Anti-leukemic Activity of the TYK2 Selective Inhibitor NDI-031301 in T-cell Acute Lymphoblastic Leukemia

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ABSTRACT

We previously found that activation of tyrosine kinase 2 (TYK2) contributes to the aberrant survival of T-cell acute lymphoblastic leukemia (T-ALL) cells (Sanda *et al. Cancer Discov* 2013), suggesting that molecular therapies targeting TYK2 would be a promising strategy for treatment of T-ALL. In this study, we investigated the therapeutic potential of a novel TYK2 kinase inhibitor NDI-031301 in T-ALL. NDI-031301 showed potent and selective inhibitory activity against TYK2 in a cellular context, because this compound strongly inhibited the growth of TYK2-transfomed Ba/F3 cells whereas Ba/F3 cells transformed by other tyrosine kinases showed decreased sensitivity. NDI-031301 induced growth inhibition and apoptosis in multiple human T-ALL cell lines. We found that treatment with 3 μM of NDI-031301 resulted in reduction of STAT1 Tyr-701 phosphorylation and BCL2 levels in KOPT-K1 T-ALL cell line, consistent with our previous finding that TYK2 phosphorylates STAT1 and upregulates BCL2 expression in most T-ALL cells. Surprisingly, the treatment also uniquely led to activation of three mitogen-activated protein kinases (MAPKs), resulting in phosphorylation of ERK, SAPK/JNK and p38 MAPK coincident with PARP cleavage, which was not observed with the JAK selective inhibitors tofacitinib and baricitinib. Activation of p38 MAPK occurred within 1 h of NDI-031301 treatment and was responsible for NDI-031301-induced T-ALL cell death, because pharmacologic inhibition of p38 MAPK by SB203580 partially rescued apoptosis induced by TYK2 inhibitor. Finally, daily oral administration of NDI-031301 at 100mg/kg BID to immunodeficient mice engrafted with KOPT-K1 cells was well tolerated, and led to decreased tumor burden and a significant survival benefit. Thus, our findings clearly support TYK2 inhibition with NDI-031301 or a related compound as a potential therapeutic strategy for patients with T-ALL, and also raise the possibility that enhancing p38 MAPK activation in T-ALL cells may be an approach to accentuate its anti-leukemic activity.

OBJECTIVES

To analyze the antitumor potency of a novel TYK2 kinase inhibitor NDI-031301 against T-ALL and elucidate the mechanisms through which this compound induces apoptosis in the cells.

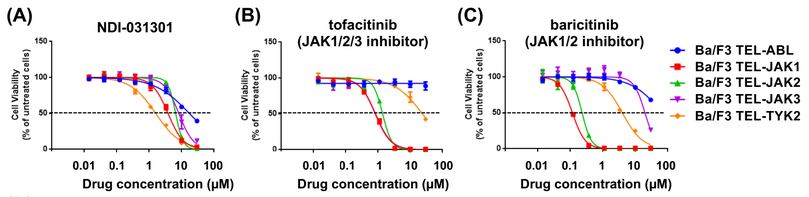
MATERIALS

- ✓ **Cells**
  Ba/F3 cells transformed by each of constitutively active JAK family kinases (TEL-JAK1, TEL-JAK2, TEL-JAK3 and TEL-TYK2) or by an alternative tyrosine kinase, TEL-ABL

  4 Human T-ALL cell lines
  DU.528, KOPT-K1, HPB-ALL and SKW-3
- ✓ **Reagents**
  NDI-031301 is a novel TYK2 kinase inhibitor from Nimbus Therapeutics. Tofacitinib and baricitinib are JAK kinase inhibitors under current clinical trials.

RESULTS

NDI-031301 is a potent and selective inhibitor of TYK2 tyrosine kinase

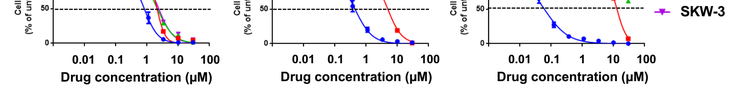


(D)

Assay IC_{50}	NDI-031301 (μM)	tofacitinib (μM)	baricitinib (μM)
Ba/F3 TEL-ABL	17.4	No response	67.36
Ba/F3 TEL-JAK1	3.809	0.8223	0.1157
Ba/F3 TEL-JAK2	6.571	1.386	0.23
Ba/F3 TEL-JAK3	8.21	0.8372	19.87
Ba/F3 TEL-TYK2	1.583	23.89	3.98

Fig 1. Anti-proliferative activity of NDI-031301 **(A)**, tofacitinib **(B)** or baricitinib **(C)** on transformed Ba/F3 cells. The indicated Ba/F3 derivatives were cultured with graded concentrations of each inhibitor for 72 h. Cell viability values were assessed with Cell Titer Glo assay. **(D)** IC_{50} values for NDI-031301, tofacitinib and baricitinib in Fig 1A-C.

