Identification of ILK as a novel therapeutic target for acute and chronic myeloid leukemia

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Abstract

Current treatment options as well as clinical efficacy are limited for chronic myelogenous leukemia (CML), Ph+ acute lymphoblastic leukemia (ALL), and acute myeloid leukemia (AML). In response to the pressing need for more efficacious treatment approaches and strategies to override drug resistance in advanced stage CML, Ph+ ALL, and AML, we investigated the effects of inhibition of ILK as a potentially novel and effective approach to treatment of these challenging malignancies. Using the small molecule ILK inhibitor, Cpd22, and ILK knockdown, we investigated the importance of ILK in the growth and viability of leukemia. Our results suggest that the ILK inhibition may be an effective treatment for CML, Ph+ ALL, and AML as a single therapy, with ILK expression levels positively correlating with the efficacy of ILK inhibition. The identification of ILK as a novel target for leukemia therapy warrants further investigation as a therapeutic approach that could be of potential clinical benefit in both acute and chronic myeloid leukemias.

Keywords

Chronic myelogenous leukemia; acute myeloid leukemia; ILK inhibitor; Compound 22; targeted therapies

1. INTRODUCTION

The underlying cause of chronic myelogenous leukemia (CML) is the chimeric BCR-ABL protein, which has constitutively activated ABL tyrosine kinase activity and is encoded by the gene product of a reciprocal t(9;22) Philadelphia (Ph) chromosomal translocation [1–3]. For accelerated and blast crisis phase CML and Ph+ acute lymphoblastic leukemia (ALL), the prognosis is poor due to the development of drug resistance; this justifies the need for

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new, more efficacious treatments. Current treatment options as well as clinical efficacy are also limited for acute myeloid leukemia (AML), a disease characterized by a complete or partial block in differentiation and aberrant proliferation of myeloid progenitor cells (reviewed in Messner and Griffin, 1986) [4]. As with advanced-stage CML and Ph+ALL, the identification of novel molecular targets that could lead to development of new, more clinically effective, therapeutic approaches is similarly warranted for AML.

As the expression and activity of the signaling molecule, integrin-linked kinase (ILK), an intracellular protein serine-threonine kinase, are upregulated in several types of cancers, including leukemia [5–7], we were interested in investigating ILK as a potential novel therapeutic target for AML and CML. ILK interacts with the cytoplasmic domains of integrin beta1 and beta3 subunits; its kinase activity is modulated by cell–extracellular matrix (ECM) interactions [8]. ILK appears to be involved in several signaling pathways via the activation of protein kinase B (PKB/Akt) and inhibition of glycogen synthase kinase-3 (GSK-3); it mediates the phosphorylation of a variety of signaling proteins, including Akt at Ser-473, GSK-3 β , and myosin light chain (MLC), and oncogenesis-related events, including suppression of apoptosis and promotion of cell survival, as well as cell migration, invasion, and epithelial-mesenchymal transition [5–7, 9, 10]. Based on these findings, ILK has attracted considerable interest as therapeutic target for the prevention of tumor progression [5, 6, 11].

Compound 22 (Cpd22, N-Methyl-3-(1-(4-(piperazin-1-yl)phenyl)-5-(4'-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)-1H-pyrazol-3-yl)propanamide) is a cell-permeable, tri-substituted pyrazol compound that acts as a potent and targeted ILK inhibitor. Cpd22 has shown potent inhibitor activity against prostate and breast cancer cell lines (IC₅₀, 1 – 2.5 μ M) via autophagy and apoptosis, while normal epithelial cells were unaffected [12]. In the present study, we investigated ILK as a putative novel target for treatment of CML, Ph+ ALL, and AML, and Cpd22 as a potential novel anti-leukemia agent.

2. METHODS

2.1. Chemical compounds and biologic reagents

Imatinib and nilotinib were synthesized by Novartis Pharma AG, Basel, Switzerland. Compound 22 (Cpd22) [12], a tri-substituted pyrazol compound that reportedly inhibits ILK with an IC₅₀ of 600 nM, was purchased from EMD Millipore (Billerica, MA) (Figure 1A). Compounds were initially dissolved in DMSO to make 10 mM stock solutions, and then were serially diluted to obtain final concentrations for in vitro experiments. Propidium iodide (PI), RNAase and MTT solution were purchased from Sigma-Aldrich (Saint Louis, MO), mAbs for Western blotting from Cell Signaling Technologies (Danvers, MA), and Annexin-V/PI assay from BD Biosciences (San Jose, CA).

