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Research Article

TYK2 Activating Alterations in Acute Lymphoblastic Leukemia: Novel Driver Oncogenes with Potential Avenues for Precision Medicine?

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Abstract

Utilization of Next Generation Sequencing (NGS) and advances in genomic profiling have led to identification of new lesions in acute lymphoblastic leukemia (ALL) cases. *TYK2* alterations are among those that warrant an in-depth characterization of the underlying mechanisms that result in leukemogenesis, targetability potential and drug response. The current literature around the functional significance and clinical importance of these alterations in driving hematological cancer (in particular, leukemia) is limited. This review focuses on recent findings demonstrating the leukemogenic potential of *TYK2* alterations. Specifically, the molecular consequences of aberrant *TYK2* levels are detailed and the effects of *TYK2* deficiency or dysregulated activation are explored in carcinogenesis and leukemogenesis. In addition, the functional role of *TYK2* in JAK/STAT signaling, possible cross talk to other cancer-related pathways and overarching avenues for

pharmacological intervention in *TYK2*-altered ALL are also described.

1. Introduction

Acute lymphoblastic leukemia (ALL) is a hematological malignancy most commonly occurring in children [1, 2]. This disease is divided into two key groups: B-cell (B-ALL) and T-cell (T-ALL) lineage, which respectively account for 75-85% and 15-25% of cases depending on the age group (childhood- adult ALL) [3-6]. Advances in the treatment of childhood ALL, by improvements in hematopoietic stem cell transplantation (SCT), CNS directed treatment and optimisation of chemotherapy regimens through risk stratification, have resulted in increases in 5-year event free survival rate from approximately 60% in the 1970s to 85% in the 1990s [5]. However, relapsed or refractory ALL still occurs in approximately 20% of childhood cases, and for these patients, outcomes are poor [5, 7, 8]. Therefore, this malignancy remains a leading cause of non-traumatic death in children. In addition, the outcome for adult patients remains extremely poor with an overall 5-year survival rate of approximately 40% and of these, nearly 7% experience a subsequent relapse [9-11]. To overcome the limitations of current chemotherapy and treatment regimens (including SCT), the ultimate approach is personalised medicine that targets specific driver lesions and pathways in individuals. Precision medicine may ultimately improve outcome for these patients and decrease the risk of treatment failure by increasing the anti-leukemic efficacy of treatment and reducing drug associated toxicities. The targeting of *BCR-ABL1+* leukaemias with ABL tyrosine kinase inhibitors (ABLi) are testament to this approach, and efficacy has also been demonstrated in patients with other *ABL1* and *PDGFRB* fusions [12-17].

ALL is a heterogenous disorder and based on the presence and functional consequence of the various lesions identified in leukemic cells, is divided into subtypes with diverse pathological and prognostic outcome [18, 19]. Technologies such as gene expression profiling, single nucleotide polymorphism (SNP) analysis and next generation sequencing (NGS) have drastically improved our understanding of the genomic basis of ALL. Genome-wide profiling studies have enabled identification of novel alterations, refinement of genomic classification and definition of genetically high risk (HR) ALL subgroups. Notably, HR ALL subtypes are characterized by alterations that activate cytokine receptor, tyrosine kinase and/or JAK/STAT signaling and are associated with poor outcome [10, 20-23]. These subtypes are of clinical importance due to potential targetability by small molecule inhibitors (SMIs) [16, 19, 22, 24-30]. *TYK2* gain of function alterations, with the potential to activate the JAK/STAT pathway have only recently been described in ALL. However, the functional role of alterations involving *TYK2* in leukemia development and targetability are not, to date, well understood.

2. Pathology of ALL

ALL is generally thought to be the result of deregulated transcription and maturation arrest of lymphoid lineage cells in the BM [3]. This phenomenon is caused by the acquisition of initiating lesions, including gene translocations that confer self-renewal, differentiation arrest and epigenetic reprogramming of lymphoid progenitors [1]. Accumulation of additional secondary mutations and genomic alterations, affecting multiple cellular pathways, then contribute to the clinical manifestation of the disease [1, 2]. Perturbed pathways comprise those governing lymphoid development, cell cycle regulation, tumour

suppression, transcriptional regulation, epigenetic modification, Janus family of tyrosine kinases (JAK)/signal transducer and activator of transcription (STAT) signaling, phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) and RAS signaling (Figure 1). In the case of *TYK2*-altered disease, perturbations in JAK/STAT, PI3K/mTOR, RAS and ERK have been reported [31-35]. JAK/STAT is one of the most frequently mutated signaling pathways in cancer; recognised as one of the twelve core cancer pathways [36, 37]. In ALL, *JAK* activating alterations are recurrent and account for approximately 10% of HR ALL, 25% of T-ALL cases and 20% of a HR subtype of B-ALL (also known as Ph-Like ALL) [22, 23, 38, 39].

