Targeting Spleen Tyrosine Kinase (SYK) for Treatment of Human Disease

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Abstract

Spleen tyrosine kinase (SYK) plays a crucial role in the coordination of immune recognition receptors and orchestrates multiple downstream signaling pathways in various hematopoietic cells. In addition to its well-known function in transducing Fcγ receptor- and B cell receptor-mediated events, SYK signals downstream of a growing list of immunoreceptor pathways that modulate the innate and adaptive immune responses. SYK has been implicated in the pathogenesis of hematological malignancies including B-lineage leukemias and lymphomas. Inhibition of SYK promotes apoptosis in leukemia and lymphoma cells. Notably, SYK-dependent functions in various cell types are important in the etiology of autoimmune, allergic and neoplastic disorders. Rationally designed small molecule inhibitors targeting the ATP or the substrate binding P site of SYK have been developed with multifunctional properties. Several ATP-binding site inhibitors are under clinical trial in patients with rheumatoid arthritis, autoimmune thrombocytopenic purpura, B-cell lymphomas, allergic rhinitis and asthma. Preclinical as well as early clinical evidence shows the therapeutic potential of targeting SYK in pediatric and adult B-lineage leukemias and lymphomas. Notably, a P site SYK inhibitor C-61 was found to overcome the radiochemotherapy resistance of leukemic cells. Targeting SYK with nanotechnology-enabled delivery of C-61 to leukemia cells may overcome resistance to radiation therapy and chemotherapy as well as broaden the therapeutic window.

Keywords: Spleen tyrosine kinase, lymphomas; β-integrins

Introduction

Spleen tyrosine kinase (SYK) and Zeta-chain-associated protein kinase 70 (ZAP-70) are members of the SYK family of non-receptor tyrosine kinases (TKs) and share a similar domain organization [1-6]. The SYK protein contains tandem NH2 terminal Src homology 2 (SH2) domains, multiple tyrosine phosphorylation sites, and a COOH terminal tyrosine kinase domain. The SH2 domains bind phosphorylated immunoreceptor tyrosine-based activation motifs (ITAM) and couple activated immunoreceptors to multiple downstream signaling pathways [2]. SYK is essential for lymphocyte development and function as well as signal transduction via a variety of membrane receptors in nonlymphoid cells, such as mast cells [3,4].

In hematopoietic cells, SYK binds to the cytoplasmic tails of B-cell receptors and Fc receptors. SYK also associates with several other cell-surface receptors, such as β-integrins, Toll-like receptor 4, tumor necrosis factor (TNF) receptor, and the collagen receptor glycoprotein V1 [7-11]. SYK undergoes autophosphorylation and phosphorylation by Src kinases. Activated SYK phosphorylates downstream signaling proteins that mediate diverse cellular responses including proliferation, differentiation, cellular adhesion, migration, phagocytosis, and cytokine production [10-13]. ZAP-70 appears to play a similar role in signaling from the T-cell receptor (TCR) [14]. Recent reports indicate that SYK also mediates other, unexpectedly diverse biological functions, including innate immune recognition, osteoclast maturation, platelet activation and vascular development -- biological functions that figure prominently in the pathophysiology of neoplastic, autoimmune as well as chronic inflammatory disorders [15,16]. With discovery of specific inhibitors, SYK has recently emerged as a pharmaceutical target for the treatment of neoplastic, autoimmune and allergic disorders.

Regulatory Functions of SYK in Normal and Aberrant Signal Transduction

Role of SYK as a key component of the B-Cell Receptor (BCR) signaling pathway

SYK plays an essential role in the development of lymphocytes and activation of immune cells. In particular, SYK is a key component of the BCR signaling pathway [5,17-19]. Normal B cells are activated by antigen ligation of Ig-α (CD79a) and Ig-β (CD79b) on the B-cell surface that triggers BCR signaling [2,6] (Figure 1). In brief, the ligation activates the Src family kinase that subsequently phosphorylates the ITAM motifs of Ig-α and Ig-β [20-22]. Phosphorylated Ig-α and Ig-β recruit SYK kinase from cytoplasm to the perimembrane location to become associated with the BCR signalosome [23-26]. SYK is then phosphorylated and activated, and subsequently interacts with and catalyzes the phosphorylation of several other signaling molecules, including phospholipase Cγ2 (PLCγ2), a lipase, Bruton’s tyrosine kinase (BTK, a protein tyrosine kinase that also activates PLCγ2), and B cell linker protein (BLNK, an adaptor molecule) [27,28]. Activated PLCγ2 cleaves phosphatidylinositol 4,5-bisphosphate

Figure 1: Role of SYK in BCR signaling pathways. See text for discussion.
(PIP2) into diacylglycerol and inositol triphosphate, which leads to calcium (Ca++) mobilization and activation of several downstream pathways such as AKT, mitogen-activated protein (MAP) kinase, and nuclear factor-kB (NFkB) [29-32]. Through these pathways, several transcriptional factors are activated and cells eventually undergo metabolic adaptation leading to increased cell survival, cell proliferation, differentiation into plasma or memory B cells, and antibody production [33-35].

SYK has an important role in the transition of pro-B cells into pre-B cells, and is essential for immature B cells to pass a Rac-dependent checkpoint that controls their entry into the splenic white pulp [36,37]. Disruption of the SYK gene leads to perinatal lethality, a petechiated in utero appearance and the lack of mature B cells [36,38]. Crosslinking of BCR in SYK−/− B cells fails to induce cell activation [39]. Analysis of SYK-deficient B-cells from homozygous SYK-mutant mouse showed that disruption of SYK gene impairs B cell differentiation at the pro-B to pre-B transition and at the maturation of immature B cells into circulating B cells [29]. SYK-deficient mice develop embryonic hemorrhaging and arteriovenous shunting due to blood-lymphatic connections that remain after the failure of emerging lymphatic vessels to separate from blood vessels [31]. Also, irradiated mice reconstituted with SYK-deficient fetal liver cells show a block in B-cell development and maturation [38].

Role of SYK as a mitotic checkpoint kinase and a stabilizer of microtubular cytoskeleton

SYK is a microtubule-associated protein that has a critical function for the stability of the microtubular cytoskeleton [40-43]. SYK colocalizes with native tubulin within the microtubular network of the cytoskeleton [42]. Inhibition of SYK in transfected human tumor cells expressing an active SYK-green fluorescent protein fusion protein caused distortion of the microtubule cytoskeleton, disrupted SYK-microtubule interactions and destroyed SYK-dependent stability of the microtubule cytoskeleton. Confocal imaging after SYK inhibition revealed an aberrant SYK topology characterized by cytoplasmic clustering of SYK in the perinuclear region [44].

