TCABSE-J Mini-Research Article
ISSN: 2583- 2557, *Volume 1, Issue 4, pp35-40. 2022.*

TCABSE-J
Interdisciplinary Sciences Journal

Survey of the janus kinase-1 (JAK-1) transcript variants and protein isoforms of JAK-1 to determine its druggability for acute lymphoid leukemia treatment.

[1, 2]Ramani S. Thokala & [1, 3]Ravikiran S. Yedidi*

[1]The Center for Advanced-Applied Biological Sciences & Entrepreneurship (TCABS-E), Visakhapatnam 530016, AP; [2]Department of Biotechnology and [3]Department of Zoology, Andhra University, Visakhapatnam 530003, AP. (*Correspondence to RSY: tcabse.india@gmail.com).

Acute lymphoblastic leukemia (ALL) has been one the major childhood cancers across the world causing high morbidity including in adults. The precursor lymphoblasts of both B- and T-cell lines developing into leukemia due to abnormal gene fusions has been well established by various research groups. Among the non-receptor tyrosine kinases that are responsible for ALL, the janus kinase-1 (JAK-1) has been implicated due to amino acid substitutions. Especially, the V658F mutation in JAK-1 is one of the most commonly observed amino acid substitutions in clinical samples of ALL. In this study the mutant JAK-1 (V658F) has been chosen for detailed analysis on its druggability. Our results suggest that the mutation containing flap/loop of JAK-1 has a 3.1 Å deviation in the C-alpha atoms of Val658 (wild type) and Phe658 (mutant). This structural deviation gives us enough selectivity for designing small molecule inhibitors that would specifically target only the mutant form of JAK-1 sparing the wild type as a potential therapeutic approach for ALL treatment. The V658F mutant JAK-1 inhibitor is currently under evaluation for future testing.

Keywords: Acute lymphoblastic leukemia, janus kinase-1, JAK1, clinical mutation, tyrosine kinase, kinase inhibitors, microRNA therapeutics.

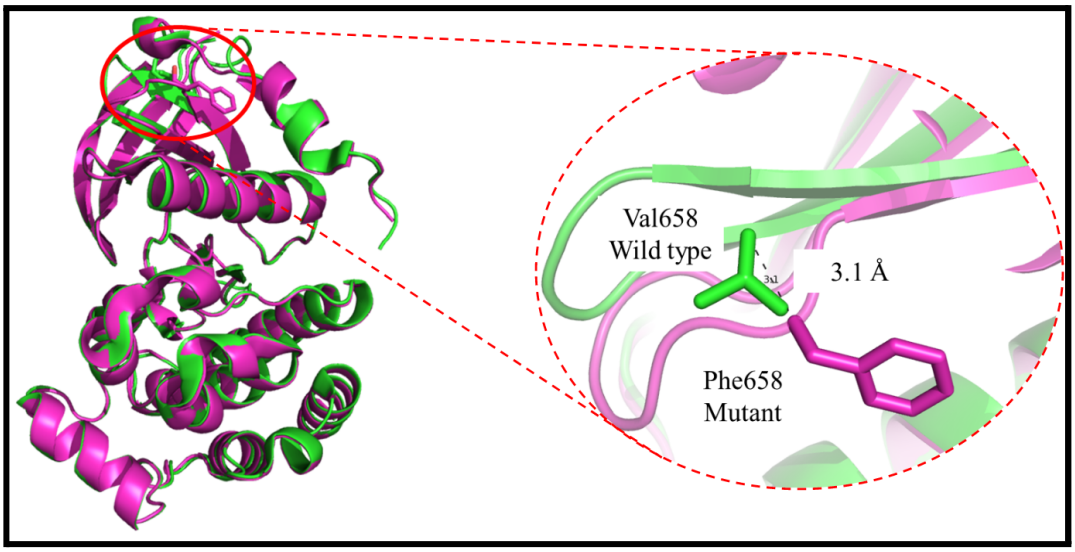

Figure 1. Conformational differences between the wild type and the V658F mutant of JAK1 provide selectivity for designing small molecule inhibitors that target the mutant form sparing the wild type.

Citation: Thokala, R. S. and Yedidi, R. S. (2022). Survey of the janus kinase-1 (JAK-1) transcript variants and protein isoforms of JAK-1 to determine its druggability for acute lymphoid leukemia treatment. *TCABSE-J*, Vol. 1, Issue 4: 35-40. Epub: Oct 5th, 2022.