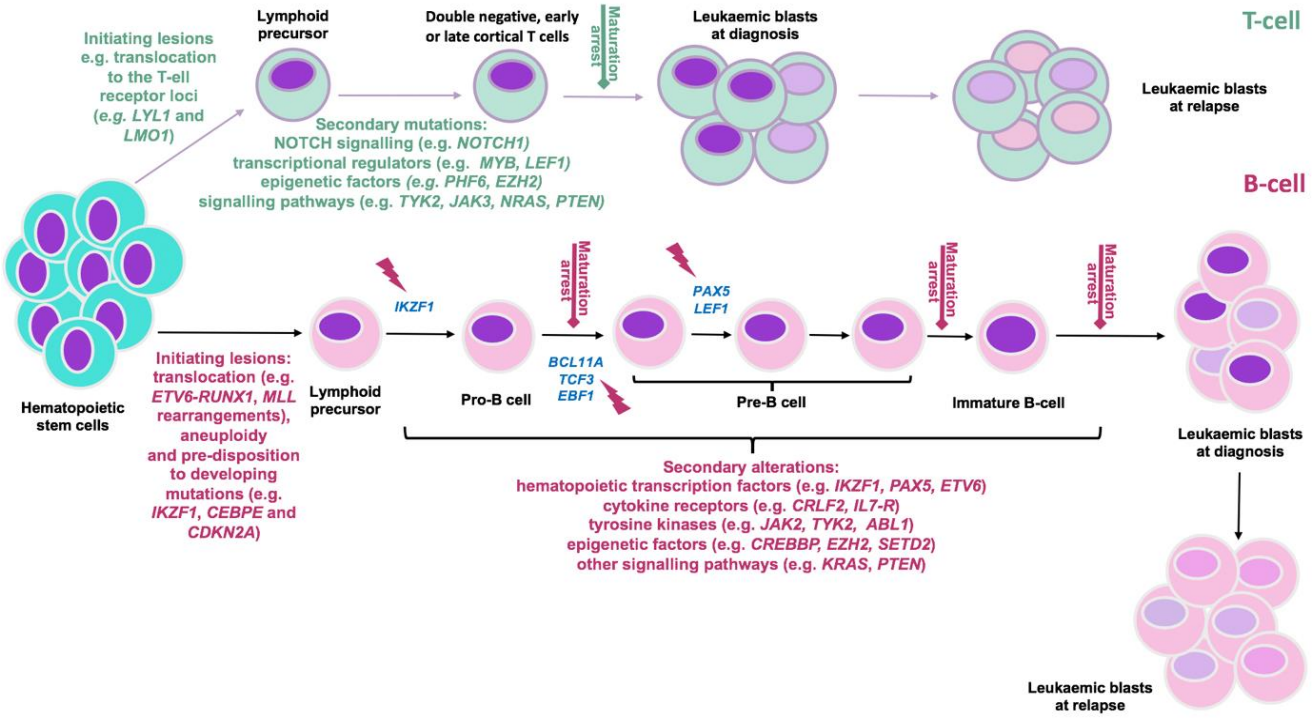

Figure 1: Predisposition and consequent development of ALL can be due to chronological acquisition of deleterious genomic alterations. The differentiation of hematopoietic cells into B- and T-lineage (represented in pink and green respectively) and their maturation is strictly regulated by transcription factors re-enforcing commitment to either fate. T-ALL initiation is mainly due to rearrangement of oncogenic transcription factors (e.g. *LYL1, LMO1)* into a position adjacent to T-cell receptor loci. In the B-ALL setting, changes in chromosome number (aneuploidy); acquisition of chromosomal rearrangements including translocations of genes that 1. control lymphoid development (e.g *ETV6*, *RUNX1*) 2. activate kinase signaling (e.g. *ABL1*) or oncogenes (e.g. *MYC*) 3. control epigenetic regulation (e.g. *MLL*(*KMT2A*)); and mutations in B-cell transcriptional regulator genes (e.g. *IKZF1, PAX5, EBF1, CEBPE*) and tumour suppressor genes (e.g, *CDKN2A/2B*) confer developmental arrest on lymphoid progenitors at various stages based on the altered genes (indicated by red flash). Subsequently, acquisition of additional co-operating events (as indicated) contribute to development of a genetically polyclonal disease. Selection or acquisition of further mutations can result in resistance to therapy and relapse. Adapted from [1, 2, 38].