SYK has also been identified as a mitotic kinase that localizes to the centrosomes, interacts with the key centrosomal component γ-tubulin, and affects mitotic progression [45,46]. It has been proposed that SYK regulates the initial stages of microtubule formation by forming a complex with γ-tubulin and modulating the γ-tubulin-mediated microtubule nucleation [45,46]. Genetic evidence in B-cells demonstrated that SYK plays a key role in regulation of cell cycle progression in G2/M phase via p38-dependent and p38-independent pathways [46]. In accord with these important regulatory functions of SYK as a mitotic checkpoint kinase and stabilizer of the microtubule cytoskeleton, it has been reported that SYK is required for mitotic activity and proliferation of B-lineage lymphoid cells. Enhanced SYK kinase activity induces growth factor-independent proliferation of pre-B cells and deregulated SYK activity results in pre-B cell transformation [41]. SYK also preferentially phosphorylates the α-tubulin subunits of microtubules, which has been proposed to regulate the ability of the microtubule cytoskeleton to function as a scaffold for the assembly of signaling complexes [42].

Role of SYK in autoimmunity and allergic diseases

In addition to its well-known function in transducing FcγR- and BCR-mediated events, SYK signals downstream of a growing list of immunoreceptor pathways that modulate the innate and adaptive immune responses [47]. SYK-dependent functions in various cell types are important in the etiology of autoimmune and allergic diseases. In preclinical rodent studies, inhibition of SYK has been shown to ameliorate autoimmunity in models of asthma [48,49], arthritis [50], and lupus nephritis [51]. Early clinical studies with a chemical inhibitor of SYK have demonstrated efficacy in immune thrombocytopenic purpura (ITP) [52] a disease in which Abs are the ultimate effectors, and in rheumatoid arthritis (RA) [53], a disease in which autoimmune T cells are the predominant effectors. Though normal T cells lack SYK expression, recent data indicate that SYK is overexpressed and functionally active in T cells from patients with systemic lupus erythematosis (SLE) [54]. SYK signaling in these cells occurs through preferential binding to FcγRy, resulting in altered calcium flux and actin polymerization. SYK inhibitor R784 has exerted potent therapeutic efficacy against autoimmune and allergic diseases such as RA, bronchial asthma and ITP. Moreover, SYK blockade prevented the development of skin and kidney lesions in lupus-prone mice, however the mechanism of action is unclear. SYK-mediated BCR-signaling is prerequisite for optimal induction of toll-like receptor (TLR)-9, thereby allowing efficient propagation of CD40- and TLR-9 signaling in human B cells. Inhibition of SYK has a potential to regulate B-cell mediated inflammatory diseases such as SLE. Colonna et al. [55], evaluated the potential for targeting SYK in APCs in animal models of autoimmune diabetes using genetic and pharmacologic approaches. SYK deficiency in dendritic cells results in impaired Ab-mediated crospriming in vivo, establishing the necessity of the activating pathways downstream of SYK in the induction of Ab-mediated T cell autoimmunity and minimizing the importance of FcγR-mediated effector cell activation in this model of T cell-mediated autoimmunity [56].

 Mast cells are crucial effector cells in the allergic cascade. SYK plays a pivotal role in IgE receptor signaling in mast cells and basophils and is involved in integrin signaling, responsible for neutrophil effector functions [57]. The cross-linking of the high affinity IgE receptor (FcεRI) activates mast cells and basophils. SYK is positioned upstream of the IgE receptor signal transducing pathway and may represent an important target for the treatment of nasal inflammatory diseases [58]. SYK−/− mast cells are unable to induce Ca++ mobilization, degranulate, phosphorylate MAP kinases, and activate NFAT or NFκB; but transfection of these cells with SYK reconstitutes their activation [59-61]. SYK inhibitors block the release of mediators such as histamine, the production of prostaglandins and leukotrienes, and the secretion of cytokines by mast cells. In addition, SYK is an important enzyme in various inflammation pathways relevant to respiratory diseases and, therefore, it is a key target for a novel antiasthmatic therapy. In human cord blood-derived mast cells (CBDMCs), the SYK inhibitor NVP-QAB205 prevented degranulation assessed by measurement of histamine release and the production of leukotrienes (LTC4/LTD4/LTE4) and prostaglandin D2 (PGD2). Furthermore, the SYK inhibitor was similarly able to significantly inhibit the release of these granules and newly synthesized mediators by nasal polyp mast cells in a dose dependent manner. The inhibition of SYK may represent an important therapeutic strategy for the treatment of upper airway disease with mast cell involvement, such as allergic rhinitis.


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Role of SYK in acute myeloid leukemia (AML)

AML is a heterogeneous disease that accounts for approximately 20% of acute leukemias in children and adolescents. Despite the lack of targeted therapy for most subtypes and a dearth of new agents, survival rates have reached approximately 60% for children treated on clinical trials in developed countries. Using a gene expression–based high-throughput screen, constitutive SYK activation has also been implicated in AML [62]. The exact pathobiology is unclear, but it has been proposed that SYK contributes to an uncoupling of proliferation and differentiation in myeloid cells. SYK activation can also lead to the upregulation of anti-apoptotic genes. A role for SYK in the pathogenesis of myeloid malignancies is supported by the reported fusion of the TEL to the SYK gene in myelodysplasia (MDS) with (9;12)(q22;p12) translocation [63]. Importantly, this TEL-SYK fusion transformed the interleukin-3 dependent murine hematopoietic cell line Ba/F3 to growth factor independence [64]. SYK mRNA transcript has been reported to be abundantly expressed in primary AML blasts [65] and its expression correlated with response to treatment with the anti-AML drug gemtuzumab ozogamicin.

Role of SYK in lymphoid malignancies

SYK acts as a master regulator of anti-apoptotic signaling pathways in leukemia and lymphoma cells: Notably, the anti-apoptotic NFκB, PI3-K/akt, and STAT3 pathways are regulated by SYK-mediated tyrosine phosphorylation events [66-72] (Figure 2). Constitutive activation and anti-apoptotic function of SYK kinase have been documented for several B-lineage lymphoid malignancies [73,74]. The identification of SYK as a master regulator of multiple anti-apoptotic pathways in B-lineage lymphoid cells prompts the hypothesis that rationally designed inhibitors targeting SYK may overcome the resistance of neoplastic cells to apoptosis and thereby provide the foundation for more effective multi-modality treatment regimens for poor prognosis leukemia and lymphoma patients.

