7].

The known mechanism of *JAK2* activation in acute lymphoblastic leukemia (ALL) involves a specific chromosomal translocation, t (9;12) (p24;p13), which leads to the formation of a fusion gene called *Tel–Jak2*. This fusion gene is believed to be responsible for the activation of JAK2 signaling pathways in ALL [23,24].

However, the results of this study mentioned indicate that there was no *JAK2* mutation detected in the cases of ALL that were examined. This finding aligns with previous research by Sulong and his group[25] , Ruiz-Argüelles and his team [26] found no cases of the JAK2V617F mutation in either acute myeloid leukemia (AML) or ALL cases.

Additionally, Badrawy and Ibrahim [17], which also reported the absence of *JAK2* mutations in ALL patients.
It is important to note that these findings suggest that *JAK2* mutations are not a common occurrence in ALL and may not play a significant role in the development or progression of the disease. However, it's essential to consider that these results are specific to the studies mentioned and may not represent the entire population of ALL patients. Further research and studies are needed to gain a more comprehensive understanding of the genetic alterations and molecular mechanisms involved in ALL.

Conclusion

Our study showed scarcity or complete absence of the JAK2V617F mutation in acute leukemia, this indicate the existence of other pathways other than JAKs can lead to STAT activation in the pathogenesis of acute leukemia.

Acknowledgement

Much obligation was given to the Iraqi Center for Hematology, which is a clinical center in the City of Medicine in Baghdad province for the collection of blood samples from leukemia patients. My thanks and respect to all leukemia patients and normal people for their consent to draw blood samples from them.

Funding Information

No funding was available.

Author's Contributions

MAD designed the study and performed the statistical analysis. M carried out the molecular genetic work. MAD participated in the sequence alignment and drafted the manuscript. M write the manuscript. All authors read and approved the final manuscript.

Ethics

The study was performed under the rules of the Ministry of higher education and scientific experiments all the patients from which samples were obtained were awarding of the purpose of the study and were agree for giving samples.

References

1. **1.** Khan, I. A., Vattam, K. K., Jahan, P., Hasan, Q., & Rao, P. (2015). Importance of glucokinase-258G/A polymorphism in Asian Indians with post-transplant and type 2 diabetes mellitus. *Intractable & Rare Diseases Research, 5*(1), 25-30. https://doi.org/10.5582/irdr.2015.01040

2. Steensma, D. P., McClure, R. F., Karp, J. E., Tefferi, A., Lasho, T. L., Powell, H. L., ... & Kaufmann, S. H. (2006). JAK2 V617F is a rare finding in de novo acute myeloid leukemia, but STAT3 activation is common and remains unexplained. *Leukemia, 20*(6), 971-978. https://doi.org/10.1038/sj.leu.2404206

3. Vijayakrishnan, L., Venkataramanan, R., & Gulati, P. (2011). Treating inflammation with the Janus kinase inhibitor CP-690,550. *Trends in Pharmacological Sciences, 32*(1), 25-34. https://doi.org/10.1016/j.tips.2010.10.004

4. Khwaja, A. (2006). The role of Janus kinases in haemopoiesis and haematological malignancy. *British Journal of Haematology, 134*(4), 366-384. https://doi.org/10.1111/j.1365-2141.2006.06206.x

5. Lai, S. Y., & Johnson, F. M. (2010). Defining the role of the JAK-STAT pathway in head and neck and thoracic malignancies: Implications for future therapeutic approaches. *Drug Resistance Updates, 13*(3), 67-78. https://doi.org/10.1016/j.drup.2010.04.001

6. Baxter, E. J., Scott, L. M., Campbell, P.

J., East, C., Fourouclas, N., Swanton, S., ... & Green, A. R. (2005). Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *The Lancet*, *365*(9464), 1054-1061. https://doi.org/10.1016/S0140-6736(05)71142-9

7. Levine, R. L., Wadleigh, M., Cools, J., Ebert, B. L., Wernig, G., Huntly, B. J., ... & Gilliland, D. G. (2005). Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell*, *7*(4), 387-397. https://doi.org/10.1016/j.ccr.2005.03.023

8. James, C., Ugo, V., Le Couédic, J. P., Staerk, J., Delhommeau, F., Lacout, C., ... & Vainchenker, W. (2005). A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature*, *434*(7037), 1144-1148. https://doi.org/10.1038/nature03546

9. Tefferi, A., Thiele, J., & Vardiman, J. W. (2009). The 2008 World Health Organization classification system for myeloproliferative neoplasms: Order out of chaos. *Cancer: Interdisciplinary International Journal of the American Cancer Society*, *115*(17), 3842-3847. https://doi.org/10.1002/cncr.24440

10. Grünebach, F., Bross-Bach, U., Kanz, L., & Brossart, P. (2006). Detection of a new JAK2 D620E mutation in addition to V617F in a patient with polycythemia vera. *Leukemia*, *20*(12), 2210-2211. https://doi.org/10.1038/sj.leu.2404419

11. Schnittger, S., Bacher, U., Kern, W., Schröder, M., Haferlach, T., & Schoch, C. (2006). Report on two novel nucleotide exchanges in the JAK2 pseudokinase domain: D620E and E627E. *Leukemia*, *20*(12), 2195-2197. https://doi.org/10.1038/sj.leu.2404325

12. Swerdlow, S. H., Campo, E., & Harris, N. L. (2017). *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues* (Revised 4th ed.). Lyon, France: IARC.