3. *TYK2* mediates cytokine signaling and activation of JAK/STAT pathway

Four human JAKs comprising JAK1, JAK2, JAK3 and *TYK2* have been reported and are associated with activation of type I and II cytokines including both interleukin (IL) and interferon (IFN) receptors [40, 41]. *TYK2* was the first member of the JAK family of tyrosine kinases to be described and linked to cytokine signaling and the downstream JAK/STAT pathway (Figure 2) [42, 43]. Cytokine signaling is associated with cross-specificity in activation of overlapping JAKs and STATs (44). *TYK2* mediated cytokine signaling has been demonstrated, including type I, II and III IFNs (e.g. IFNα, β and *λ*) and ILs (e.g IL-6, IL-10, IL-12, IL-22 and IL-23) [45, 46], but importantly the capability of *TYK2* to activate all STAT proteins has also been defined (46). Apart from homo-dimerization of JAK family proteins, hetero-dimerization of these proteins can also lead to activation of the JAK/STAT pathway [45, 47]. Various studies have reported *TYK2* signaling upon dimerization with JAK1 and JAK2 in response to cytokines (Figure 2) [43, 45, 47-50] and the association of *TYK2* with JAK1 and/or JAK2 has also been reported in hematological malignancies [32, 35, 51, 52]. Here, constitutive *TYK2* auto- and trans-phosphorylation due to activating *TYK2* genomic alterations predominantly results in activation of STAT1, 3 and 5 [31-34, 53]. Therefore, dependent on the specific cell types, cytokines present and also the disease, multiple JAK and STAT family proteins can be activated in response to *TYK2* perturbation.

4. *TYK2* mediates crosstalk with other oncogenic signaling pathways

JAK signaling and STAT family protein activation can interact with, and induce activation of, several other signaling pathways such as PI3K/mTOR and RAS [46, 54]. RAS (family of small GTPases) and PI3K/mTOR signaling pathways are responsible for signal transmission from cytokine, B-cell receptors and tyrosine kinases [55]. These signaling pathways are crucial for lineage commitment and development of B-cells in the bone marrow [55, 56]. Crosstalk with other oncogenic pathways, has been demonstrated in the setting of B- and T-cell ALL. For example, the aberrant expression of the *ETV6-JAK2* fusion gene results in constitutive activation of RAS, PI3K and also NF-κB signaling pathways in the B-ALL setting [57]. In T-ALL patients, 2 of 6 *TYK2* activating point mutations were reported to induce activation of extracellular signal-regulated kinase (ERK) signaling, in addition to JAK/STAT activation [33]. In other hematological malignancies, activating mutations in *TYK2*, resulted in aberrant signaling through additional pathways such as PI3K/mTOR, RAS and also PIM (proto-oncogene serine/threonine-protein kinases) [34, 35]. Taken together, these results demonstrate the capacity of the *TYK2* protein to induce activation of additional oncogenic signaling pathways, depending on the specific cell type, mode of activation and disease context.

5. The interplay in regulation and stabilisation of *TYK2* and STAT proteins

The activity of JAK family proteins including *TYK2* is negatively regulated by multiple intrinsic and extrinsic factors. The first intrinsic inhibitory feature includes the regulatory ability of the pseudokinase domain (JH2), to control the kinase domain activity [58-60]. In addition, extrinsic negative regulation of the JAK/STAT pathway relies mainly on the SH2 domain-containing suppressors of cytokine signaling (SOCSs) proteins and proteases (e.g. protein tyrosine phosphatases PTPN1, PTPN6 and PTPN11) [45, 58]. The SOCS family proteins promote

ubiquitination and degradation of JAK family proteins while proteases dephosphorylate activated JAKs and cytokine receptors; both actions lead to attenuation of JAK/STAT signaling [58]. Apart from the global role of these negative regulators, previous studies have demonstrated that deactivation of *TYK2* signaling and acceleration of *TYK2* protein degradation is largely due to direct protein-protein interaction of SOCS1/3, PTPN6 and PTPN1 with activated *TYK2* [50, 61-64]. JAK and STAT family proteins are clients of heat shock proteins (HSP), in particular HSP90 [65]. The HSP90 chaperone protein plays an important role in maturation, stabilisation, folding and function of JAK and STAT proteins [65-67]. Interestingly, HSP90 is identified as the chaperone for various oncogenes [68, 69]. It promotes the functional stability of malignant cells that would otherwise be disrupted due to acquired alterations and increases their adaptability to environmental factors such as treatment [68, 69]. This function of HSP90 is also vital in JAK/STAT dependent hematological malignancies and *CRLF2*-rearranged and *JAK2*- and *JAK1*-mutated B-ALL [65, 70-72]. In these cases, inhibition of HSP90 by small molecule inhibitors (SMIs) or RNA interference resulted in attenuation of STATs phosphorylation and degradation of JAK proteins [70-72]. Proteomic analysis further confirmed the direct interaction of HSP90 with *TYK2* protein, enhancing its stability [46, 73]. In addition, HSP90 inhibition resulted in *TYK2* degradation and signal reduction in T-ALL cells harbouring wildtype and mutant *TYK2* [74].