Targeting SYK in lymphomas and leukemias with aberrant BCR signaling: SYK is necessary for BCR signaling and contributes to the survival and growth of B-cells after BCR stimulation [2,5,17-19]. In specific contexts, BCR signaling via SYK can be activated in an uncontrolled manner which has been implicated in the pathogenesis of B-lineage lymphomas and leukemias. B-lineage lymphoid malignancies, including diffuse large B cell lymphomas (DLBCL), chronic lymphocytic leukemias (CLL), mucosa-associated lymphoid tissue (MALT) lymphomas, follicular lymphoma (FL), Helicobacter pylori–associated gastric mucosa-associated lymphatic tissue lymphomas, and certain indolent lymphomas associated with chronic hepatitis C virus infection all present with aberrant antigen receptor signaling pathways [75-85]. The reported primary role of SYK in B-lineage lymphomas is to relay ITAM-mediated tonic BCR signaling. In addition, SYK also contributes to the survival of B-lineage lymphoma cells through processing of BCR-independent adhesive and homing signals [77]. Given the vital role of SYK in BCR signaling, pharmacological or genetic targeting of SYK has been pursued as a therapeutic strategy for the treatment of NHL [41,68,86-93]. SYK inhibition by using a chemical SYK inhibitor or by siRNA results in potent inhibition of mTOR activity in mantle cell lymphoma, large cell lymphoma, and Burkitt’s lymphoma [67]. Targeting BCR signaling pathways via SYK inhibition appears to be a highly promising therapeutic strategy in B cell lymphomas.

Murine models of NHL show tumor regression or remission when SYK is targeted pharmacologically or by shRNA [89]. Pharmacological inhibition of SYK resulted in apoptosis of lymphoma cells in vitro [77,87,89,91-94]. Moreover, SYK inhibition was efficacious in patients with relapsed/refractory NHL [95]. These studies have implicated the phospholipase Cγ2, PI3K/Akt, and Erk/MAPK downstream signaling pathways and a number of downstream targets, including Bcl-xL, Bad, and Mcl-1, as mediators of the SYK survival signal.

Role of SYK in diffuse large B-Cell lymphoma (DLBCL): DLBCL is one of the most common types of aggressive NHL. Recently, BCR signaling has been recognized as a key pathway in the pathogenesis of DLBCL [96]. Using gene-expression profiling and unsupervised consensus clustering for analysis, Shipp et al. [97] identified a subset of lymphoma that demonstrates a BCR/proliferation signature (BCR-type DLBCL). These lymphomas express high levels of SYK mRNA, along with other components of the BCR signaling pathway. Chen et al. [98] described a constitutive high level of SYK phosphorylation at Y348/Y352 in DLBCL, supporting the concept of “tonic BCR” in this disease. Compelling preclinical data using NHL patient samples demonstrated that inhibition of SYK could lead to proliferation arrest and apoptosis, both of which were associated with the degree of overexpression of SYK [98]. Using a tissue microarray, Cheng et al. [99] demonstrated that the SYK protein is phosphorylated, localized to the cell membrane, and therefore activated in 44% of human DLBCL tissues. The specific cellular effect caused by SYK inhibition is cell-cycle arrest. Furthermore, pharmacologic inhibition of SYK induced apoptosis in murine B-cell lymphomas in vitro and resulted in regression of NHL-like B-cell lymphomas [74]. Taken together, these findings suggest that SYK expression and function are necessary for the survival of NHL cell lines [74] and highlight the potential use of SYK inhibition in the treatment of NHL.

Role of SYK in lymphoproliferative diseases (LPD): In addition to BCR activation, SYK can also be activated by other endogenous receptors and ITAM-containing viral proteins, including K1 of Kaposis sarcoma herpesvirus and protein latent membrane 2a (LMP2a) of Epstein-Barr virus (EBV) [100]. The EBV LMP2a, a functional mimic of the BCR, provides constitutive survival signals for latently infected cells through SYK activation [101-105]. LMP2a is a 54 kDa protein expressed during the latent cycle of EBV infection. SYK inhibition using small molecule inhibitors and siRNA strategies has been shown to reduce proliferation and induce apoptosis of posttransplant lymphoproliferative disorder (PTLD)-derived EBV-B
cell lines. In EBV+ PTLD-derived B cell lines, SYK is activated either by the BCR or LMP2a. SYK directly activates the PI3K/Akt pathway, leading to IL-10 production. SYK inhibition reduces autocrine IL-10 production. Although SYK inhibition attenuates signaling through both the PI3K/Akt and Erk pathways, only PI3K/Akt inhibition causes apoptosis of PTLD-derived cell lines. Loss of the endogenous caspase inhibitor XIAP is observed after SYK or PI3K/Akt inhibition [73]. The loss of XIAP and apoptosis that results from SYK or PI3K/Akt inhibition is reversed by inhibition of the mitochondrial protease HtrA2. These findings suggest that SYK drives EBV+ B cell lymphoma survival through PI3K/Akt activation, which prevents the HtrA2-dependent loss of XIAP and apoptosis induced by SYK and Akt inhibition.

Role of SYK in mantle cell lymphoma (MCL): Rinaldi et al. [90] reported differential overexpression of SYK in mantle cell lymphoma (MCL) cells at DNA, RNA, and protein levels. After treatment with plicatannol, cell cycle arrest and apoptosis were observed and were correlated to the degree of SYK overexpression [90]. A comparison of the single-cell signaling profiles of follicular lymphoma (FL) cells with those of nonmalignant B cells indicates that BCR-mediated signaling through SYK occurs to a greater degree and for a longer duration in neoplastic cells than in nonmalignant B cells [106]. FL cells display greater levels of BCR-induced phosphorylation of SYK (Tyr527), Btk, and MAPK [107]. Thus, FL cells represent models suitable for evaluating pharmacologic targeting of BCR signaling. These preclinical observations formed the basis for clinical investigation of SYK inhibition in NHL.

Role of SYK in B-lineage ALL: B-lineage ALL is the most common form of cancer in children and adolescents [108]. Contemporary multimodality treatment for ALL can cure approximately 80% of children with the disease [109,110]. Currently, the major challenge in the treatment of B-lineage ALL is to cure patients who have relapsed (~20%) despite intensive multigene chemotherapy [111-115]. The standard approach to the treatment of these high-risk patients has been salvage chemotherapy to achieve a second remission and subsequent use of very intensive treatment regimens including high dose “suprarethral” chemotherapy, often combined with TBI, followed by hematopoietic stem cell transplantation (SCT). Laboratory evidence indicates that primary clonogenic blasts from a significant portion of B-lineage ALL patients are among those reported as the most radiation-resistant human tumor cells [116,117]. Furthermore, clonogenic leukemia cells from over 2/3 of the ALL patients exhibit a substantial capacity to repair sublethal radiation damage [118]. Resistance of B-lineage leukemia cells to the pro-apoptotic effects of radiation-induced oxidative stress hampers the attempts to improve the survival outcome of patients with B-lineage ALL undergoing TBI and SCT and only ~20% of high risk B-lineage ALL patients become long-term disease-free survivors even after TBI/SCT with substantial short-term and long-term morbidity and mortality [111,112]. These preclinical and clinical observations in relapsed B-lineage ALL emphasize the urgent need for identification of new drug candidates capable of potentiating the anti-leukemic potency of pre-SCT radiochemotherapy regimens.