TCABSE-J Mini-Research Article
ISSN: 2583- 2557, *Volume 1, Issue 4, pp35-40. 2022.*

Leukemia is a type of blood cancer in the bone marrow that results in the production of a high number of abnormal blood cells called blasts or leukemia cells. Some symptoms include bleeding and bruising, bone pain, fatigue and fever and these symptoms mainly occur due to lack of normal blood cells.In general diagnosis is usually done either by blood tests or bone marrow biopsy [1, 2]. The exact cause of leukemia is unknown [3]. Combination of some genetic factors and environmental factors are believed to play a role in causing leukemia [4, 5]. Risk factors mainly include smoking, radiation, chemicals and also prior chemotherapy. People with a family history of leukemia are at a higher risk. There are four main types of leukemia which include: acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL) and chronic myeloid leukemia (CML). Leukemias and lymphomas both belong to a broader group of tumors that affect the blood, bone marrow which are known as tumors of the hematopoietic and lymphoid tissues [6, 7]. Treatment involves chemotherapy, radiation therapy, targeted therapy, and bone marrow transplant. In addition to these, supportive care is needed. The success rate of treatment depends upon the type of leukemia and also the age of the person. The survival rate for a five-year old is 57% in the United States. In children under 15, the five-year old survival rate is greater than 60% or even about 90%, depending upon the type of leukemia. In children with acute leukemia who are cancer-free after five years, there is a chance of cancer likely to return [8].

In 2015, leukemia was present in almost 2.3 million people worldwide and caused nearly 353,500 deaths [9, 10]. It is the most common type of cancer in children, about three-quarters of leukemia cases in children are of acute lymphoblastic type. However, about 90% of all leukemias are diagnosed in adults, with CLL and AML and it is most common in adults [11]. Clinically, leukemia is subdivided into a large variety of groups. Generally they are classified into acute and chronic types [12].

ALL is a cancer of the lymphoid line of blood cells characterized by the development of large numbers of immature lymphocytes. Symptoms mainly include tiredness, pale skin color, fever, easy bleeding or bruising, enlarged lymph nodes, or sometimes even leads to bone pain. In acute leukemia, ALL increases rapidly and slowly becomes fatal within weeks or months if it is left untreated [13]. In most cases, the cause for acute lymphoblastic leukemia is unknown. Genetic risk factors may include Down syndrome, Li-Fraumeni syndrome. Environmental risk factors may include significant radiation exposure or prior chemotherapy. The mechanism involves multiple genetic mutations which results in rapid cell division. The excessive immature lymphocytes in the bone marrow interfere with the production of new red blood cells, white blood cells, and platelets. Diagnosis is generally based upon blood tests and bone marrow examination [14]. ALL is typically treated initially with chemotherapy is aimed at bringing about remission.Which is further followed by chemotherapy over a number of years. Treatment usually includes intrathecal chemotherapy since systemic chemotherapy can have limited penetration into the central nervous system as the central nervous system is a common site for the relapse of acute lymphoblastic leukemia. Treatment includes radiation therapy if spread to the brain, Stem cell transplantation may be used if the disease reoccurs then we need to follow the standard treatment [15]. Additional treatments such as Chimeric antigen receptor T (CAR-T) cell immunotherapy are also in use.

Janus kinase (JAK) is a family of intracellular, non-receptor tyrosine kinases (120-140 kDa) that transduce cytokine-mediated signals via the JAK-STAT pathway [16]. Janus kinase consists of two domains in which one domain exhibits the kinase activity, while the other domain negatively regulates the kinase activity of the first [17]. The four JAK family members are:

JAK-1, 2, 3 and Tyrosine kinase-2 (TYK-2). The JAK autophosphorylation induces a conformational change within itself, enabling it to transduce the intracellular signal by further phosphorylating and activating transcription factors called STATs (Signal Transducer and Activator of Transcription, or Signal Transduction And Transcription). The activated STATs dissociate from the receptor and form dimers before translocating to the cell nucleus, where they regulate transcription of selected genes [18]. Disrupted JAK-STAT signaling may lead to a variety of diseases, such as skin conditions, cancers, and disorders affecting the immune system [19-28]. JAK inhibitors (tofacitinib, baricitinib, upadacitinib and filgotinib) are used in the treatment of atopic dermatitis, rheumatoid arthritis, psoriasis, polycythemia vera, alopecia, essential thrombocythemia, ulcerative colitis, myeloid metaplasia with myelofibrosis and vitiligo [29-31].