HSP90 functions through association with several proteins and co-chaperones and is subject to multiple regulatory mechanisms [66, 67]. One of these negative regulatory mechanisms involves acetylation of lysine residues on HSP90 that subsequently inhibits the binding of client and co-chaperone proteins leading to aggregation of JAK proteins [67, 75]. Therefore, histone deacetylases (HDACs) including HDAC1, 6 and 10 play an important role in facilitating HSP90 activity [67, 75]. Immunoprecipitation and western blot analysis, have demonstrated HDAC6 to be the main enzyme that stabilizes the HSP90 complex in multiple leukemia cell lines [76]. Inhibition of HDAC6 in these cells resulted in degradation of driver oncogenes such as *BCR-ABL1*, mutated *FLT3* and subsequent reduction of their downstream signaling [76-78].

Moreover, STAT proteins interact with epigenetic co-factors such as histone acetyltransferases (HATs) and HDACs to modulate transcription of target genes [44]. Historically, HDACs activity was believed to regulate transcriptional repression only, however, recent studies revealed that both HDACs and HATs can act as activators and/or repressors of STAT-mediated transcription [44, 79-81]. HDAC mediated deacetylation of target gene transcriptional activation sites upon, STAT1, 2 and 5 binding to DNA, is essential to recruit the transcription machinery (e.g. RNA polymerase II) and initiate transcription [79, 82-86]. Hence, silencing of HDAC activity through SMIs or small interfering RNAs results in decreased STATs phosphorylation levels and expression of target genes [79, 82-86]. However, this is not without conjecture as activation of STAT3 mediated transcription, for instance, is reported to be associated with either acetylation in response to IFN stimulation in the normal setting [87, 88] or deacetylation in B-cell lymphomas [89]. It is important to note that the effect of acetylation/deacetylation on transcription can be cell type or condition specific. The exact mechanism by which chromatin modifiers interact with STATs in normal versus malignant cells remains unclear. However, the data

highlighting a positive regulatory effect of HDAC is in agreement with the growing evidence of HDAC inhibitors (HDACi) efficacy in hematological malignancies and other cancers [80, 90].

The JAK/STAT signaling network is complex (Figure 2). The *TYK2* protein can induce signaling redundancy, alternate signaling through other pathways and interact with positive or negative regulatory components. This interplay enables multiple therapeutic targets and emphasises the importance of careful analysis to find the appropriate inhibitors for each patient and disease context, discussed in further detail below.