NDI-031301 induces growth inhibition and apoptosis in human T-ALL cell lines



(D)

Assay IC_{50}	NDI-031301 (μM)	tofacitinib (μM)	baricitinib (μM)
DU.528	0.8186	0.4414	0.06095
KOPT-K1	2.092	4.964	12.99
HPB-ALL	2.143	79.69	32.91
SKW-3	2.38	No response	32.81

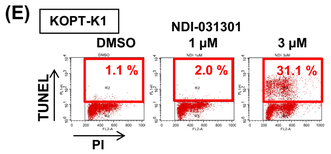


Fig 2. Anti-proliferative activity of NDI-031301 **(A)**, tofacitinib **(B)**, or baricitinib **(C)** on human T-ALL cell lines. The cells were cultured with graded concentrations of each inhibitor for 72 h. Cell viability values were assessed with Cell Titer Glo assay. **(D)** IC_{50} values in Fig 2A-C. **(E)** KOPT-K1 cells were cultured in 0, 1 μM, or 3 μM of NDI-031301 for 48 h. Cells were fixed, and assessed for apoptosis and cell-cycle distribution by flow cytometric analysis after TUNEL / PI double labeling. The panels show two-dimensional dot plots with the percentage of TUNEL-positive cells in each sample.

TYK2 inhibition leads to activation of the MAPK signaling pathways as well as downregulation of phospho-STAT1 in T-ALL cells

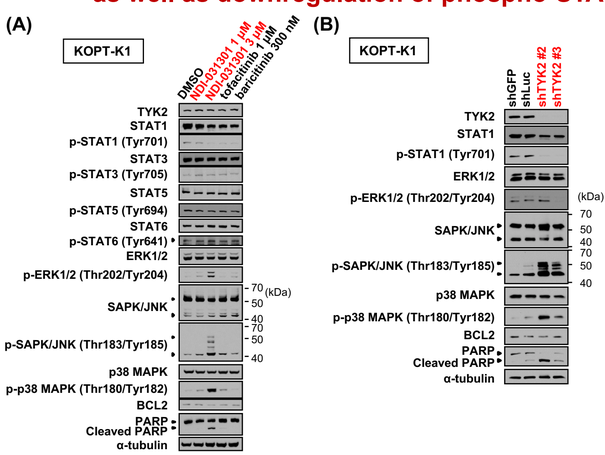


Fig 3. (A) KOPT-K1 cells were treated with the indicated concentrations of NDI-031301, tofacitinib or baricitinib for 24 h, and subjected to immunoblot analysis with each specified antibody. **(B)** KOPT-K1 cells were lentivirally transduced with *TYK2*-targeting shRNAs (shTYK2 #2 and #3) or control shRNAs targeting GFP (shGFP) or luciferase (shLuc). Whole-cell extracts were analyzed by immunoblotting with each specified antibody.

Activation of p38 MAPK is involved in NDI-031301-induced apoptosis in T-ALL cells

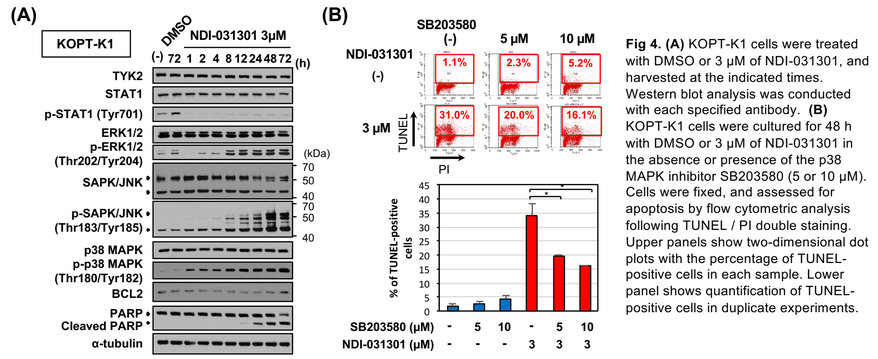
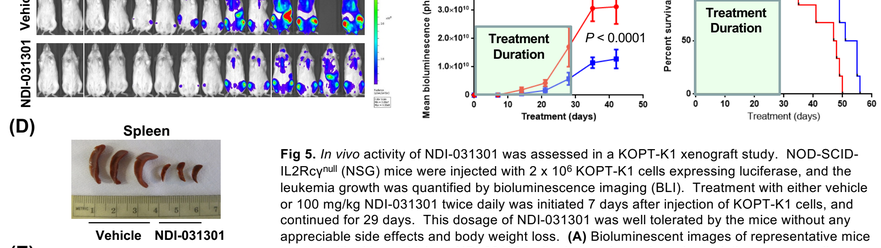