In this study, we performed an extensive Bioinformatics and Computational survey of all the transcript variants of JAK-1 gene and the 3-dimensional structural analysis, respectively with a goal to target all the variants with a single small molecule inhibitor with reasonable selectivity and possibly lowest side effects. This potential therapeutic approach may result in new drugs for ALL treatment in the future.

Materials & Methods:

NCBI search & sequence alignment: The nucleotide sequences of 8 JAK-1 variants were searched for using NCBI RefSeq. The FASTA sequences of all the 8 variants were then aligned against each other using the Clustal Omega server in order to understand the length and conserved domains within these sequences. This multiple sequence alignment gives us an understanding about how all the 8 variants were differentially spliced.

Computing the RNA secondary structure: RNAfold web server will predict secondary structures of single stranded RNA or DNA sequences. The FASTA sequences were pasted into the box in order to build the secondary structures.

Secondary structure analysis of protein: The 3-D models for the two JAK-1 isoforms were built by using the SWISS MODEL server. These models were then downloaded and analyzed using PyMOL.

Results and Discussion:

NCBI search revealed 8 variants of JAK-1: The RefSeqGene for human JAK-1 with accession no: NG_023402.2 was taken as the original gene sequence that is unspliced in order to understand the full length of the gene. The full length gene contains 2,41,524 base pairs and is located on chromosome number 1. A search was performed using NCBI databases such as Gene and Nucleotide, which resulted in a list of 8 variants of JAK-1. Variants 4 and 5 are the longest (Table 1). These 8 variants were further sequence aligned using Clustal omega server.

NCBI Accession Number	Length (bp)
NM_002227.4	5,092
NM_001320923.2	5,015
NM_001321852.2	5,018
NM_001321853.2	5,277
NM_001321854.2	5,193
NM_001321855.2	5,176
NM_001321856.2	4,931
NM_001321857.2	5,089