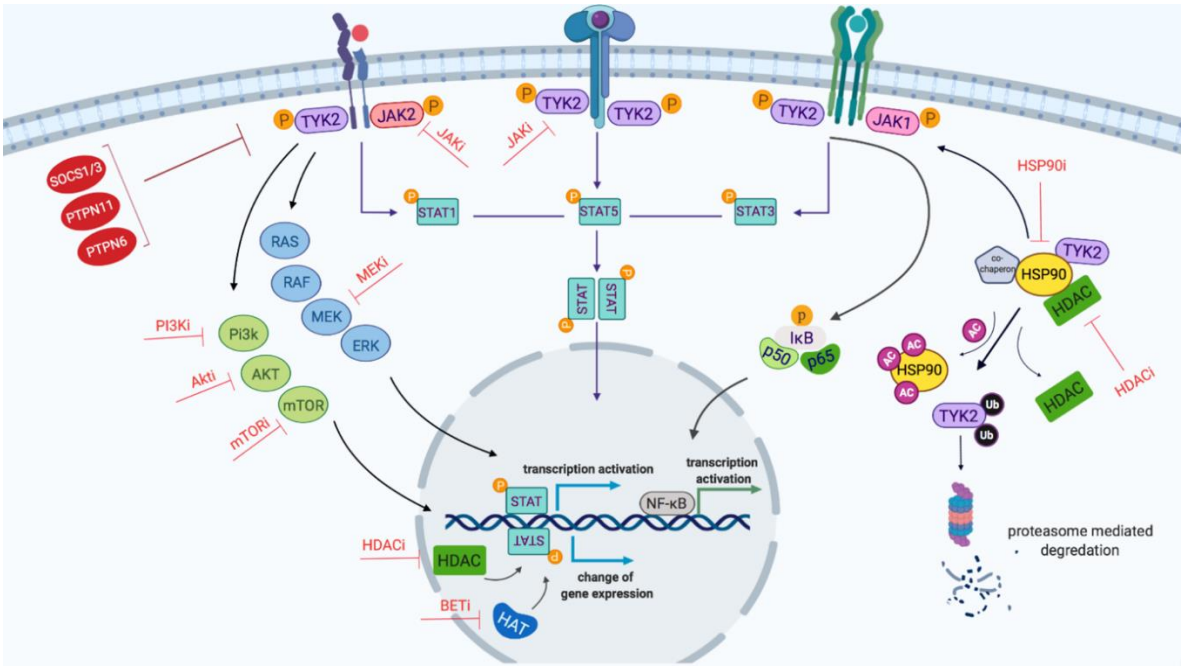

Figure 2: Schematic representation of *TYK2*-mediated JAK/STAT signaling network created by Biorender.com. Binding of cytokine to the cytokine receptor complex results in receptor dimerization, which consequently phosphorylates JAK proteins as the cytokine receptor itself lacks intrinsic biological activity. Activated JAKs induce the phosphorylation of STATs which, following dimerization, translocate into the nucleus and stimulate gene expression. STAT family proteins consist of seven members: STAT1-4, 5a, 5b and 6. STATs bind to the enhancer region of genes and by recruiting epigenetic modifiers (HDAC and HAT) to modulate the transcription of genes. JAKs activate other downstream signaling cascades including PI3K/mTOR, RAS and NF-κB. Furthermore, HSP90 and its co-chaperones such as HDAC proteins play an important role in facilitating signaling and JAK protein stabilisation. Red proteins (e.g SOCS1/3) are pathway regulators. Potential inhibitors of the proteins and pathways are indicated with red T-shaped lines. Abbreviations: AC, acetyl group; BET, Bromodomain and Extra-Terminal motif; HATs, histone acetyltransferases; HDAC, histone deacetylates; Ub, Ubiquitin; P, phosphorylation.

6. Can aberrant *TYK2* levels cause leukemia?

The involvement of JAK1-3 in inducing cancer have been intensively studied while *TYK2* has primarily been studied in the setting of auto-immune and inflammatory diseases [91, 92]. Impaired type I IFN and IL signaling due to *TYK2* deficiency have been reported in several mouse models [93] but there is limited data in human cases [94, 95]. Subsequent genome wide association analyses linked *TYK2* deficiency with auto-immune and inflammatory diseases [50]. Furthermore, *ex vivo* analysis on bone marrow cells from wild type and *TYK2* deficient mice, demonstrated a reduced inhibition of B-cell lymphopoiesis upon IFNα stimulation in *TYK2* deficient cells [96]. IFNα signaling can also inhibit B-cell differentiation and induce apoptosis in a normal setting [97]. In addition, reduced STAT3 signaling and response to IFNβ-mediated apoptosis have been reported in *TYK2* deficient pro-B cells [98]. Collectively, these findings highlighted a possible role for *TYK2* in the regulation of B-cell apoptosis that may be related to B-cell leukemia. Another study suggested an increased susceptibility of *TYK2* deficient mice to the development of B-cell lymphoid leukemia/lymphoma and T-ALL induced by Abelson murine leukemia virus and *ETV6-JAK2* fusion gene, respectively [99]. The increased incidence of disease in *TYK2* deficient mice compared to wild type controls however, may be explained by the tumour surveillance properties of *TYK2*, as *TYK2*-deficient animals also demonstrated reduced cytotoxic activity of T- and natural killer cells [50, 99]. Interestingly, the immunosurveillance properties of *TYK2* are demonstrated to be independent of its canonical kinase activity. *TYK2* deficient mice expressing kinase inactive *TYK2* protein (harbouring *TYK2* p.K923E mutation) exhibited normal development of natural killer cells in bone marrow [100]. The cytotoxic activity of these natural killer cells against a variety of tumour cells was also restored upon expression of kinase inactive *TYK2* protein [107]. These results highlight the potential benefit of *TYK2* inhibitors (*TYK2*i) to treat cancers exhibiting higher *TYK2* levels and would not lead to impairment of tumour surveillance.

TYK2 overexpression and activation has been demonstrated to be associated with oncogenesis in various cancer cell lines and patient samples (breast, prostate and ovarian cancer) [97, 101-104]. For instance, a study by Ide *et al.* (2008) demonstrated increased invasiveness of prostate tumour cells as a result of increased *TYK2* expression [102]. Another study demonstrated invasion of malignant cells into the liver upon *TYK2* protein expression using *in vivo* transgenic mouse models of B-cell lymphoma co-expressing the *c-MYC* oncogene [102]. The significance of increased *TYK2* expression in hematological malignancies was only recently highlighted in T-ALL (approximately 80% and 60% of lines and cases screened in the cohort, respectively) and anaplastic large cell lymphoma (ALCL) [33, 105]. *TYK2* gene knock down by small interfering RNA, resulted in impaired growth and increased cell apoptosis in more than 60% of patient derived T-ALL cells and cell lines [105]. This observation was not reported in other *JAK* family genes and appeared specific to *TYK2* [105]. In addition, *TYK2* deletion in mouse models of *NPM-ALK*-induced ALCL and human primary ALCL cells demonstrated prolonged survival in mouse models and reduced growth/increased apoptosis in primary cells [33]. Data from both studies also demonstrated the dependency of T-ALL and ALCL cell lines and patient samples on *TYK2* activation that leads to upregulation of STAT1/3 and consequently members of anti-apoptotic BCL2 family [33, 105].