2.2. Cell lines and cell culture

SUP-B15, KU812F, K562, and THP cells were purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). NOMO-1 and K052 cells were obtained from Dr. Gary Gilliland. LAMA84S and LAMA84R cells were a gift from Dr. Junia Melo. MOLM14

[13] was provided by Dr. Scott Armstrong, Dana Farber Cancer Institute (DFCI), Boston, MA. All cells were cultured at 37°C, 5% CO₂, in RPMI1460 media (Corning CellGro, Mediatech, Manassas, VA) with 10% fetal bovine serum (FBS) and supplemented with 2% L-glutamine and 1% penicillin/streptomycin.

Human Bone Marrow Mononuclear Cells (BM MNCs) were purchased from STEMCELL Technologies (Vancouver, BC, Canada). Cells were cultured at 37°C, 5% CO₂, in RPMI1460 media (Corning CellGro) with 10% FBS and supplemented with 2% L-glutamine and 1% penicillin/streptomycin. Peripheral blood mononuclear cells (PBMCs) were isolated from pheresis leukopaks from the Siteman Cancer Center, Washington University in Saint Louis. 1X RBC Lysis Buffer (BioLegend, San Diego, CA) was added to whole blood, gently vortex and incubated at room temperature, protected from light, for 10–15 minutes. Cells were washed and cultured in RPMI completed media. Informed consent was obtained from all patients with an approval from the Washington University Medical School IRB committee and in accord with the Declaration of Helsinki.

2.3 Primary Patient Samples

All samples from leukemia patients were obtained under approval of the Dana-Farber Cancer Institute Institutional Review Board.

AML patient samples—AML patient 1, male, 67 years old, had 10–20% bone marrow blasts and 21% peripheral blood blasts. Cytogenetics: Heterozygote: Cyto: Some cells with three copies Chromosome 21, some with 4 copies Chromosome 21. Treatment history: 3+7 (ida/cytarabine) X2, clofarabine/cytarabine. AML patient 2, male, 37 years old, had 90% bone marrow blasts and 36% peripheral blood blasts. Cytogenetics: PML-RARA by PCR. Cyto-t(15;17). Treatment history: No prior treatments.

Ph+ ALL patient samples—ALL patient 1, female, 60 years old, had 95% bone marrow blasts and 17% peripheral blood blasts. Cytogenetics: Ph+ ALL (hyperdiploidy). Treatment history: Diagnosed with metastatic hormone receptor positive breast cancer (lymph node positive), and thus for prior therapy had lumpectomy followed by cyclophosphamide, methotrexate, and 5-FU, and then tamoxifen, Lupron, before switching to anastrozole and then to exemestane before changing to fulvestrant. No anti-leukemic therapy prior to this. ALL patient 2, male, 40 years old, had 95% bone marrow blasts and 95% peripheral blood blasts. Cytogenetics: Ph+ ALL (hyperdiploidy, der22t(9;22). Treatment history: No prior therapy. ALL patient 3, male, 60 years old, had 95% bone marrow blasts and 78% peripheral blood blasts. Cytogenetics: Ph+ ALL (Ph+ (9;22). 1st clone had Trisomy 28, Trisomy 21. 2nd clone had Ph+ tetrasomy 8, Trisomy 6. Treatment history: ALK negative advanced anaplastic T cell Non-Hodgkins lymphoma treated with CHOP followed by DHAP (dexamethasone, high dose Ara-c and platinum) given 6 years prior to presentation.

CML patient samples—Samples were obtained from patients with newly diagnosed CML. Samples were de-identified and no additional clinical information was available.

2.4. Expression of ILK

The expression of ILK in CML, AML, and Ph+ ALL cell lines, primary patient cells, and BM MNCs was analyzed. Cells were retrieved from cultures by pipetting, washed, fixed and permeabilized (BD Cytofix/Cytoperm), incubated with mouse anti-ILK antibody (Clone: 3A9, dilution 1:200, Thermo Scientific) for 1 hour at room temperature, then incubated with AlexaFluor488-goat-anti-mouse IgG (H+L) (dilution 1:2000, Invitrogen) 30 minutes at room temperature, and analyzed by flow cytometry. MFI of AlexaFluor488 was detected and normalized to MFI of BM MNCs. As a control, secondary antibody incubation (AlexaFluor488 mouse IgG1, BD Pharmingen, BD Biosciences) was included.