Table 1. List of 8 JAK-1 transcript variants from NCBI.

```
NP_001308785.1: MQYLNIKEDCNAMAFCAKMRSSKKTEVNLEAPEPGVEVIFYLSDREPLRLGSGEYTAEELCIRAAQACRISPLCH   75
NP_001308786.1: MQYLNIKEDCNAMAFCAKMRSSKKTEVNLEAPEPGVEVIFYLSDREPLRLGSGEYTAEELCIRAAQACRISPLCH   75
                ********************************************************************************

NP_001308785.1: NLFALYDENTKLWYAPNRTITVDDKMSLRLHYRMRFYFTNWHGTNDNEQSVWRHSPKKQKNGYEKKKIPDATPLL  150
NP_001308786.1: NLFALYDENTKLWYAPNRTITVDDKMSLRLHYRMRFYFTNWHGTNDNEQSVWRHSPKKQKNGYEKKKIPDATPLL  150
                ********************************************************************************

NP_001308785.1: DASSLEYLFAQGQYDLVKCLAPIRDPKTEQDGHDIENECLGMAVLAISHYAMMKKMQLPELPKDISYKRYIPETL  225
NP_001308786.1: DASSLEYLFAQGQYDLVKCLAPIRDPKTEQDGHDIENECLGMAVLAISHYAMMKKMQLPELPKDISYKRYIPETL  225
                ********************************************************************************

NP_001308785.1: NKSIRQRNLLTRMRINNVFKDFLKEFNNKTICDSSVSTHDLKVKYLATLETLTKHYGAEIFETSMLLISSENEMN  300
NP_001308786.1: NKSIRQRNLLTRMRINNVFKDFLKEFNNKTICDSSVSTHDLKVKYLATLETLTKHYGAEIFETSMLLISSENEMN  300
                ********************************************************************************

NP_001308785.1: WFHSNDGGNVLYYEVMVTGNLGIQWRHKPNVVSVEKEKNKLKRKKLENKHKKDEEKNKIREEWNNFSYFPEITHI  375
NP_001308786.1: WFHSNDGGNVLYYEVMVTGNLGIQWRHKPNVVSVEKEKNKLKRKKLENKHKKDEEKNKIREEWNNFSYFPEITHI  375
                ********************************************************************************

NP_001308785.1: VIKESVVSINKQDNKKMELKLSSHEEALSFVSLVDGYFRLTADAHHYLCTDVAPPLIVHNIQNGCHGPICTEYAI  450
NP_001308786.1: VIKESVVSINKQDNKKMELKLSSHEEALSFVSLVDGYFRLTADAHHYLCTDVAPPLIVHNIQNGCHGPICTEYAI  450
                ********************************************************************************

NP_001308785.1: NKLRQEGSEEGMYVLRWSCTDFDNILMTVTCFEKSEQVQGAQKQFKNFQIEVQKGRYSLHGSDRSFPSLGDLMSH  525
NP_001308786.1: NKLRQEGSEEGMYVLRWSCTDFDNILMTVTCFEKSE-VQGAQKQFKNFQIEVQKGRYSLHGSDRSFPSLGDLMSH  524
                ************************************ ************************************

NP_001308785.1: LKKQILRTDNISFMLKRCCQPKPREISNLLVATKKAQEWQPVYPMSQLSFDRILKKDLVQGEHLGRGTRTHIYSG  600
NP_001308786.1: LKKQILRTDNISFMLKRCCQPKPREISNLLVATKKAQEWQPVYPMSQLSFDRILKKDLVQGEHLGRGTRTHIYSG  599
                ********************************************************************************

NP_001308785.1: TLMDYKDDEGTSEEKKIKVILKVLDPSHRDISLAFFEAASMMRQVSHKHIVYLYGVCVRDVENIMVEEFVEGGPL  675
NP_001308786.1: TLMDYKDDEGTSEEKKIKVILKVLDPSHRDISLAFFEAASMMRQVSHKHIVYLYGVCVRDVENIMVEEFVEGGPL  674
                ********************************************************************************

NP_001308785.1: DLFMHRKSDVLTTPWKFKVAKQLASALSYLEDKDLVHGNVCTKNLLLAREGIDSECGPFIKLSDPGIPITVLSRQ  750
NP_001308786.1: DLFMHRKSDVLTTPWKFKVAKQLASALSYLEDKDLVHGNVCTKNLLLAREGIDSECGPFIKLSDPGIPITVLSRQ  749
                ********************************************************************************

NP_001308785.1: ECIERIPWIAPECVEDSKNLSVAADKWSFGTTLWEICYNGEIPLKDKTLIEKERFYESRCRPVTPSCKELADLMT  825
NP_001308786.1: ECIERIPWIAPECVEDSKNLSVAADKWSFGTTLWEICYNGEIPLKDKTLIEKERFYESRCRPVTPSCKELADLMT  825
                ********************************************************************************

NP_001308785.1: RCMNYDPNQRPFFRAIMRDINKLEEQNPDIVSEKKPATEVDPTHFEKRFLKRIRDLGEGHFGKVELCRYDPEGDN  900
NP_001308786.1: RCMNYDPNQRPFFRAIMRDINKLEEQNPDIVSEKKPATEVDPTHFEKRFLKRIRDLGEGHFGKVELCRYDPEGDN  899
                ********************************************************************************

NP_001308785.1: TGEQVAVKSLKPESGGNHIADLKKEIEILRNLYHENIVKYKGICTEDGGNGIKLIMEFLPSGSLKEYLPKNKNKI  975
NP_001308786.1: TGEQVAVKSLKPESGGNHIADLKKEIEILRNLYHENIVKYKGICTEDGGNGIKLIMEFLPSGSLKEYLPKNKNKI  974
                ********************************************************************************

NP_001308785.1: NLKQQLKYAVQICKGMDYLGSRQYVHRDLAARNVLVESEHQVKIGDFGLTKAIETDKEYYTVKDDRDSPVFWYAP 1050
NP_001308786.1: NLKQQLKYAVQICKGMDYLGSRQYVHRDLAARNVLVESEHQVKIGDFGLTKAIETDKEYYTVKDDRDSPVFWYAP 1049
                ********************************************************************************
NP_001308785.1: ECLMQSKFYIASDVWSFGVTLHELLTYCDSDSSPMALFLKMIGPTHGQMTVTRLVNTLKEGKRLPCPPNCPDEVY 1125
NP_001308786.1: ECLMQSKFYIASDVWSFGVTLHELLTYCDSDSSPMALFLKMIGPTHGQMTVTRLVNTLKEGKRLPCPPNCPDEVY 1124
                ********************************************************************************

NP_001308785.1: QLMRKCWEFQPSNRTSFQNLIEGFEALLK 1154
NP_001308786.1: QLMRKCWEFQPSNRTSFQNLIEGFEALLK 1153
                ****************************
```

Figure 2. Clustal omega alignment of JAK-1 isoforms.