7. *TYK2* alterations occur in ALL patients

Reports on gain of function or activating *TYK2* mutations and alterations in hematological malignancies have only recently emerged. In 2013, the first activating *TYK2* mutations (Table 1) were reported in T-ALL cell lines and were demonstrated to have transformative ability; enabling IL-3 dependent pro-B murine Ba/F3 cells harbouring these mutations cytokine independent growth [33]. A year later, the first case of a *TYK2* fusion gene was reported in HR subtype of B-ALL (also termed Ph-like ALL; *MYB-TYK2*) [22]. Subsequently, two more 5' partners for *TYK2* rearrangements in HR Ph-like ALL (*MYB*, *SMARCA4* and *ZNF340*) were reported [22, 26]. *TYK2* rearrangements have variable 5' partners and breakpoints, yet retain an in-frame kinase domain (also known as JH1; Figure 3), potentially resulting in constitutive activation of the fusion protein. Each fusion may, or may not, contain a disrupted pseudokinase domain (termed JH2), responsible for auto-inhibition of JAK kinase domain [22, 40, 106]. In addition, the fusions lack the FERM (four-point one, erzrin, radixin, moesin) and SH-like (Src-homology) domains, which are involved in protein and cytokine receptor binding, respectively [40, 107]. Despite the diversity of the 5' partner, 5' genes commonly harbour a DNA binding domain and facilitate the dimerization and subsequent activation of *TYK2* [22, 106].

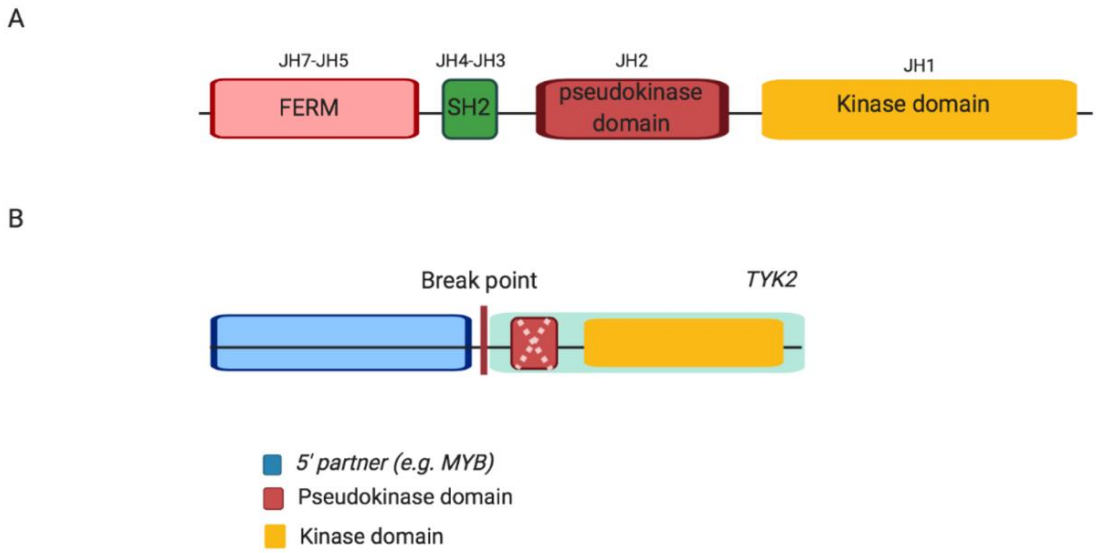

Figure 3: Schematic structure of wild type Jak protein, JAK2 and *TYK2* alterations in Ph-like ALL. (A) Wild type Jak, each Jak protein consists of four domains including FERM, SH2, pseudokinase and kinase. (B) *TYK2* alterations, *JAK*-class fusions exhibit an intact kinase domain with either a disrupted or absent (as indicated by dashed cross) pseudokinase domain fused to variable 5' partner genes. Abbreviation: JH, Jak homology domain.