2.5. Cell viability assays

CML cell lines (K562, Ku812F, LAMA84S and LAMA84R) and PBMCs were cultured with ILK inhibitor, Cpd22 (0 – 1000 nM) for 3 days. Toxicity of Cpd22 (0 – 500 nM) toward BM MNCs and primary patient cells (CML, ALL, and AML) was assessed. Cell viability assay was assessed by MTT assay as previously described [14]. Briefly, the MTT solution was added to the cells at day 3 after starting the treatment, and 2 – 4 hours later the stop solution was added and the absorption in wells was read at 570 nm. IC50 values were calculated based on polynomial trend-line for each cell line.

The Trypan Blue exclusion assay has been previously described [15] and was used for quantification of cells for seeding for the CellTiter Glo assay (Promega, Madison, WI), which was used for synergy testing and carried out according to manufacturer instructions. Cell viability is reported as percentage of control (untreated) cells, and error bars represent the standard error of the mean for each data point.

2.6. Cell cycle analysis

K562 cells (1×10^6 cell/ml) were cultured with ILK inhibitor (0, 400 nM) for 3 days as previously described [16]. Briefly, cells were fixed with 70% ethanol on ice 30 minutes and washed, RNA was degraded by 10 µg/ml RNAase incubation for 30 minutes at 37°C, then the DNA was stained with 5 µg/ml PI solution for 10 minutes, and cells were analyzed by flow cytometry.

2.7. Apoptosis assay

K562 cells (1×10^6 cell/ml) were cultured with ILK inhibitor (0, 400 nM) for 3 days as previously described [14]. Cells were suspended in 1X Annexin-binding buffer, incubated with Annexin-V for 30 minutes and with PI for 15 minutes on ice, and then analyzed with flow cytometry.

2.8. Analysis of proteins relevant to Cpd22 target specificity, effects on cell cycle progression, and effects on apoptosis

To test the effect of the ILK inhibitor, Cpd22, on proliferation, cell cycle progression, and apoptosis signaling, K562 cells were treated with ILK inhibitor (0, 400 nM) for 3 days. Cells were washed and lysed with 1X PMSF for 15 minutes. Protein concentration in the cell lysates was assessed by Quick Start™ Bradford dye reagent (BioRad, Hercules, CA),

normalized and 50 µg of protein was loaded per lane. Electrophoresis was performed using NuPAGE 4% – 12% Bis-Tris gels (Novex, Life Technologies, Grand Island, NY) and transferred to a nitrocellulose membrane using iBlot (Invitrogen, Life Technologies). Membranes were blocked with 5% milk in Tris-Buffered Saline/Tween20 (TBST) buffer and incubated with primary antibodies overnight at 4°C for validation of target specificity of Cpd22 with phospho-AKT and phospho-GSK-3, for cell cycle analysis with phospho-retinoblastoma tumor suppressor protein Rb (pRb) and p27 Kip1, and for apoptosis analysis with cleaved-Caspase-3, cleaved-Caspase-8, cleaved-Caspase-9, and cleaved-PARP. α-Tubulin was used as loading control. The membranes were washed with TBST for 30 minutes, incubated for 1 hour at room temperature with HRP-conjugated secondary antibody, washed, and developed using Novex ECL Chemiluminescent Substrate Reagent Kit (Invitrogen).

Antibodies were purchased from Cell Signaling Technology (Danvers, MA). Phospho-AKT (S473) (D9#) (rabbit mAb, #4060), Phospho-GSK-3 (Ser9) (D85E12) (rabbit mAb #5558), pRb (Ser807/811) (rabbit mAb #9308), p27 Kip1 (D69C12) (rabbit mAb #3686), cleaved-Caspase-3 (Asp175) (5A1E) (rabbit mAb #9664), cleaved-Caspase-8 (Asp391) (rabbit mAb #9496), cleaved-Caspase-9 (Asp330) (D2D4) (rabbit mAb #7237), cleaved-PARP (Asp214) (D64E10) (rabbit mAb #5625), and α-Tubulin (11H10) (rabbit mAb #2125) were used at a dilution of 1:1000.