The pentapeptide mimic C-61, targeting the substrate binding P-site of SYK tyrosine kinase acts as a potent inducer of apoptosis in chemotherapy-resistant SYK-expressing primary leukemic B-cell precursors taken directly from relapsed B-lineage ALL patients, exhibits favorable pharmacokinetics in mice and non-human primates, and eradicates in vivo clonogenic leukemia cells in SCID mouse xenograft models of chemotherapy-resistant human BPL at dose levels non-toxic to mice and non-human primates [72,93,119]. These in vitro and in vivo findings provide unprecedented proof of principle for effective treatment of chemotherapy-resistant BPL by targeting SYK-dependent anti-apoptotic blast cell survival machinery with a SYK P-Site inhibitor [93]. Further development of C-61 may provide the foundation for therapeutic innovation against chemotherapy-resistant B-lineage ALL. Our recent studies also provided experimental evidence that the SYK-STAT3 signaling network plays an important and indispensable role in resistance of B-lineage ALL cells to OS-induced apoptosis [72]. We discovered the pentapeptide mimic 1,4-Bis (9-O dihydroquinidinyl) phthalazine/hydroquinidine 1,4-phthalazinediyl diether (C-61) as a TK inhibitor targeting the substrate binding P-site of SYK as a novel drug candidate against B-lineage ALL [72,93]. Disruption of the SYK-STAT3 network with C-61 at nanomolar concentrations augmented OS-induced apoptosis of human B-lineage leukemia cells in vitro [72]. C-61 has the potential to improve the treatment outcomes of ALL patients by killing chemotherapy- and radiation therapy-resistant leukemia cells through inactivation of their SYK kinase-dependent survival mechanism as well as by improving the efficacy of radiation therapy through radiosensitization.

We used a highly radiation-resistant subclone of the murine B-lineage leukemia cell line BCL-1 in a syngeneic BMT model to investigate whether the use of C-61 in combination with TBI would help achieve a selective radiosensitization of B-lineage leukemia cells in vivo without significant increase in the toxicity of TBI. Our results provided evidence that C-61, when administered at one-fifth of its nonobservable adverse effect level (NOAEL), which does not produce detectable single-agent activity against BCL-1 leukemia in vivo, markedly enhances the antileukemia effect of 7 Gy TBI in the context of syngeneic BMT. C-61 prevented radiation-induced activation of SYK and markedly augmented radiation-induced apoptosis of BCL-1 cells and enhanced the anti-leukemic potency of TBI in the context of syngeneic BMT [119]. Based on this preclinical proof-of-principle study, we hypothesize that the incorporation of C-61 into the pre-HSCT TBI regimen of patients with recurrent or high-risk B-lineage ALL will help overcome the radiochemotherapy resistance of their neoplastic B-cell precursors and thereby improve their treatment response and survival outcome after hematopoietic SCT.

Role of SYK in chronic lymphocytic leukemia (CLL): CLL is an incurable lymphoproliferative disorder, characterized by the accumulation of mature CD5+ B-cells [120] that shows resistance to apoptosis. BCR signaling has a central role in the pathomechanism, reflected by the prognostic impact of the somatic hypermutational status of BCR immunoglobulin heavy chain genes (IGHV) [121,122] and biased IGHV gene usage [123]. The best complete remission rate in previously untreated patients remains ~50% and median progression-free survival is, at most, 4 years with increasing intensity of combination chemotherapy plus monoclonal antibody [124]. Nonmalignant accessory cells in the tumor microenvironment, that is, stromal cells in the bone marrow (BM) and lymph nodes, are known to induce up-regulation of anti-apoptotic molecules in CLL cells and to protect them thereby from spontaneous and chemotherapy-induced apoptosis [125]. The latter phenomenon has been termed cell adhesion–mediated drug resistance (CAM-DR) [126]. Residual
leukemic cells in protective microenvironmental niches potentially contribute to disease relapse after cytotherapeutic therapy [125]. SYK kinase is constitutively active in CLL. Gene expression profiling studies combined with Western blot analyses documented that SYK is overexpressed in CLL [87]. Elevated SYK expression by CLL cells results in activation of its downstream targets including PLCgamma2, Erk and Akt. Selective SYK inhibitors BAY-61-3606 [88] and R406 [87,127] induced apoptosis of CLL cells. siRNA knockdown suggested that constitutively active SYK maintained high levels of antiapoptotic Mcl-1 via protein kinase C, which blocked proteasomal degradation of Mcl-1 [81]. Downregulation of Mcl-1 in CLL cells treated with SYK inhibitors [122] is consistent with this hypothesis. The susceptibility of CLL cells to apoptosis induction by SYK inhibitors correlated with levels of SYK expression. SYK is involved in the chemotaxis and adhesion of B-cell CLL cells and in polarization of a pro-B cell line [128]. SWAP-70 is phosphorylated by SYK and this modulates SWAP-70 F-actin binding in vitro and is required for normal B-cell polarization in vitro and migration in vivo. The chemokine CXCL12 (SDF1α) is known to direct homing of CLL cells toward protective niches, and competitive CXCR4 antagonists reduce CAM-DR in CLL in vitro cultures [129,130]. CXCL12 is an important survival axis: SYK inhibition antagonizes CXCL12-induced Akt activation, actin polymerization, and chemotaxis. Likewise, inhibition of VCA-M1 binding to very late antigen-4 (VLA-4; α4β1 integrin; CD49d/CD29) partially prevents stroma-mediated protection of apoptosis in CLL [131,132]. Furthermore, enhanced expression of the α chain of VLA4, CD49d, correlates with poor overall survival in CLL [133]. Inhibition of SYK decreases CLL cell migration toward chemokines such as CXCL12, thereby reducing the numbers of CLL cells that benefit from protective effects of stroma [77]. Moreover, SYK inhibition leads to decreased BCR-dependent secretion of the chemokines CCL3 and CCL4 by CLL cells. CLL cell migration beneath stromal cells, and ERK phosphorylation after BCR triggering [92]. siRNA-mediated SYK knockdown confirms SYK involvement in the VCA-M1 and CXCL12 signaling pathway. The SYK inhibitor R406 abrogates cell adhesion–mediated drug resistance in CLL. Inhibition of SYK by R406 reduces stromal cell–induced Mcl-1 expression in CLL cells. These in vitro observations have also been confirmed in the Eμ-TCL-1 transgenic mouse, an in vivo model of CLL [133].

