Structural modeling of *JAK1* mutations in T-cell acute lymphoblastic leukemia reveals a second contact site between pseudokinase and kinase domains

With great interest we read the extensive review on JAK kinase targeting in hematologic malignancies by Springuel, Renauld and Knoops.[1] It provides a clear overview of the plethora of cytokine receptor complexes and downstream signaling adapter molecules that are mutated in hematologic malignancies. In particular, Janus kinase family members play a clear role in both normal hematopoiesis and malignancies. Four JAK kinases (JAK1, JAK2, JAK3, and TYK2) transmit extracellular signals from ligand-activated cytokine receptors to downstream transcriptional effectors such as STATs to regulate gene transcription. Myeloid development is predominantly driven by various cytokine receptors that recruit JAK2, the likes of which then interacts with the intracellular domains of said receptors. In addition to activating mutations in receptors such as TPO-R and CSF3R, myeloid

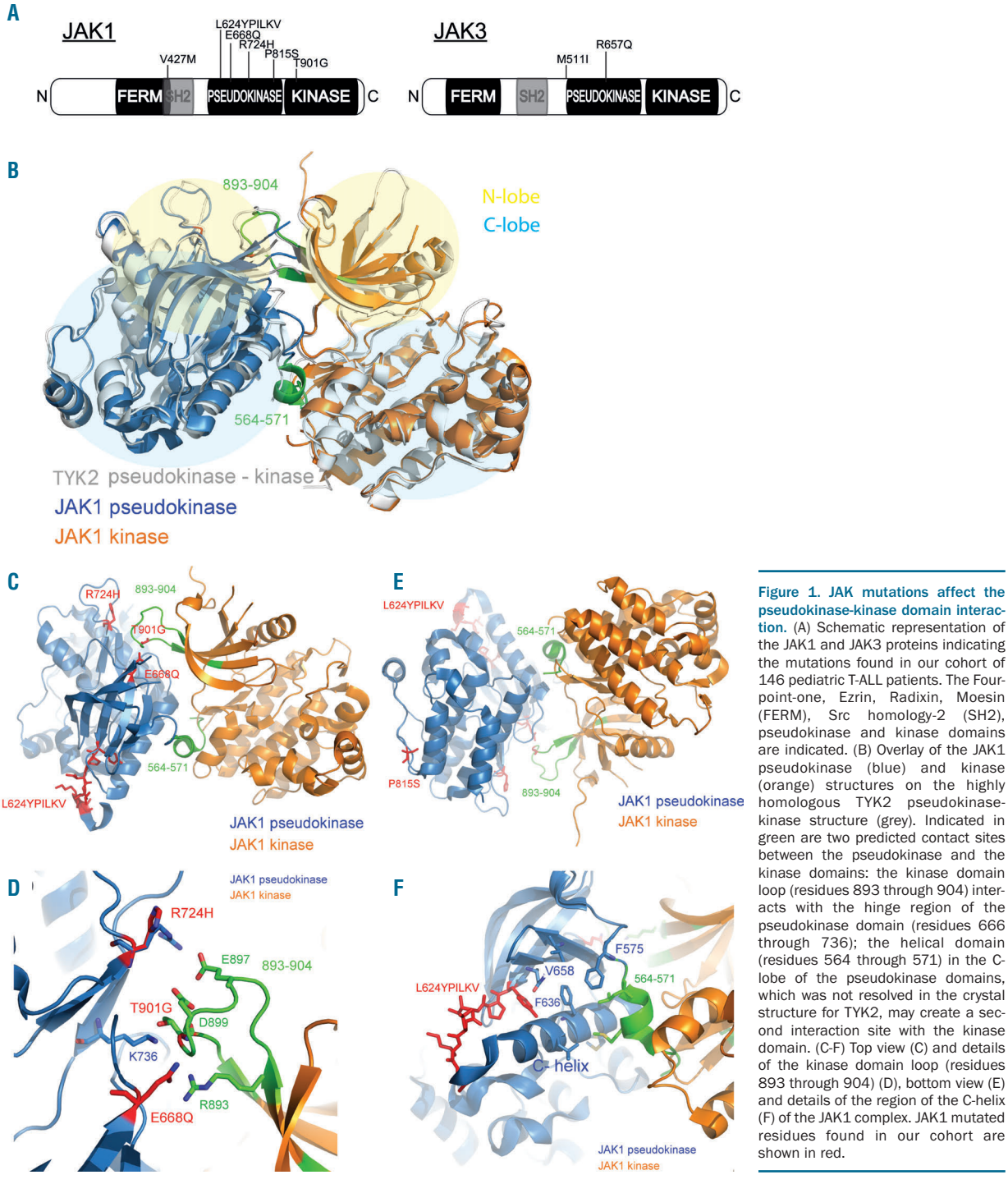

Figure 1. JAK mutations affect the pseudokinase-kinase domain interaction. (A) Schematic representation of the JAK1 and JAK3 proteins indicating the mutations found in our cohort of 146 pediatric T-ALL patients. The Four-point-one, Ezrin, Radixin, Moesin (FERM), Src homology-2 (SH2), pseudokinase and kinase domains are indicated. (B) Overlay of the JAK1 pseudokinase (blue) and kinase (orange) structures on the highly homologous TYK2 pseudokinase-kinase structure (grey). Indicated in green are two predicted contact sites between the pseudokinase and the kinase domains: the kinase domain loop (residues 893 through 904) interacts with the hinge region of the pseudokinase domain (residues 666 through 736); the helical domain (residues 564 through 571) in the C-lobe of the pseudokinase domains, which was not resolved in the crystal structure for TYK2, may create a second interaction site with the kinase domain. (C-F) Top view (C) and details of the kinase domain loop (residues 893 through 904) (D), bottom view (E) and details of the region of the C-helix (F) of the JAK1 complex. JAK1 mutated residues found in our cohort are shown in red.