2.9. Knockdown (KD) of genes by shRNA

pLKO.1puro lentiviral shRNA vector particles against ILK were purchased from Sigma-Aldrich (St. Louis, MO). Cells were incubated with the viral particles in the presence of 8 µg/ml Polybrene for 24 hours, and the cells were selected with 1–2 µg/ml puromycin for 72 hours. Following selection, cells were used for the studies described.

The sequences of shRNA are follows:

Control GFP: ACAACAGCCACAACGTCTATA

ILK shRNA hp2:

CCGGGCAATGACATTGTCGTGAAGGCTCGAGCCTTCACGACAATGTC
ATTGCTTTTTG

ILK shRNA hp4:

CCGGTCAGAGCTTTGTCACTTGCCACTCGAGTGGCAAGTGACAAAGC
TCTGATTTTTG

ILK shRNA hp5:

CCGGCGACCCAAATTTGACATGATTCTCGAGAATCATGTCAAATTTG
GGTCGTTTTTG

While KD of ILK inhibited the growth of leukemia cells, as compared to control shRNA, it did not inhibit growth to the extent of prohibiting validation of gene knock down by western analysis. Protein lysate preparation and immunoblotting for validation of knockdown efficiency were carried out as previously described [15]. Antibodies were purchased from Cell Signaling Technology (Danvers, MA). The ILK1 antibody (rabbit, 3862S) was used at a

dilution of 1:200. Phospho-Akt (Ser473) (D9E) (rabbit, 4060) and phospho-GSK-3 α/β (Ser21/9) (rabbit, 9331) were used at a dilution of 1:500. The GAPDH (14C10) antibody (rabbit mAb, 2118S) was used at a dilution of 1:1000.

2.10. Statistical analysis

Experiments were performed in triplicates. Results were analyzed using student *t*-test for statistical significance, and were considered significantly different for *p* value less than 0.05.

3. RESULTS

3.1. Compound 22 does not affect the growth and viability of BM MNCs and PBMCs

Integrin-linked kinase (ILK) is usually upregulated in several types of cancers; we therefore investigated ILK expression due to its potential as a novel therapeutic target for CML, AML, and ALL. ILK expression in normal BM MNCs was compared to primary CML patient cells. The highest levels of ILK expression were found in the primary CML samples while normal BM MNCs expressed ILK at a lower level, as evidenced by a clear shift to the right in the ILK expression histogram (Figure 1Bi). The ILK inhibitor, Cpd22, was tested on normal bone marrow mononuclear cells (BM MNCs) as well as normal peripheral blood mononuclear cells (PBMCs). Cpd22 did not kill normal BM MNCs (Figure 1Bii), or normal PBMCs (Figure 1Biii).

3.2. Compound 22 inhibits CML proliferation

ILK expression was investigated in human CML cell lines, K562, KU812F, LAMA84S (imatinib-sensitive), and LAMA84R (imatinib-resistant). Flow cytometry histograms showed that all CML cell lines expressed ILK (Figure 2Ai). Cpd22 inhibited the proliferation of the human CML cell lines tested at concentrations that did not kill normal BM MNCs and PBMCs (Figure 2Aii). The relative sensitivity of LAMA84S versus LAMA84R to imatinib is shown in Figure 2Aiii. Representative flow cytometry histogram showed that LAMA84R had increased ILK expression compared to LAMA84S (Figure 2Aiv). Furthermore, we validated the target specificity of Cpd22 (400 nM, three days) in CML cells by immuno-blotting and investigating inhibitor effects on signaling molecules downstream of ILK. Cpd22 potently suppressed the phosphorylation levels of Ser-473-Akt (pAKT) and another ILK substrate, (pGSK-3) (Figure 2Av). ILK expression was also investigated in three primary CML patient cells. These cells showed a 10-fold increase in ILK expression as compared to BM MNCs (Figure 2Bi). The effect of Cpd22 on proliferation was studied in the same primary CML patient cells. Cpd22 inhibited the proliferation of the human CML patient cells in the same manner (Figure 2Bii).

3.2. Compound 22 inhibits cell cycle progression of CML cells and induces apoptosis

In an attempt to better understand the mechanism underlying Cpd22 inhibition of leukemia cell proliferation, we examined the effects of ILK inhibition on K562 cell cycle progression. We observed cell cycle arrest in Cpd22-treated K562 cells, specifically accumulation of apoptotic cells in subG1 and a decrease in G2/M with no change in S-phase following 3 days of drug treatment (Figure 3Ai). Cell cycle histograms revealed an important increase in subG1 and decrease in G1 (Figure 3Aii), which was associated with down-regulation of the

expression of proteins involved in cell cycle transition from G1 to S phase, such as pRb, while the cell cycle inhibitor p27 was increased (Figure 3B).