Role of SYK in peripheral T-Cell lymphomas (PTCL): PTCL constitutes a rare but highly aggressive group of lymphomas that respond poorly to standard chemotherapy regimens, with long-term survival of <30% [134]. Normal T lymphocytes lack SYK expression and utilize a SYK homologue, Zap-70, in receptor signaling. SYK was shown to have an important role in pre-TCR signalling, which occurs during the transition from the double negative 3 (DN3) to the DN4 stage of early thymocyte development [14]. However, aberrant expression of activated SYK has been found in >90% of PTCL [135] suggesting the possibility that this signaling pathway is either dominant in this malignancy or the t(5;9) translocation is an alternative mechanism for this aberrant expression [136]. A recurrent translocation has been identified in a subset of PTCL that fuses the inducible T cell kinase (ITK) gene on chromosome 5 with the SYK gene on chromosome 9 to form the ITK-SYK fusion gene [137]. The t(5;9)(q33;q22) is found in ~18% of all PTCL–not otherwise specified (NOS) cases. ITK-SYK is a catalytically active TK that constitutively engages the antigen receptor signaling machinery in T cells. Conditional expression of ITK-SYK in vivo induces highly aggressive T cell lymphomas with 100% penetrance and clinical and pathological features of the human PTCL. Silencing SYK induces apoptosis and blocks proliferation in PTCL cells. The pathways responsible for the functional effects of SYK in PTCLs are unknown. Furthermore, in addition to promoting cell growth and survival, SYK may have other functional effects in PTCLs; for example, SYK regulates tumor cell migration and cytokine production in various solid tumors [138]. These findings suggest that SYK represents a novel therapeutic target for patients with PTCL.

Role of SYK in retinoblastoma: Retinoblastoma (RB) is an aggressive childhood cancer of the developing retina that is initiated by the biallelic loss of RB1 [139] with an incidence of 1 in 15–20 000 births. It accounts for 11% of all infant cancers and 3% of cancers developing in children younger than 15 years old [140,141]. Except for the hereditary forms, its causes are not well-known. Studies have recently suggested an increased risk of RB among children born after assisted reproductive technology [142]. Tumors progress very quickly following RB1 inactivation but the underlying mechanism is not known. With early detection, survival probability is approximately 90% in developed countries; in developing countries, it is only about 50%. The goal of retinoblastoma treatment is to preserve vision without compromising long-term survival and minimizing side effects. Zhang et al. [143], recently reported epigenetic deregulation as a possible mechanism for the rapid progression of malignancy-related changes, using an integrative approach involving chromat immunoprecipitation (ChIP), DNA methylation and gene expression data analysis. Of 104 genes that showed differences in expression between fetal retina tissue and retinoblastoma, 15 known cancer genes were shown to have significant differences. Among these, SYK, which has no known function in eye development, was found to be expressed at high levels in retinoblastoma. SYK is required for tumor cell survival and suppression of the SYK protein by small molecule inhibitors caused the degradation of MCL1 and caspase-mediated cell death in retinoblastoma cells in culture and in vivo indicating ‘oncogene addiction’ to this kinase in the retinoblastoma cells. SYK is a promising new target for treating retinoblastoma.

Role of SYK in melanoma and breast cancer: Accumulating evidence suggests that SYK functions as a tumor suppressor against melanoma and breast cancer. The role of SYK as candidate tumor suppressor has been well documented in breast cancer [144-146]. Loss or reduced expression of SYK in human breast cancers was associated with a higher degree of malignancy and poor prognosis [145]. The tumor-suppressive activity of SYK in breast cancer cells has been associated with abnormal mitotic progression and cell death [145,146]. SYK localizes to the centrosomes and negatively affects mitotic progression [45]. Epigenetic silencing through hypermethylation of critical CpG islands was proposed to be involved in the loss of SYK in a significant fraction of breast tumors [147]. More recently, a similar loss of SYK expression has been documented in melanoma cells, SYK levels are frequently low or undetectable in melanoma cells and primary tumors. The loss of expression occurs at transcriptional level as a result of DNA hypermethylation [148]. Notably, reintroduction of SYK was shown to restrict tumor growth and metastasis in vivo [148,149]. Re-expression of SYK in melanoma cells at levels similar to that seen in melanocytes results in suppression of chemotaxis, sustained cell cycle arrest, and senescence accompanied by molecular events associated with activation of the
p53 tumor suppressor pathway. Hence, SYK is not an appropriate therapeutic target for melanoma or breast cancer.

Small molecules as multifunctional SYK kinase inhibitors: The validity of SYK as a novel pharmaceutical target for the treatment of allergic, autoimmune and neoplastic disorders has progressed to clinical trials in the last decade (Figure 3). Majority of SYK inhibitors undergoing preclinical and clinical development target the conserved ATP binding site within the catalytic domain of the kinase. Because of the similarities of the ATP pocket structures among different kinases, the ATP-binding site inhibitors of SYK affect multiple TKs such as RET, KIT, JAK 1-3, FLT3, PDGFR-α, Aurora, LCK, KDR as well as adenosine A3 receptor and have off-target effects that lead to undesirable side effects such as hypertension, myelosuppression, teratogenicity, neutropenia or autoimmune diseases. Moreover, because SYK is widely distributed in different cell types, inhibiting its catalytic activity bears the risk of unwanted consequences on various physiological functions such as cell differentiation, adhesion and proliferation [150]. SYK inhibitors block the release of mediators such as histamine, the production of prostaglandins and leukotrienes, and the secretion of cytokines by mast cells. In addition, SYK is an important enzyme in various inflammation pathways relevant to respiratory diseases and, therefore, it is a key target for a novel antiasthmatic therapy. The lead SYK inhibitors in clinical trials include R112, R343, R406, R788, CGI14979 and PRT062607. Several of these ATP binding site SYK inhibitors are under clinical trials in patients with rheumatoid arthritis, autoimmune thrombocytopenic purpura [151], B-cell lymphomas, allergic rhinitis and asthma [152,153]. In addition, more specific small molecule substrate binding (P-site) SYK inhibitors as well as antisense oligonucleotides and siRNAs have also been developed to selectively knock down SYK as a molecular target [44,154-156]. The availability of both ATP-binding site inhibitors (e.g. R-406) and P-site inhibitors (e.g. C-61) of SYK may be particularly helpful in patients with SYK mutations that might influence inhibitor binding to ATP- and/or substrate-binding sites. Recent studies have established SYK kinase as a potential therapeutic target in NHL [89,157], CLL [77,87,127,158-160], and AML [161].