An *in vitro* study demonstrated the ability of the *MYB-TYK2* fusion gene to induce cytokine independent growth in pro-B murine Ba/F3 cells [26]. Only recently, additional investigations by our group demonstrated the predominant activation of JAK/STAT signaling and constitutive phosphorylation of *TYK2* due to the expression of MYB-*TYK2* fusion protein [108]. The leukemogenic ability of the *MYB-TYK2* fusion gene was also confirmed where the resultant gene fusion induced an aggressive B-ALL in mouse models [108]. These findings provided evidence, for the first time, of the leukemogenic potential of *TYK2* fusion genes. Furthermore, *TYK2* alterations have been reported in other blood cancers including acute myeloid leukemia (AML), CD30-positive lymphoproliferative disorder (LPD) and ALCL [31, 32, 34, 109]. *In vitro* analysis of *NPM1-TYK2* and *NFKB2-TYK2* fusion genes, detected only in LPD and ALCL patients respectively, demonstrated constitutive auto- and trans-phosphorylation of *TYK2* and downstream activation of STAT family proteins [31, 32]. However, the oncogenesis potential of these *TYK2* fusion genes in *in vivo* models is unknown. Table 1 highlights the key *TYK2* alterations and the current *in vitro* functional consequences in ALL.

Table 1: Key *TYK2* mutations and rearrangements detected in ALL. Modified from Woss et.al (2019) (46) and St. Jude PeCan Data portal [110].

***TYK2* alterations**	**Disease**	**Known functional status**	**Domain**	**References**
TYK2 p.G271S	B-ALL	n.d. *	FERM	[111]
TYK2 p.W327R	B-ALL (Ph-Like)	n.d. *	FERM	[22]
TYK2 p.V678L	B-ALL	n.d. *	Pseudokinase (JH2)	[111]
TYK2 p.P760L	B-ALL	increased *TYK2* autophosphorylation/ STATs activation	Pseudokinase (JH2)	[53]
TYK2 p.G909S	B-ALL	n.d.	Kinase (JH1)	[110]
TYK2 p.A1156V	B-ALL (Ph-like)	n.d. *	Kinase (JH1)	[110].
MYB-TYK2	B-ALL (Ph-like)	cytokine independent growth (*in vitro)* $^{+}$STATs activation/ increased *TYK2* autophosphorylation/ induced B-ALL in mouse models	Intact kinase (JH1)	[22, 26]
SMARCA4-TYK2	B-ALL (Ph-like)	n.d. *	Intact kinase (JH1)	[10]
ZNF340-TYK2	B-ALL (Ph-like)	n.d. *	Intact kinase (JH1)	[10]

TYK2 p.V15A	T-ALL	n.d.	FERM	[110]
TYK2 p.A35V	T-ALL	n.d.	FERM	[110]
TYK2 p.G36D	T-ALL	cytokine independent growth (*in vitro)*	FERM	[33]
TYK2 p.S47N	T-ALL	cytokine independent growth (*in vitro)*	FERM	[33]
TYK2 p.R425H	T-ALL	failed cytokine independent growth (*in vitro)*	FERM	[33]
TYK2 p.C192Y	T-ALL	n.d.	FERM	[110]
TYK2 p.R243W	T-ALL	n.d.	FERM	[110]
TYK2 p.R274H	T-ALL	n.d.	SH2	[110]
TYK2 p.A375V	T-ALL	n.d.	SH2	[110]
TYK2 p.P494S	T-ALL	n.d.	SH2	[110]
TYK2 p.V678L	T-ALL	n.d.	Pseudokinase (JH2)	[110]
TYK2 p.V731I	T-ALL	cytokine independent growth (*in vitro)*	Pseudokinase (JH2)	[33]
TYK2 p.G761V	T-ALL	increased *TYK2* autophosphorylation/ STATs activation	Pseudokinase (JH2)	[53]
TYK2 p.G937A	T-ALL	n.d.	Kinase (JH1)	[110]
TYK2 p.E957D	T-ALL	cytokine independent growth (*in vitro)* / Weak STATs activation	Kinase (JH1)	[33, 53]
TYK2 p.M926V	T-ALL	no STATs activation	Kinase (JH1)	[53]
TYK2 p.Y955H	T-ALL	n.d.	Kinase (JH1)	[110]
TYK2 p.R1027H	T-ALL	cytokine independent growth (*in vitro)* /Weak STATs activation	Kinase (JH1)	[33]

*These alterations are speculated to be gain of function and activating mutations and rearrangements, since no functional analyses are available. [+]unpublished data (Tavakoli *et al*, 2021, under revision). Abbreviations: n.d.=no data