Consistent with the observed increase in the subG1 fraction in the cell cycle studies, Annexin/PI staining showed a 3-fold increase in the fraction of apoptotic cells staining positive for Annexin and PI in Cpd22 (400 nM)-treated cells (Figure 4Ai–ii). Apoptosis was confirmed by immuno-blotting: While the extrinsic (death receptor mediated) pathway was not affected (there was no expression of cleaved caspase 8), caspase activation through the intrinsic (mitochondrial mediated) pathway was detected by increased cleaved caspase 3 and 9, in addition to increase PARP cleavage (Figure 4B).

3.3. Cpd22 inhibits Ph+ ALL proliferation

ILK expression was tested on the human Ph+ ALL cell line SUP-B15. A flow cytometry histogram showed ILK expression on SUP-B15 (Figure 5Ai). Cpd22 inhibited the proliferation of the human ALL cell lines at a concentration (500 nM) that did not kill normal BM MNCs and PBMCs (Figure 5Aii). ILK expression was then investigated in three primary ALL patient cells, which showed a 5 to 13-fold increase in ILK expression as compared to BM MNCs (Figure 5Bi). The effect of Cpd22 on proliferation was then studied in the same primary ALL patient cells; Cpd22 inhibited the proliferation of the three human ALL patient cells: Specifically, the higher the ILK expression the more sensitive to Cpd22 the patients cells were (Figure 5Bii).

Interestingly, Cpd22 was observed to potentiate the inhibitory effects of both imatinib and nilotinib against SUP-B15 cells (Supplementary Figure 1). Specifically, the combination of either imatinib or nilotinib across a range of concentrations with Cpd22 (held steady at 500 nM) led to more SUP-B15 cell killing than Cpd22 (500 nM) alone, which killed less than 40% of cells. The drug combination was also more effective than either imatinib or nilotinib alone across a range of concentrations (as evidenced by a downward shift in the dose-response curve), with the majority of cells killed at higher concentrations combined. These data suggest that ILK inhibition may potentiate the effects of BCR-ABL-targeted tyrosine kinase inhibition in Ph+ ALL.

3.4. Compound 22 decreases AML proliferation

In addition to CML and ALL, ILK expression was also investigated in human AML cell lines, K052, NOMO-1, THP, and MOLM14. Flow cytometry histogram showed that all AML cell lines expressed ILK (Figure 6Ai). Cpd22 inhibited proliferation of AML cell lines with an IC50 lower than 1000 nM (Figure 6Aii).

Furthermore, ILK expression was examined in two primary AML patient cells. These samples showed a 5 to 20-fold increase in ILK expression as compared to BM MNCs (Figure 6Bi). Cpd22 was observed to inhibit the proliferation of the two human AML patient samples: Specifically, the higher the ILK expression, the more sensitive to Cpd22 the patients cells were (Figure 6Bii).

3.5. Knockdown of ILK in human CML and AML cell lines leads to inhibition of cell proliferation

The importance of ILK for the growth of leukemia cells was demonstrated by knockdown of ILK in KU812F and K562 cells, which led to decreases in growth (Figure 7A–C). Similarly, knockdown of ILK in human AML cell lines, THP and NOMO-1, also resulted in decreases in cell proliferation (Figure 7D–F). pAkt and pGSK-3 in ILK KD cells of THP cell lines is shown in Figure 7G.

4. DISCUSSION

In response to the pressing need for more efficacious treatment approaches and strategies to override TKI resistance in advanced stage CML, Ph+ ALL, and AML, we investigated the effects of inhibition of ILK as a potentially novel and effective approach to treatment of these challenging malignancies. ILK is an intracellular adaptor and kinase that links the integrins, cell-adhesion receptors, and growth factors to a range of signaling pathways [8]. It has been shown that inhibition of ILK expression and activity is anti-tumorigenic, which makes ILK an attractive target for cancer therapeutics [6, 9, 11].