Multiple sequence alignment of JAK-1 mRNA transcript variants: The nucleotide sequences of all 8 JAK-1 variants were aligned against each other using the CLUSTAL-OMEGA server in order to understand the length and conserved domains within these sequences. Variants 4 and 5 are the longest (Table 1) and showed proper alignment from the 5'-end to the 3'-end while the other variants were not properly aligned at the 5'-end but were absolutely aligned at the 3'-end. This multiple sequence alignment gives us an understanding about how the 8 variants were differentially spliced post-transcription.

Variant	MFE (kcal/mol.)	Diversity
1	-1608.32	1340.92
2	-1533.00	1368.23
3	-1534.05	1231.20
4	-1655.12	1304.88
5	-1627.81	1536.48
6	-1629.69	1187.29
7	-1514.48	1357.97
8	-1602.21	1337.11

Table 2. Mean free energy (MFE) values of variant mRNAs.

Secondary structure analysis of mRNA transcript variants of JAK-1: The secondary structure analysis of all the 8 mRNA transcript variants of JAK-1 was performed to understand their structural diversity as well as their stability. As shown in Table 2, the thermodynamic minimum free energy values of each variant along with their corresponding ensemble diversity values were compared and analyzed. Among the 8 variants, variant 4 shows higher stability relatively and variant 7 shows the least stability relatively with an energy difference of 141 kcal/mol. between the two variants. Based on our studies in this article, we conclude that all the 8 mRNA transcript variants of JAK-1 possess similar profiles of stability and the two protein isoforms have more than 99% amino acid sequence homology. However, the structural analysis of wild type vs. the V658F mutant of JAK-1 confirmed that the structural deviation of 3.1 Å can be leveraged for the design of selective inhibitors that bind to the V658F mutant form of JAK-1 sparing the wild type. It is noteworthy to mention that the selective inhibitors will only work if the patient is heterozygous for mutant JAK-1 so that if the mutant form is inhibited, the wild type can function. On the other hand, if the patient is homozygous for mutation, then the inhibitor may not work as expected. Currently we are in the process of synthesizing a library of analogs based on a lead molecule that was identified *in silico*. The structure-activity relationship studies in this regards will be published in the future issues of TCABSE-J.

References

1. Devine SM, Larson RA. Acute leukemia in adults: recent developments in diagnosis and treatment. CA Cancer J Clin. 1994 Nov-Dec;44(6):326-52. doi: 10.3322/canjclin.44.6.326. PMID: 7953914.
2. "What You Need To Know About™ Leukemia". *National Cancer Institute*. 23 December 2013. Archived from the original on 6 July 2014. Retrieved 18 June 2014.
3. Hutter, JJ (June 2010). "Childhood leukemia". *Pediatrics in Review*. 31 (6): 234–41. doi:10.1542/pir.31-6-234. PMID 20516235.
4. "A Snapshot of Leukemia". *NCI*. Archived from the original on 4 July 2014. Retrieved 18 June 2014.
5. Cordo V, Meijerink J (January 2021). "T-cell Acute Lymphoblastic Leukemia: A Roadmap to Targeted Therapies". *Blood Cancer Discovery*. 2: 19–31. doi:10.1158/2643-3230.BCD-20-0093. ISSN 2643-3230. PMC 8447273.
6. Vardiman, JW; Thiele, J; Arber, DA; Brunning, RD; Borowitz, MJ; Porwit, A; Harris, NL; Le Beau, MM; Hellström-Lindberg, E; Tefferi, A; Bloomfield, CD (30 July 2009). "The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes". *Blood*. 114 (5): 937–51. doi:10.1182/blood-2009-03-209262. PMID 19357394. S2CID 3101472.
7. Cătoi, Alecsandru Ioan Baba, Cornel (2007). *Comparative oncology*. Bucharest: The Publishing House of the Romanian