malignancies frequently have *JAK2* mutations. Early B-cell development depends on signaling through the thymic stromal-derived lymphopoietin (TSLP) receptor, a heterodimeric receptor composed of a CRLF2 chain and an IL7Ra chain. The TSLP receptor recruits JAK2 and JAK1 upon ligand binding. In addition to mutations in the CRLF2 and IL7Ra chains and chromosomal rearrangements of JAK2, precursor B-cell acute lymphoblastic leukemia (BCP-ALL) frequently has activating *JAK1* and *JAK2* mutations.[2-4] Early lymphoid and T-cell development rely on signaling through the IL7 receptor, which is a dimer composed of an IL7Ra chain and a common-γ chain. Upon binding IL7, this receptor recruits and activates both JAK1 and JAK3. Consistent with a pivotal role in normal T-cell development, mutations in *IL7Ra, JAK1,* and *JAK3* (but not *JAK2*) are commonly found in T-ALL.[2,5-7]

Herein, we expand on the structural consequences of *JAK* mutations as briefly discussed by Springuel *et al*.[1] The interaction between the N-lobes of the pseudokinase and kinase domains is believed to prevent aberrant (*i.e.*, ligand-independent) kinase activity. This interaction predominantly relies on residues between the hinge region in the pseudokinase domain and the kinase domain loop (Figure 1). Thus, mutations in the N-lobes of either domain may directly affect the interaction between the two domains, thereby triggering a conformational change that induces ligand-independent activation. We screened our cohort of 146 pediatric T-ALL patients and identified five novel JAK1 mutations (plus one previously identified mutation, R724H[5,8]); the majority of these mutations are located in N-lobe interaction sites between the pseudokinase and kinase domains (Figure 1A). To better understand the impact of these mutations on the function of JAKs, we superimposed these JAK1 pseudokinase[9] and kinase domain[10] mutants on the TYK2 crystallographic structure[11] (Figure 1B). This model is consistent with the interaction between the hinge region in the pseudokinase domain (residues V666 through K736) and the kinase domain loop (residues R893 through E904); this interaction is supported by strong salt bridges formed between the following four pairs of amino acid residues: E668-R893, D709-H1015, R724-E897, and K736-D899 (Figure 1B-D). Mutations in these residues, or nearby residues, were identified in our T-ALL cohort and include E668Q, R724H (corresponding to the R657Q mutation in JAK3) and T901G (Figure 1C-D). It is likely that these mutations disrupt the pseudokinase-kinase domain interaction, leading to constitutive JAK1 activation.