ILK expression was initially evaluated in normal BM MNCs and CML, AML, and ALL primary patient cells and cell lines. Subsequently, the effects of the ILK inhibitor, Cpd22, in all cells were assessed. Normal BM MNCs showed ILK expression, but expression was lower than in cell lines and primary samples from CML, AML, and ALL. Cpd22 inhibited the proliferation of CML cell lines with an IC₅₀ of approximately 235 – 500 nM, while none of three normal PBMC and three normal BM MNCs controls were affected by the same concentration range after three days of treatment with Cpd22 as a single agent. Interestingly, Cdp22 was more potent against imatinib-resistant LAMA84R cells than imatinib –sensitive LAMA84S cells, which could be explained by a higher expression of ILK in the imatinib-resistant LAMA84R cells. ILK expression in primary ALL patient cells correlated with the efficacy of Cpd22: Specifically, the higher the ILK expression, the more sensitivity to Cpd22. ILK expression in primary AML patient cells also correlated with efficacy of Cpd22: Specifically, the higher the ILK expression, the more sensitivity to Cpd22.

ILK phosphorylates Akt on Ser473, which, together with Thr308 phosphorylation by phosphoinositide-dependent kinase (PDK)1, activates Akt. Both ILK and Akt phosphorylate and inhibit the activity of GSK-3 β , which results in activation of β -catenin/LEF-1 and AP-1 transcription factors. AKT also regulates signaling pathways that activate NF- κ B, leading to suppression of apoptosis [5, 7, 17]. Therefore, ILK promotes cell survival by inhibiting GSK-3 β and inhibits apoptosis by activating AKT. Specific small-molecule inhibitors of ILK could inhibit these activities. In accordance, the target specificity of Cpd22 was validated in CML cells by demonstration of potent inhibition of the aforementioned ILK substrates; Cpd22 induced Ser-473-Akt dephosphorylation, which was accompanied by a parallel decrease in the phosphorylation levels of GSK3 β , a downstream target of ILK. It has previously been shown that the antiproliferative effects of Cpd22 are, at least in part, attributable to inactivation of Akt signaling (Ser-473-Akt dephosphorylation) [12]. This suggests that Cpd22 could potentially serve as a therapeutic target in CML. This is in

contrast to findings suggesting that ILK is dispensable for survival of other hematopoietic malignancies, such as multiple myeloma [18].

We found that Cpd22 induces apoptosis in CML cells. This is consistent with the demonstrated ability of Cpd22 to induce apoptosis in prostate cancer [7, 11]. Immunoblotting confirmed cell cycle arrest by decreased pRb and increased cell cycle inhibitor p27; immunoblotting also confirmed drug induction of apoptosis through the caspase intrinsic pathway by demonstrating increased cleavage of caspase-3 and caspase-9. Our data show that inhibition of ILK in CML cells affects cell cycle and apoptosis, implying that inhibition of ILK may provide a novel strategy of inhibiting the growth and progression of CML.

The importance of ILK for the growth of leukemia cells was demonstrated by knockdown of ILK in CML and AML cell lines, which led to decreases in cell proliferation. These results suggest a partial dependency of acute and chronic myeloid leukemia cells on ILK for growth, and are consistent with results obtained with the ILK inhibitor, Cpd22. Complete or almost complete (robust) inhibition of ILK expression by three hairpins in THP cell lines correlated with suppression of cell growth to varying degrees (with ILK hp2 growth suppression consistently being the most pronounced, and ILK hp4 and hp5 growth suppression consistently apparent but comparatively more modest).

In conclusion, our findings suggest that the ILK inhibition may be an effective treatment for BCR-ABL-positive leukemia and AML as a single therapy. In addition, ILK inhibition could potentially potentiate the effects of TKIs in Ph⁺ leukemia. The identification of ILK as a novel target for leukemia therapy warrants further investigation as a therapeutic approach that could be of potential clinical benefit in both acute and chronic myeloid leukemias.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. ILK inhibition does not affect normal mononuclear cells

A) Chemical structure of Cpd22. **B) i)** Representative flow cytometry histogram showing ILK expression in BM MNCs and CML primary cells. BM MNCs (dashed line shaded gray), primary CML patient cells (black line shaded gray) ILK expression and fluorescence of matched secondary antibody control-stained cells (black line shaded white) are shown. **ii)** The effect of Cpd22 (0, 500 nM) for three days on proliferation of normal BM MNCs analyzed by MTT. **iii)** The effect of Cpd22 (0 – 1000 nM) for three days on proliferation of normal PBMCs.