It is noteworthy however that SYK is also required for recognition of infectious agents and innate immune response against them mediated by Dectin-1 and Toll-like receptors [162]. SYK is required for monocyte/macrophage chemotaxis to the delta subclass chemokine CX3CL1 (fractalkine) and inhibition of SYK results in impairment in activation and migration of macrophages [163]. SYK is critical for FcyRs signaling in macrophages and neutrophils. SYK- macrophages are defective in phagocytosis induced by Fcγr but show normal phagocytosis in response to complement. SYK- neutrophils are incapable of generating reactive oxygen intermediates in response to Fcγr engagement [164]. Therefore, use of SYK inhibitors may increase the risk of infection in immunocompromised lymphoma patients.

SYK Inhibitor Pipeline

ATP-binding Site Inhibitors of SYK

R112 (Rigel Pharmaceuticals; 3,3’-[(5-Fluoro-2,4-pyrimidinediyl) diimino] bis-phenol) has the ability to completely inhibit all three IgE-induced mast cell functions: degranulation, lipid mediator production, and cytokine production [165,166]. The safety and tolerability of R112 has been evaluated in an allergen challenge model of allergic rhinitis (AR) in a double blinded, randomized, placebo-controlled, crossover trial [165]. Single-dose intranasal dose of R112 among 20 out-of-season volunteers with AR was well-tolerated and AEs were similar between R112 and vehicle treatments. R112 significantly reduced the release of PGE2, but not histamine or tryptase, in response to allergen challenge in subjects with AR. However, no differences were found in symptoms or in acoustic rhinometry between treatment groups. In the second study, Meltzer et al. [166] demonstrated that intranasal R112 was effective in seasonal AR. R112 provided rapid amelioration of clinical symptoms in patients with seasonal AR widely attributed to IgE-- mast cell-triggered airway inflammation.

R343: R343 (Rigel Pharmaceuticals; (4S)-[(2,2-difluoro-4H-benzo [1,4] oxazin-3-one)-6-yl]-5-fluoro-N (2)-[(3-methylaminocarbon- methyl)ethylenecxy]-2,4-pyrimidinediamine xinafoate) is an inhaled SYK kinase inhibitor designed to bind to the SYK in mast cells. R343 interrupts the signal from the IgE receptors and blocks the major pathways triggered by asthma [167]. A Phase I clinical trial of R343 in normal healthy adults and in asthmatic adults interrupts the signal from the IgE receptors and blocks the major pathways triggered by asthma was well tolerated and induced improvement in both the early and late phase asthmatic responses following an allergen challenge. R343’s ability to inhibit SYK potentially prevents or stops the immune response to the allergen and may be effective in the short and long-term control of allergic asthma.

R406: R406 (Rigel Pharmaceuticals; [N4-(2,2-dimethyl-3-oxo-4Hpyrid[1,4]oxazin-6-y1)-5-fluoro-N2-(3,4,5-trimethoxyphenyl)-2,4-pyrimidinediamin]) is an oral SYK inhibitor that blocks SYK-dependent FcγR-mediated activation of monocytes/macrophages and neutrophils and BCR-mediated activation of B lymphocytes [3,59,91,98,168]. R406 lacks selectivity for SYK [169,170]. DLBCL cell lines and primary DLBCL cells with both tonic and induced BCR-signaling are highly sensitive to R406-mediated apoptosis [98]. R406 reduced inflammation mediated by the immune complex in a reverse-passive Arthus reaction in two mouse models of antibody-induced arthritis [171]. In rat models of RA, treatment with R406 led to the inhibition of inflammation, as measured by reduction of cytokines in the synovial fluid and cartilage oligomeric matrix protein in the serum [50]. R406 is a potent inhibitor of platelet signaling and...
functions initiated by FcγRIIA cross-linking by specific antibodies or by sera from heparin-induced thrombocytopenia (HIT) patients [172]. R406 strongly inhibited FcγRIIA-induced platelet aggregation, granule secretion and microparticles production with therapeutic potential in the treatment of HIT by reducing HIT antibodies-mediated platelet activation and monocyte procoagulant activity. However, development of R406 has been discontinued in favor of its prodrug, R788.

R788: R788 (fostamatinib disodium; Rigel Pharmaceuticals; N4-(2,2-dimethyl-3-oxo-4-pyridin-1-oxazin-6-yl)-5-fluoro-N2-(3,4,5-trimethoxyphenyl)-2,4-pyrimidinediamine) is an oral prodrug of R406 under development for lymphoid malignancies and RA [52,53,158]. R788 reversibly blocks signaling in multiple cell types involved in inflammation and tissue degradation and has already been successfully tested in RA, ITP, and B-cell lymphomas in both rodent models and in clinical phase II trials in humans [56,152,153]. In the RA models, R788 suppressed clinical arthritis, bone erosions, pannus formation and synovitis. R788 was shown to prevent the development of renal disease in established murine lupus nephritis in lupus-prone NZB/NZW mice via inhibition of FcγRIIa and BCR signaling [51]. R788 was also active in the treatment of Ab-mediated glomerulonephritis in Wistar–Kyoto rats [173]. Since R788 is not entirely specific for SYK, it is thought to have several potential off target effects.

R788 is under clinical development for the treatment of RA, ITP, and B-cell lymphoid malignancies [52,174]. Genovese et al. [174] evaluated the efficacy and safety of R788 in patients with active RA who failed biologic therapies. A total of 219 patients with active RA were enrolled in a 3-month double-blind, placebo-controlled Phase II trial. The primary endpoint was the percentage of patients meeting the ACR20 response at month 3. Results showed that the primary outcome, the ACR 201 response, as well as the ACR 50 and 70 responses, were not significantly different between the group receiving R788 and the placebo group. However, in patients who entered the trial with an elevated C-reactive protein (CRP) level, analysis suggested a meaningful difference in the ACR 20 responses between the R788 (42%) and placebo (26%) groups. Additionally MRI results demonstrated improvement in joint inflammation in those patients with the greatest disease activity. Weinblatt et al. evaluated the efficacy and safety of R788 in 457 patients with active RA despite receiving methotrexate therapy in a 6-month, double-blind, placebo-controlled Phase II trial ([NCT00665925] [53,175]. In this study, patients continued to receive methotrexate in addition to active treatment with R788. Significantly more patients in the R788 groups than in the combined placebo group met the criteria for American College of Rheumatology (ACR) 20 response. ACR 20 response rates were 67% for patients treated with R788 at a dose of 100 mg BID, 57% for patients treated with a dose of 150 mg once daily, and 35% for the placebo group (P<0.001). ACR 50 response rates, defined by at least a 50% improvement, were 43%, 32%, and 19% for the 100 mg, 150 mg and control groups, respectively. R788 therapy was also seen to have a significant effect on the ACR 70 response rates and the rates of DA528 remission. AEs included diarrhea, neutropenia, elevated liver enzyme levels, hypertension, infection, and neutropenia. Since SYK has been reported to have a role in hematopoiesis, neutropenia side effects can be explained by the inhibition of SYK. The active form of this kinase inhibitor (R406) targets Lyn, Lck as well as SYK. Since Lck is a critical kinase in T-cell lineage commitment, inhibition of Lck might suppress immunity, resulting in a high risk of infection [176]. Furthermore, since suppression of Lyn has been reported to play a role in an antibody-mediated autoimmune disease resembling SLE in mice, inhibition of Lyn might increase the risk of the development of lupus [177]. Podolanczuk et al. [52] evaluated the efficacy of R788 in 16 adult patients with chronic refractory ITP. In this open-label, single-arm cohort dose-escalation Phase I trial, patients received doses of 75 mg of R788, which were escalated to 175 mg BID. Sustained response to treatment with oral R788 was achieved in 50% of patients, and 75% achieved at least a transient increase in platelet count. The efficacy of R788 as a TK inhibitor was associated with GI toxicity. Other toxicities observed were elevation in liver function tests, elevations in systolic blood pressure, and weight gain.