A second mutation hotspot in JAK molecules involves residues that form the so-called F-F-V triad in the C-lobe of the pseudokinase domain. This conserved triad (F575-F636-V658 in JAK1 and F537-F595-V617 in JAK2) is believed to act as a structural switch that controls the catalytic activity of JAK kinases.[9] Mutations in this triad, including the JAK1 V658F mutation in T-ALL[6] (and the equivalent JAK2 V617F mutation in polycythemia vera patients), disrupt the structural switch, leading to constitutive kinase activity. In JAK3, the M511I mutation is prevalent in T-ALL[12,13] and is two amino acids away from residue F513, which corresponds to the F575 residue in JAK1. The L624YPILKV mutation is located within a loop that connects a β-strand to the N-terminus of the C-helix (Figure 1F). These inserted residues extend the loop upstream of the C-helix and may compromise the F-F-V triad, thereby leading to de-repression of the kinase domain (Figure 1C-F). Our model of the JAK1 pseudokinase-kinase complex predicts a second interaction site between the C-lobes of the pseudokinase and kinase domains; this interaction is formed by a helical domain (residues 564 through 571) in the pseudokinase domain, which was not resolved in the crystallographic structure of TYK2 (Figure 1B). As modeled in Figure 1B, this helical domain has significant van der Waals clashes with the kinase domain, indicating that the helical domain may actually fold into the catalytic pocket of the kinase domain, thereby repressing kinase activity. The helical domain lies just upstream of the F575 residue in the F-F-V triad. Our results support a model in which the triad-controlled position of the C-helix functions as a switch that can alter the interaction between the helical domain (residues 564 through 571) and the kinase domain.

A second point of discussion concerns the frequency of *TYK2* mutations in T-ALL: Springuel *et al*.[1] cited an incorrect frequency of *TYK2* mutations in 21% of T-ALL patients based on findings by Sanda *et al*.,[14] who reported five activating *TYK2* mutations in four out of 17 T-ALL cell lines, but none in 45 primary T-ALL patient samples. In contrast to frequent mutations in *JAK1, JAK2,* and *JAK3* in hematological malignancies,[2,6] incidental mutations in *TYK2* have been reported to date,[15,16] with only two out of 93 T-ALL cases reported by Kalender *et al*.[17] Neither of these mutations revealed TYK2 autophosphorylation or increased transforming activity compared to wild-type TYK2 in Ba/F3 cells, so it remains unclear whether these are activating mutations or not. In support of other large sequencing screens,[13,18] we found no mutations in *TYK2* in 146 pediatric T-ALL patient samples. Interestingly, the *TYK2* mutations in four T-ALL cell lines identified by Sanda *et al*.[14] are different from those in the same cell lines reported by Kalender *et al*.,[17] who provide evidence that *TYK2* mutations arise during culture over time. Therefore, it is likely that the *TYK2* mutations acquired in T-ALL cell lines during culture do not represent mutations in T-ALL patients.

Despite a lack, or very low frequency, of activating *TYK2* mutations in T-ALL, TYK2 activity could be an important factor. The survival of primary T-ALL cells seems to require TYK2 signaling, which activates STAT1 and BCL2.[14] Therefore, TYK2-specific inhibitors may have therapeutic potential, as suggested by Sanda *et al*.[14] In a commentary on that report, Fontan and Melnick[19] proposed two possible mechanisms to explain TYK2 dependency in T-ALL: i) a somatic activating mutation occurs in TYK2 itself, or ii) a signaling pathway upstream of TYK2 becomes constitutively active.[19] Since *TYK2* mutations are so rare in T-ALL patients, the second explanation seems more likely. In general, given the high frequency of *JAK1, JAK2*, and *JAK3* mutations, the very low frequency of TYK2 aberrations in the entire spectrum of hematologic malignancies is highly intriguing. Additional research into the role of TYK2 in normal hematopoiesis may reveal why it is rarely a target for mutagenesis during malignant transformation.

Kirsten Canté-Barrett,[1] Joost C.M. Uitdehaag,[2] and Jules P.P. Meijerink[1]

[1]Department of Pediatric Oncology/Hematology, Erasmus MC Rotterdam-Sophia Children's Hospital, Rotterdam; and [2]Netherlands Translational Research Center B.V., Oss, The Netherlands

Funding: the authors thank the following sources for financial support: the Children Cancer Free Foundation, Stichting Kinderen Kankervrij KiKa2008-29 and KiKa2013-116 (KC-B), and the European Commission Horizon 2020 SME instrument 662461 project (JCMU). We thank English Editing Solutions for editing support.