Figure 2. ILK inhibitor inhibits CML cell proliferation

A) i) Representative flow cytometry histogram showing ILK expression in CML cells lines, K562, KU812, LAMA84S and LAMA84R. CML cell line (black line shaded gray) ILK expression and fluorescence of matched secondary antibody control-stained cells (black line shaded white) are shown. ii) The effect of Cpd22 (0 – 1000 nM) for three days on proliferation of K562, KU812, LAMA84S and LAMA84R cells analyzed by MTT. iii) Approximately three-day treatment of LAMA84S and LAMA84R cells with imatinib. IC50 values of imatinib in both cell lines are shown. iv) Representative flow cytometry histogram

showing ILK expression on LAMA84S and LAMA84R. LAMA84R (dotted black line shaded gray) and LAMA84S (dotted gray line shaded white) ILK expression are shown. v) The effect of approximately three day treatment with Cpd22 (0, 400 nM) on pAKT and pGSK-3 in K562 cells as determined by immunoblotting. **B**) i) ILK expression in three primary CML patient cells. MFI of anti-ILK-AF488 is shown as fold of base level of BM MNCs. ii) The effect of Cpd22 (0, 500 nM) for three days on proliferation of the three primary CML patient cells analyzed by MTT. *p<0.05.

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Figure 3. Cpd22 treatment of CML cells leads to cell cycle arrest

A) The effect of Cpd22 (0, 400 nM) for approximately 3 days on i) cell cycle progression of K562 cells, shown as a bar graph. ii) Flow cytometry cell cycle graphs. **B)** The effect of approximately 3 day treatment with Cpd22 (0, 400 nM) on cell cycle –associated molecules, pRb and p27, in K562 cells, measured by immunoblotting.

Figure 4. Cpd22 treatment of CML cells leads to induction of apoptosis and autophagy

A) i) The effect of Cpd22 (0, 400 nM) for 3 days on apoptosis of K562 cells, shown as a bar graph. **ii)** The effect of Cpd22 (0, 400 nM) for 3 days on apoptosis of K562 cells, shown as flow cytometric apoptosis dot plots. * $p < 0.05$. **B)** The effect of Cpd22 treatment (0, 400 nM) for approximately three days on protein levels of cleaved caspase 3, cleaved caspase 8, cleaved caspase 9, and cleaved PARP in K562 cells.

Figure 5. Cpd22 inhibits Ph+ ALL proliferation

A) i) Representative flow cytometry histogram showing ILK expression in Ph+ ALL cell line, SUP-B15. SUP-B15 (black line shaded gray) ILK expression and fluorescence of matched secondary antibody control-stained cells (black line shaded white) are shown. ii) The effect of Cpd22 (0, 500 nM) for three days on proliferation of SUP-B15 cells analyzed by MTT. * $p < 0.05$. **B**) i) ILK expression in three primary ALL patient cells. MFI of anti-ILK-AF488 is shown as fold of base level of BM MNCs. ii) The effect of Cpd22 (0, 500 nM) for three days on proliferation of the three primary ALL patient cells analyzed by MTT.

Figure 6. Treatment of AML cells with Cpd22 leads to inhibition of cell proliferation

A i) Representative flow cytometry histogram showing ILK expression in AML cell lines, K052, NOMO-1, THP, and MOLM14. AML cell line (black line shaded gray) ILK expression and fluorescence of matched secondary antibody control-stained cells (black line shaded white) are shown. ii) Approximately 3-day treatment of human AML cell lines with Cpd22. **B** i) ILK expression in two primary AML patient cells. MFI of anti-ILK-AF488 is shown as fold of base level of BM MNCs. ii) The effect of Cpd22 (0, 500 nM) for 3 days on proliferation of the two primary AML patient cells analyzed by MTT. * $p < 0.05$.

Figure 7. Knockdown of ILK in human CML and AML cell lines leads to inhibition of cell proliferation

(A–B) Approximately 3-day proliferation of human CML cell lines, K562 and KU812F, or (D–E) human AML cell lines, THP and NOMO-1, incubated with pLKO.1puro lentiviral shRNA vector particles against ILK. “SCR” refers to cells incubated with pLKO.1 lentiviral shRNA vector particles containing a scrambled sequence as a control. Hairpins “2,” “4” and “5” were used for knockdown experiments. (C, F) Analysis of ILK protein expression as a measure of knockdown efficiency. (G) pAkt and pGSK-3 in ILK KD cells.