Fig 4. (A) KOPT-K1 cells were treated with DMSO or 3 μM of NDI-031301, and harvested at the indicated times. Western blot analysis was conducted with each specified antibody. **(B)** KOPT-K1 cells were cultured for 48 h with DMSO or 3 μM of NDI-031301 in the absence or presence of the p38 MAPK inhibitor SB203580 (5 or 10 μM). Cells were fixed, and assessed for apoptosis by flow cytometric analysis following TUNEL / PI double staining. Upper panels show two-dimensional dot plots with the percentage of TUNEL-positive cells in each sample. Lower panel shows quantification of TUNEL-positive cells in duplicate experiments.

NDI-031301 suppresses the proliferation of T-ALL cells *in vivo*



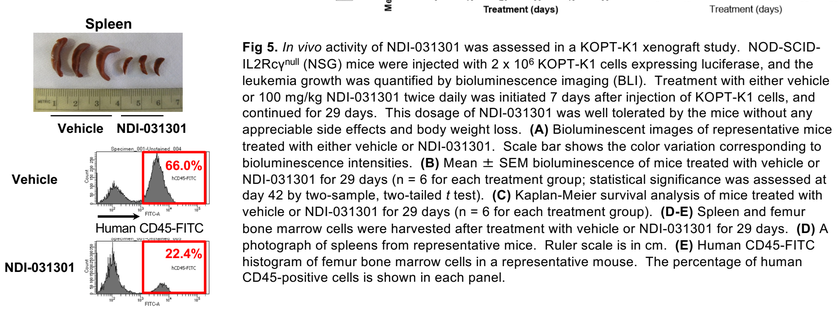


Fig 5. *In vivo* activity of NDI-031301 was assessed in a KOPT-K1 xenograft study. NOD-SCID-IL2Rcγ^{null} (NSG) mice were injected with 2 x 10^6 KOPT-K1 cells expressing luciferase, and the leukemia growth was quantified by bioluminescence imaging (BLI). Treatment with either vehicle or 100 mg/kg NDI-031301 twice daily was initiated 7 days after injection of KOPT-K1 cells, and continued for 29 days. This dosage of NDI-031301 was well tolerated by the mice without any appreciable side effects and body weight loss. **(A)** Bioluminescent images of representative mice treated with either vehicle or NDI-031301. Scale bar shows the color variation corresponding to bioluminescence intensities. **(B)** Mean ± SEM bioluminescence of mice treated with vehicle or NDI-031301 for 29 days (n = 6 for each treatment group; statistical significance was assessed at day 42 by two-sample, two-tailed *t* test). **(C)** Kaplan-Meier survival analysis of mice treated with vehicle or NDI-031301 for 29 days (n = 6 for each treatment group). **(D-E)** Spleen and femur bone marrow cells were harvested after treatment with vehicle or NDI-031301 for 29 days. **(D)** A photograph of spleens from representative mice. Ruler scale is in cm. **(E)** Human CD45-FITC histogram of femur bone marrow cells in a representative mouse. The percentage of human CD45-positive cells is shown in each panel.

CONCLUSIONS

1. **A novel TYK2 kinase inhibitor NDI-031301 induced cytotoxicity in human T-ALL cell lines tested, consistent with our previous result showing the growth and survival inhibition of these cells by silencing *TYK2* with shRNAs. The anti-leukemic activity of NDI-031301 was recapitulated in a KOPT-K1 xenograft study, indicating that TYK2 inhibitor is able to efficiently suppress the growth of human T-ALL cells *in vivo* as well as *in vitro*.**
2. **TYK2 inhibition by NDI-031301 led not only to suppression of STAT1 phosphorylation, but also activation of MAPK signaling pathways in KOPT-K1 T-ALL cells.**
3. **Pharmacologic inhibition of p38 MAPK partially rescued apoptosis induced by NDI-031301, indicating involvement of the p38 MAPK pathway in TYK2-mediated survival of T-ALL cells.**

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Conflict of interest disclosure: C.E.M., W.M., J.R. and R.K. are employees of Nimbus Therapeutics, and receive compensation and hold equity in the company. The remaining authors declare no conflict of interest.