Correspondence: j.meijerink@erasmusmc.nl

doi:10.3324/haematol.2015.138248

Key words: JAK1, TYK2, structure, activating mutation, T-cell acute lymphoblastic leukemia.

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References

1. Springuel L, Renauld JC, Knoops L. JAK kinase targeting in hematologic malignancies: a sinuous pathway from identification of genetic alterations towards clinical indications. Haematologica. 2015;100(10):1240-1253.
2. Mullighan CG, Zhang J, Harvey RC, et al. JAK mutations in high-risk childhood acute lymphoblastic leukemia. Proc Natl Acad Sci U S A. 2009;106(23):9414-9418.
3. Shochat C, Tal N, Bandapalli OR, et al. Gain-of-function mutations in interleukin-7 receptor-alpha (IL7R) in childhood acute lymphoblastic leukemias. J Exp Med. 2011;208(5):901-908.
4. Yoda A, Yoda Y, Chiaretti S, et al. Functional screening identifies CRLF2 in precursor B-cell acute lymphoblastic leukemia. Proc Natl Acad Sci U S A. 2010;107(1):252-257.
5. Flex E, Petrangeli V, Stella L, et al. Somatically acquired JAK1 mutations in adult acute lymphoblastic leukemia. J Exp Med. 2008;205(4):751-758.
6. Jeong EG, Kim MS, Nam HK, et al. Somatic mutations of JAK1 and JAK3 in acute leukemias and solid cancers. Clin Cancer Res. 2008;14(12):3716-3721.
7. Zenatti PP, Ribeiro D, Li W, et al. Oncogenic IL7R gain-of-function mutations in childhood T-cell acute lymphoblastic leukemia. Nat Genet. 2011;43(10):932-939.
8. Asnafi V, Le Noir S, Lhermitte L, et al. JAK1 mutations are not frequent events in adult T-ALL: a GRAALL study. Br J Haematol. 2010;148(1):178-179.
9. Toms AV, Deshpande A, McNally R, et al. Structure of a pseudokinase-domain switch that controls oncogenic activation of Jak kinases. Nat Struct Mol Biol. 2013;20(10):1221-1223.
10. Kulagowski JJ, Blair W, Bull RJ, et al. Identification of imidazopyrrolopyridines as novel and potent JAK1 inhibitors. J Med Chem. 2012;55(12):5901-5921.
11. Lupardus PJ, Ultsch M, Wallweber H, Bir Kohli P, Johnson AR, Eigenbrot C. Structure of the pseudokinase-kinase domains from protein kinase TYK2 reveals a mechanism for Janus kinase (JAK) autoinhibition. Proc Natl Acad Sci U S A. 2014;111(22):8025-8030.
12. Degryse S, de Bock CE, Cox L, et al. JAK3 mutants transform hematopoietic cells through JAK1 activation, causing T-cell acute lymphoblastic leukemia in a mouse model. Blood. 2014;124(20):3092-3100.
13. Zhang J, Ding L, Holmfeldt L, et al. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature. 2012;481(7380):157-163.
14. Sanda T, Tyner JW, Gutierrez A, et al. TYK2-STAT1-BCL2 pathway dependence in T-cell acute lymphoblastic leukemia. Cancer Discov. 2013;3(5):564-577.
15. Roberts KG, Li Y, Payne-Turner D, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med. 2014;371(11):1005-1015.
16. Velusamy T, Kiel MJ, Sahasrabuddhe AA, et al. A novel recurrent NPM1-TYK2 gene fusion in cutaneous CD30-positive lymphoproliferative disorders. Blood. 2014;124(25):3768-3771.
17. Kalender Atak Z, De Keersmaecker K, Gianfelici V, et al. High accuracy mutation detection in leukemia on a selected panel of cancer genes. PLoS One. 2012;7(6):e38463.
18. De Keersmaecker K, Atak ZK, Li N, et al. Exome sequencing identifies mutation in CNOT3 and ribosomal genes RPL5 and RPL10 in T-cell acute lymphoblastic leukemia. Nat Genet. 2013;45(2):186-190.
19. Fontan L, Melnick A. Discovering what makes STAT signaling TYK in T-ALL. Cancer Discov. 2013;3(5):494-496.