In a Phase I/II clinical trial, Friedberg et al. [158], investigated R788 in the treatment of patients with relapsed and refractory B-cell NHL and CLL. In the Phase I part of the trial, two cohorts of six patients each received one of two dose levels, 200 mg or 250 mg, BID orally. All patients in cohort 1 had stable disease after treatment with R788 with a median duration of 5.3 months. In cohort 2, one patient with FL displayed a PR with response duration of 13.3 months. The dose-limiting toxicities in this part of the study were neutropenia, diarrhea, and thrombocytopenia. Sixty-eight patients with recurrent B-NHL were then enrolled to the Phase II study. The patients were treated with R788 with doses of 200 mg BID. The highest response rate (55%) was observed in patients with SLL/CLL. Objective response rates were also noted in DLBCL (22%), FL (10%), and MCL (11%). The median PFS was 4.1 months for all patients. Thus, differences in response may reflect variability in the inherent biology of a specific tumor. Common toxicities observed in this study included diarrhea, fatigue, and cytopenias. The authors speculated that inhibition of SYK may have disrupted the nodal microenvironment and led to increased trafficking of CLL cells out of nodal tissues and into the peripheral blood where they would then eventually die.

BAY-61-3606: BAY-61-3606 (Bayer; (2-[7-(3,4-dimethoxypyridyl)-imidazo-[1,2-c][pyrimidin-5-ylamino]-nicotinamide dihydrochloride) is an oral SYK inhibitor which exhibits a variety of actions on mast cells, basophils, B cells, eosinophils, and antigen-presenting cells. The efficacy of BAY-61-3606 on antigen-induced degranulation has been confirmed both in RBL-2H3 cells and freshly isolated rat mast cells [178]. BAY-61-3606 suppressed BCR signaling in a human B cell line, Ramos, and effectively suppressed BCR activation and receptors for the Fc portion of IgG signaling in eosinophils and monocytes. In addition, this agent significantly suppressed the antigen-induced passive cutaneous anaphylactic reaction, bronchoconstriction, and bronchial edema in rats [179].

ER-27319: ER-27319 is a synthetic acridone-related compound that inhibits rodent and human mast cell responses by inhibiting the phosphorylation and activation of SYK [180]. ER-27319 selectively suppresses the interaction of SYK with the FcεRI γ subunit phosphotyrosine and causes the abrogation of degranulation, TNF-α production, and FcεRI γ-mediated up-regulation of VLA-4-mediated basophil adhesion [180]. This agent is potentially useful in the treatment of allergic diseases.

YM 193306: YM 193306 is a 7-azaindole derivative, SYK-selective TKI that is intended for the potential treatment of allergic and autoimmune disorders [151]. YM 193306 blocks antigen-induced
airway inflammation in rats and inhibits the degradation of the RBL-2H3 cell line, which is a commonly used histamine-releasing cell line used in inflammation, allergy, and immunological research.

**PRT-318:** PRT-318, (Portola Pharmaceuticals; (2-((1R,2S)-2-aminoaclohexylamino)-4-(m-tolylamino) pyrimidine-5-carboxamide) has been shown to prevent thrombocytopenia and thrombosis in a mouse model of HIT [181] suggesting that inhibition of SYK by this agent may be a potential strategy to treat human HIT. The effect of PRT-318 was, however, not tested on platelet phosphorylation pathways nor on monocytes. PRT-2607, a more specific SYK inhibitor is currently in a Phase 1 program.

**Substrate-Binding (P-) Site Inhibitors of SYK**

Inhibitors targeting the substrate binding (P-) sites of SYK kinase are desirable for enhanced specificity and potency. The availability of both ATP-binding site inhibitors and P-site inhibitors may be helpful in patients with SYK mutations that might influence inhibitor binding to ATP and/or substrate-binding sites.

**C-61:** The pentapeptide mimic C-61 (1,4-Bis (9-O dihydroquinindyl) phthhalazine/hydroquinidine 1,4-phthalazinediyldiether) is the first inhibitor targeting the protein substrate-binding region (P-Site) of SYK [95] and the first apoptosis-promoting anti-cancer drug candidate in the cinchona alkaloid compound class [93]. C-61 has five individual molecular ring fragments representing the functional analogs of five amino acid residues, and resembles that of a tyrosine (Y)-containing pentapeptide (GDYEMN). C-61 contains the DYE motif most favored by the P-Site of SYK. C-61 was designed as candidate against B-lineage ALL [93] and acts as a potent inducer of apoptosis in chemotherapy-resistant SYK-expressing primary leukemic B-cell precursors taken directly from relapsed B-precurser ALL patients. In vitro, C-61 inhibited SYK at nanomolar concentrations in both cell-free kinase assays using recombinant SYK as well as in cellular kinase assays using the B-lineage ALL cell line NALM-6 [119]. In contrast, even at high micromolar concentrations, C-61 did not inhibit the enzymatic activity of EGF receptor kinase, TEC family tyrosine kinase BTK, SRC family tyrosine kinase HCK, SRC family tyrosine kinase LYN, Janus family tyrosine kinases JAK1, JAK2 and JAK3, or the insulin receptor kinase [93]. Treatment with C-61 at dose levels 10-times lower than those found to be safe and nontoxic in cynomolgous monkeys, was capable of destroying >99.9% of clonogenic B-lineage ALL cells in vivo and thereby improved the event-free survival outcome of SCID mice challenged with otherwise invariably fatal doses of human leukemic B-cell precursors in 3 different xenograft models of chemotherapy-resistant human B-lineage ALL [93]. Further development of C-61 may lead to therapeutic innovation against chemotherapy-resistant malignancies in which SYK contributes to the survival and growth of cancer cells.