8. Targeted therapeutic possibilities for *TYK2*-altered disease

Currently, the lack of knowledge and efficient therapeutics targeting the *TYK2* oncogenic alterations, necessitate broader screening of SMIs. The subsequent JAK/STAT signaling activation due to *TYK2* alterations, provide a rational avenue for the use of JAKi against this HR subtype. Ruxolitinib was the first JAK1/2i approved for treatment of patients with myeloproliferative disorders such as myeloproliferative neoplasms (MPN), polycythemia vera (PV) and essential thrombocythemia (ET), the majority of whom harboured the activating *JAK2* p.V617F mutation [112-115]. The significant improvement of symptoms and reduced splenomegaly, in addition to the good tolerability of ruxolitinib treatment [116, 117], accelerated the development and clinical use of JAKi as an anti-cancer

drug. The efficacy of some therapeutics against HR ALL harbouring JAK/STAT activating alterations has been demonstrated in *in vitro* and *in vivo* pre-clinical models and case studies [16, 19, 22, 24, 26-29]. The sensitivity of cell lines harbouring *JAK2* fusions (i.e. *PAX5-JAK2* and *ATF7IP-JAK2*) to ruxolitinib *in vitro*, plus its efficacy in reducing leukemic burden in mouse models of *JAK2* rearranged ALL, has also been reported [26]. In particular, a strong *in vitro* and *in vivo* anti-leukemic effect for NDI-031301 (*TYK2*-specific inhibitor) has been reported against *TYK2*-dependent T-ALL cell lines and primary cells [118]. Similarly, our group identified the JAKi, cerdulatinib, as an efficacious therapeutic agent against cells harbouring the *MYB-TYK2* fusion gene, with significantly reduced cell proliferation and decreased tumour burden in mice [108].

The protein-protein interactions and co-operation of *TYK2* with HDACs and HSPs to activate JAK/STAT signaling and possible cross talk of JAK/STAT signaling to other pathways due to *TYK2* alterations, provide an alternative kinase independent and even more attractive therapeutic targets [46, 73, 79]. Thus far, the sensitivity of the *MYB-TYK2* fusion gene has been established to the HDACi, vorinostat and the HSP90i, tanespimycin *in vitro*; anti-leukemic effects of vorinostat were also demonstrated in pre-clinical *in vivo* models of *MYB-TYK2* altered disease [119]. In addition to *TYK2*-altered cases, various SMIs (PI3K/mTORi, MEK1/2i, HSP90i and BETi) have demonstrated promising *in vitro* and *in vivo* efficacy against *CRLF2*-rearranged ALL cases exhibiting JAK/STAT hyperactivation [25, 30, 120-122]. The use of HDACi such as vorinostat alone or in combination with a chemotherapy backbone has been approved for treatment of T-cell lymphoma and demonstrated activity against other hematological malignancies such as AML [123, 124]. Interestingly, HDACi demonstrated efficacy in clinical trials for *JAK* mutated MPN and PV cases [125-127]. Recently, studies have also demonstrated HDACi efficacy in inducing apoptosis in cells and engraftment reduction of leukemic cells in *in vitro* and *in vivo* models of B-ALL (B-ALL cell lines and xenograft models of *KMT2A*-rearranged ALL and *CRLF2*-rearranged ALL), respectively [122, 128, 129].

Taken together, these findings highlight the potential benefit of therapies targeted to specific genomic alterations that may improve the response to treatment and subsequent outcomes in patients. Currently, there is an unmet need to widely investigate the efficacy of SMIs on other HR ALL subtypes including *TYK2* alterations. The data so far, supports the further exploration of the efficacy of *TYK2*i, HDACi and HSP90i in larger cohort of cases with *TYK2*-altered disease. Furthermore, the efficacy of these SMIs in combination and/or as an addition to the chemotherapeutic backbone regimen requires future investigation. In the era of precision medicine, it is essential to understand the activated pathways of each underlying genomic alteration in individual patients. In addition, it is crucial to identify therapeutics that specifically target activated pathways, such as those in *TYK-2* altered disease. This will be achieved through robust pre-clinical models to test novel therapies and facilitate more genomic alteration-specific clinical trials.

9. Future Prospects

Utilization of NGS technology has led to the identification of a growing number of *TYK2* alterations in ALL cases [10, 33, 53, 111]. Given the well-established role of *TYK2* in JAK/STAT signaling and its potential kinase-dependent oncogenic consequence, it is essential to inform effective

targeted therapeutics for ALL cases harbouring *TYK2* gain of function mutations and/or rearrangements. The prerequisite for identifying effective targeted therapies, however, relies on robust *in vitro* and *in vivo* modelling of each alteration. Aside from the recent study by our group investigating the oncogenic potential of the *MYB-TYK2* fusion gene in *in vivo* models, the significance of other *TYK2* alterations (e.g. pseudokinase or FERM mutated *TYK2* cases) as a driver oncogene in leukemogenesis is unknown. It is not clear whether all *TYK2* alterations are capable of inducing disease and activating similar pathways downstream of each specific alteration. Thus, future research should focus on the comprehensive characterization of the functional and prognostic consequence of these alterations and *TYK2* activation in each setting as well as to their therapeutic targetability. This approach will consequently elucidate a clear picture of the leukemogenic role, clinical importance and therapeutic targetability of *TYK2* alterations in ALL.

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