13. Lee, J. W., Kim, Y. G., Soung, Y. H., Han, K., Kim, S. Y., Rhim, H. S., ... & Lee, S. H. (2006). The JAK2 V617F mutation in de novo acute myelogenous leukemias. *Oncogene*, *25*(9), 1434-1436. https://doi.org/10.1038/sj.onc.1209163

14. Tai, E. W., Ward, K. C., Bonaventure, A., Siegel, D. A., & Coleman, M. P. (2017). Survival among children diagnosed with acute lymphoblastic leukemia in the United States, by race and age, 2001 to 2009: Findings from the CONCORD- 2 study. *Cancer*, *123*, 5178-5189. https://doi.org/10.1002/cncr.30899

15. Carroll, W. L., & Raetz, E. A. (2012). Clinical and laboratory biology of childhood acute lymphoblastic leukemia. *The Journal of Pediatrics*, *160*(1), 10-18. https://doi.org/10.1016/j.jpeds.2011.08.006

16. Mousa, Z. Q., & Dragh, M. A. (2023). Association of BCL11A Gene Polymorphism in Human Cells of Thalassemia Patient by Evaluation of Amplification Refractory Mutation

System (ARMS). *Journal of Medical Chemistry and Chemical Sciences*. https://doi.org/10.26655/JMCHEMSCI.2023.4.15

17. Badrawy, H., & Ibrahim, A. (2013). JAK-2V617F Mutation in Acute Leukemia (South Egypt Experience). *International Blood Research & Reviews*, *2*(1), 1-7. https://doi.org/10.9734/IBRR/2014/5340

18. Jekarl, D. W., Han, S. B., Kim, M., Lim, J., Oh, E. J., Kim, Y., ... & Han, K. (2010). JAK2 V617F mutation in myelodysplastic syndrome, myelodysplastic syndrome/myeloproliferative neoplasm, unclassifiable, refractory anemia with ring sideroblasts with thrombocytosis, and acute myeloid leukemia. *The Korean Journal of Hematology*, *45*(1), 46. https://doi.org/10.5045/kjh.2010.45.1.46

19. Lee, Y., Lee, J. Y., Lee, J. O., Bang, S. M., & Hwang, S. M. (2022). JAK2 V617F-Positive Acute Myeloid Leukemia: Clinicopathological Features of Two Cases. *Laboratory Medicine Online*, *12*(1), 53-57. https://doi.org/10.47429/lmo.2022.12.1.53

20. Farasani, A. (2022). Screening of V617F mutation in JAK2 gene with acute myeloid leukemia in the Saudi population. *Acta Biochimica Polonica*, *69*(1), 211-214. https://doi.org/10.18388/abp.2020_5945

21. Jaradat, S. A., Khasawneh, R., Kamal, N., Matalka, I., Al-Bishtawi, M., Al-Sweedan, S., & Ayesh, M. H. (2015). Analysis of JAK2V617F mutation in Jordanian patients with myeloproliferative neoplasms. *Hematology/Oncology and Stem Cell Therapy*, *8*(4), 160-166. https://doi.org/10.1016/j.hemonc.2015.07.004

22. Itzykson, R., Kosmider, O., Renneville, A., Gelsi-Boyer, V., Meggendor fer, M., Morabito, M., ... & Solary, E. (2013). Prognostic score including gene mutations in chronic myelomonocytic leukemia. *Journal of Clinical Oncology*, *31*(19), 2428-2436. https://doi.org/10.1200/JCO.2012.47.3314

23. Lacronique, V., Boureux, A., Della Valle, V., Poirel, H., Quang, C. T., Mauchauffé, M., ... & Bernard, O. A. (1997). A TEL-JAK2 fusion protein with constitutive kinase activity in human leukemia. *Science*, *278*(5341), 1309-1312. https://doi.org/10.1126/science.278.5341.1309

24. Carron, C., Cormier, F., Janin, A., Lacronique, V., Giovannini, M., Daniel, M. T., ... & Ghysdael, J. (2000). TEL-JAK2 transgenic mice develop T-cell leukemia. *Blood, The Journal of the American Society of Hematology*, *95*(12), 3891-3899. https://doi.org/10.1182/blood.V95.12.3891

25. Sulong, S., Case, M., Minto, L., Wilkins, B., Hall, A., & Irving, J. (2005). The V617F mutation in Jak2 is not found in childhood acute lymphoblastic leukaemia. *British Journal of Haematology*, *130*(6), 964-965. https://doi.org/10.1111/j.1365-2141.2005.05697.x

26. Ruiz-Argüelles, G. J., Garcés-Eisele, J., Reyes-Núñez, V., Ruiz-Delgado, G. J., Navarro-Vázquez, M., & González-Carrillo, M. L. (2006). The Janus Kinase 2 (JAK2) V617F mutation in hematological malignancies in México. *Revista de Investigación Clínica, 58*(5), 458-461.