Academy. p. Chapter 17. ISBN 978-973-27-1457-7. Archived from the original on 10 September 2017.
8. American Cancer Society (2 March 2014). "Survival rates for childhood leukemia". Archived from the original on 14 July 2014.
9. GBD 2015 Disease and Injury Incidence and Prevalence, Collaborators. (8 October 2016). "Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015". *Lancet*. 388 (10053): 1545–1602.
10. GBD 2015 Mortality and Causes of Death, Collaborators. (8 October 2016). "Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015". *Lancet*. 388 (10053): 1459–1544.
11. *World Cancer Report 2014*. World Health Organization. 2014. pp. Chapter 5.13. ISBN 978-9283204299
12. "Questions and Answers About Leukemia" (PDF). *Center for Disease Control and Prevention*. Archived (PDF) from the original on 30 July 2021. Retrieved 8 August 2021.
13. Marino BS, Fine KS (2013). *Blueprints Pediatrics*. Lippincott Williams & Wilkins. p. 205. ISBN 9781451116045.
14. Ferri, Fred F. (2017). *Ferri's Clinical Advisor 2018 E-Book: 5 Books in 1*. Elsevier Health Sciences. p. 743. ISBN 9780323529570.
15. Hunger SP, Mullighan CG (October 2015). "Acute Lymphoblastic Leukemia in Children". *The New England Journal of Medicine*. 373 (16): 1541–52.
16. Rodig SJ, Meraz MA, White JM, Lampe PA, Riley JK, Arthur CD, King KL, Sheehan KC, Yin L, Pennica D, Johnson EM, Schreiber RD (1998). "Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine -induced biologic responses". *Cell*. 93 (3): 373–83.
17. Kisseleva; Bhattacharya, S; Braunstein, J; Schindler, CW; et al. (2002-02-20). "Signaling through the JAK/STAT pathway, recent advances and future challenges". *Gene*. 285 (1–2): 1–24.
18. Kisseleva; Bhattacharya, S; Braunstein, J; Schindler, CW; et al. (2002-02-20). "Signaling through the JAK/STAT pathway, recent advances and future challenges". *Gene*. 285 (1–2): 1–24.
19. Aaronson DS, Horvath CM (2002). "A road map for those who don't know JAK-STAT". *Science*. 296 (5573): 1653–5. Bibcode:2002Sci...296.1653A.
20. Jatiani, S. S.; Baker, S. J.; Silverman, L. R.; Reddy, E. P. (2011). "JAK/STAT Pathways in Cytokine Signaling and Myeloproli -ferative Disorders: Approaches for Targeted Therapies". *Genes & Cancer*. 1 (10): 979–993.
21. Freund, P.; Kerenyi, M. A.; Hager, M.; Wagner, T.; Wingelhofer, B.; Pham, H T T.; Elabd, M.; Han, X.; Valent, P.; Gouilleux, F.; Sexl, V.; Krämer, O. H.; Groner, B.; Moriggl, R. (2017). "O-GlcNAcylation of STAT5 controls tyrosine phosphorylation and oncogenic transcription in STAT5-dependent malignancies". *Leukemia*. 31 (10): 2132–2142.
22. Schindler, Christian; Levy, David E.; Decker, Thomas (2007). "JAK-STAT Signaling: From Interferons to Cytokines". *Journal of Biological Chemistry*. 282 (28): 20059–20063.
23. Aaronson DS, Horvath CM (2002). "A road map for those who don't know JAK-STAT". *Science*. 296 (5573): 1653–5. Bibcode:2002Sci...296.1653A.
24. Kiu, Hiu; Nicholson, Sandra E. (2012). "Biology and significance of the JAK/STAT signaling pathways". *Growth Factors*. 30(2): 88–106.
25. Reich, Nancy C; Rout, M. P. (2014). "STATs get their move on". *JAK-STAT*. 2 (4): 27080.
26. Liu, L.; McBride, K. M.; Reich, N. C. (2005). "STAT3 nuclear import is independent of tyrosine phosphorylation and mediated by importin- 3". *Proceedings of the National Academy of Sciences*. 102 (23): 8150–8155.
27. Flex E *et al*. Somatically acquired JAK1 mutations in adult acute lymphoblastic leukemia. J Exp Med. 2008 Apr 14;205(4):751-8.
28. Eun Goo Jeong, Min Sung Kim, Hyo Kyung Nam, Chang Ki Min, Seok Lee, Yeun Jun Chung, Nam Jin Yoo and Sug Hyung Lee. Somatic Mutations of *JAK1* and *JAK3* in Acute Leukemias and Solid Cancers. Clin Cancer Res June 15 2008 (14) (12) 3716-3721.
29. "Search for: GLPG0634 - List Results - ClinicalTrials.gov". *clinicaltrials.gov.*
30. Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy: D. Golan et al. LWW. 2007.
31. Craiglow, B. G.; King, B. A. (2015). "Tofacitinib Citrate for the Treatment of Vitiligo: A Pathogenesis-Directed Therapy". *JAMA Dermatology*. 151(10): 1110–2.

Acknowledgements:
The authors thank T*yi*DE-Toronto, Canada for helping to write this manuscript.

Funding:
The authors thank TCABS-E, Rajahmundry, India and T*yi*DE-Toronto, Canada for financial support.

Conflict of interest:
This research article is an ongoing project currently at TCABS-E, Rajahmundry, India. However the authors welcome academic collaborations from various groups.