**Nanoparticle formulations of SYK inhibitors**

The efficient delivery of the various SYK inhibitors by leveraging nanotechnology holds particular promise. The rationally engineered nanoparticle constructs of SYK inhibitors are likely to be less toxic and more effective than free molecules. Further development of rationally designed SYK inhibitors and their nanoscale formulations may provide the foundation for therapeutic innovation against a broad spectrum of serious human diseases. Liposomal nanoparticle (LNP) therapeutics containing SYK inhibitors may provide the foundation for potentially more effective and less toxic anti-cancer treatment strategies due to their improved pharmacokinetics, reduced systemic toxicity, and increased intra-tumoral/intra-cellular delivery [182-184]. LNPs have been coated with polyethylene glycol (PEG) (i.e., PEGylated) in an attempt to render them resistant against protein adsorption, enhance their biocompatibility, and to stabilize them against agglomeration in biological environments. PEGylated LNPs with diameters around 100-nm may become long-circulating in the blood stream and have been called stealth particles since they can evade recognition by T cells and macrophages and avoid rapid clearance by the immune system [60,184,185]. LNPs that are sterically stabilized by PEG polymers on their surface and have surface charges that are slightly negative or slightly positive have minimal self-self or self-non-self interactions and improved pharmacokinetics. PEGylation of LNPs creates a hydrophilic surface and leads to increased protein solubility, reduced immunogenicity, prolonged plasma half life due to prevention of rapid renal clearance, and reduced clearance by the RES system due to decreased macrophage capture and opsonization [183-185]. The rationally engineered LNP constructs of SYK inhibitors are likely to be less toxic and more effective than free molecules.

LNPs can be functionalized with a tumor targeting moiety such as a ligand or scFv directed against a surface receptor on cancer cells in order to achieve optimal tumor targeting and site-specific drug delivery to further reduce their toxicity and improve their efficacy [186-189]. When linked with tumor targeting moieties LNPs can reach cancer cells carrying the target receptors with high affinity and precision. The targeting ligands enable LNPs to bind to cell surface receptors and enter cells by receptor-mediated endocytosis. In our own program, efforts are underway to prepare LNPs targeted against the CD19 antigen on B-lineage leukemia/lymphoma cells. We postulate that the LNP-enabled delivery of potent SYK inhibitors like C-61 to leukemia cells will require lower systemic exposure levels for therapeutic efficacy and thereby significantly broaden their therapeutic window.

Advantages of nanoparticle-based drug delivery are that it improves the solubility of poorly water-soluble drugs, prolongs the half-life of drug systemic circulation, releases drugs at a sustained rate, reduces the frequency of administration, delivers drugs in a targeted manner to minimize systemic side effect, and enables to deliver two or more drugs simultaneously for combination therapy to generate a synergistic effect and suppress drug resistance [189]. The goal for the SYK inhibitor nanoformulations in development is to minimize the unwanted side effects, while preserving the beneficial profiles. Oral, skin, nasal and pulmonary drug delivery systems require efficient transport of drugs across the epithelium to achieve high systemic bioavailability. Nanocarriers provide a means to overcome cellular and anatomical barriers to drug delivery.

**Future Perspective**

Within the next 3-5 years, we should anticipate having more clinical experience regarding the activity profile and side effects of SYK inhibitors. Most importantly, we will learn about biomarkers that will help identify those patients most likely to benefit from SYK inhibitors. While many of the SYK inhibitors show promise as drug candidates, their use as the payload of therapeutic nanoparticles is likely to further enhance their clinical potential.
Recently SYK has been identified as a dual-specificity kinase that not only phosphorylates tyrosine (Y) but also serine (S) residues [190]. Our recent results provided the first genetic and biochemical evidence for a previously unknown regulatory function of SYK as an activating partner of Ikaros, that phosphorylates Ikaros at novel serine phosphorylation sites S358 and S361, thereby augmenting its nuclear localization and sequence-specific DNA binding activity [191]. Ikaros exhibits a very important tumor suppressor function in lymphocyte precursors, which has been attributed in part to its ability to repress expression of oncogenic genes via chromatin remodeling in association with the SWI/SNF remodeling complex and recruitment of potentially oncogenic proliferation-promoting genes to pericentromeric heterochromatin (PC-HC) [192]. SYK likely plays an important tumor suppressor function during human lymphocyte ontogeny by protecting the lymphoid progenitors from a leukemogenic Casein kinase 2 (CK2)-mediated inhibition of Ikaros function [191]. Furthermore, SYK-induced serine phosphorylation is required for the ability of Ikaros to mediate the differentiation of human B-cell precursors [191]. Indeed, defective SYK expression has been implicated in the pathogenesis of infant pro-B cell acute lymphoblastic leukemia (ALL), which is thought to originate from B-cell precursors with a maturational arrest at the pro-B cell stage and is associated with poor prognosis [193]. This association between SYK deficiency and development of aggressive pro-B cell leukemia in infancy may be caused by a loss of SYK-induced phosphorylation of Ikaros on activating serine residues S358 and S361. Consequently, the use of kinase inhibitors of the conserved ATP binding site within the catalytic domain of SYK, which is required for both its tyrosine kinase activity and serine kinase activity, as are most SYK inhibitors in preclinical or clinical development [194,195] including compound R406 and its pro-drug R788 (Postamatinib disodium/FosD), may contribute to an increased risk of emergence of new leukemogenic clones and progression of leukemia, especially in pediatric leukemia patients who are subjected to DNA damaging agents as part of their multi-modality standard treatment programs [196]. Furthermore, because of the similarities of the ATP pocket structures among different kinases, most of these inhibitors affect multiple tyrosine kinases and have off-target activities [197]. Indeed hypertension, a common and potentially dangerous side effect of FosD, has been attributed to off-target inhibition of VEGFR [195]. Inhibitors targeting the substrate binding sites of tyrosine kinases are hoped to have enhanced specificity and potency [199]. Unlike available inhibitors of SYK targeting the ATP binding site, C-61 targets the tyrosine kinase substrate-binding site of SYK [93,119] and thereby provides a unique opportunity to selectively target the SYK-dependent anti-apoptotic blast cell survival machinery that is controlled by the tyrosine kinase activity of SYK, especially via tyrosine phosphorylation events leading to activation of STAT3 and PI3-Kinase [44]. The selective inhibition of anti- apoptotic tyrosine phosphorylation events by C-61-induced blocking the binding of the substrates of SYK would not cause a malfunction of Ikaros because it spares the ATP site-dependent serine kinase function of SYK.

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Conflict of Interest Statement

The authors have no conflicts of interest to disclose.

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