# Deletion of the protein tyrosine phosphatase gene *PTPN2* in T-cell acute lymphoblastic leukemia

**Maria Kleppe**[1,2], **Idoya Lahortiga**[1,2], **Tiama El Chaar**[3], **Kim De Keersmaecker**[1,2,4], **Nicole Mentens**[1,2], **Carlos Graux**[5], **Katrien Van Roosbroeck**[1,2], **Adolfo A. Ferrando**[4,6,7], **Anton W. Langerak**[8], **Jules P.P. Meijerink**[9], **François Sigaux**[3], **Torsten Haferlach**[10], **Iwona Wlodarska**[2], **Peter Vandenberghe**[2], **Jean Soulier**[3,11], and **Jan Cools**[1,2,11]

[1]Department of Molecular and Developmental Genetics, VIB, Leuven, Belgium. [2]Center for Human Genetics, K.U.Leuven, Leuven, Belgium. [3]INSERM U944 and Hematology Laboratory, Hôpital Saint-Louis, IUH, Paris, France. [4]Institute for Cancer Genetics, Columbia University Medical Center, New York, NY, USA. [5]Cliniques universitaires de Mont-Godinne, Yvoir, Belgium. [6]Department of Pathology, Columbia University Medical Center, New York, NY, USA. [7]Department of Pediatrics, Columbia University Medical Center, New York, NY, USA. [8]Department of Immunology, Erasmus MC, Rotterdam, The Netherlands. [9]Department of Pediatrics, Erasmus MC – Sophia, Rotterdam, The Netherlands. [10]Munich Leukemia Laboratory, Munich, Germany.

## Abstract

PTPN2 (protein tyrosine phosphatase non-receptor type 2, also known as TC-PTP) is a cytosolic tyrosine phosphatase that functions as a negative regulator of a variety of tyrosine kinases and other signaling proteins.[1−3] In agreement with its role in the regulation of the immune system, *PTPN2* was identified as a susceptibility locus for autoimmune diseases.[4,5] In this work, we describe the identification of focal deletions of *PTPN2* in human T-cell acute lymphoblastic leukemia (T-ALL). Deletion of *PTPN2* was specifically found in T-ALLs with aberrant expression of the *TLX1* transcription factor oncogene,[6] including four cases also expressing the NUP214-ABL1 tyrosine kinase.[7] Knockdown of PTPN2 expression increased the proliferation and cytokine sensitivity of T-ALL cells. In addition, PTPN2 was identified as a negative regulator of NUP214-ABL1 kinase activity. Our study provides genetic and functional evidence for a tumor suppressor role of *PTPN2*, and suggests that expression levels of PTPN2 may modulate response to treatment.

## Keywords

tumor suppressor gene; cancer; phosphorylation

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T-ALL is characterized by leukemic transformation of thymocytes caused by cooperative events altering proliferation, survival, cell cycle and differentiation of T-cells.[8,9] Despite major improvements in our understanding of the molecular genetics of T-ALL, the mechanisms that lead to the abnormal proliferation and/or survival of T-lymphoblasts remain largely unknown. [10] In order to identify novel oncogenes or tumor suppressor genes involved in the development

of T-ALL, we performed microarray comparative genomic hybridization (array CGH) analysis using high density oligonucleotide arrays.[11] In one individual we detected an acquired homozygous microdeletion at chromosome 18p11 (Fig. 1a, Supplementary Fig. 1 online). Fluorescence *in situ* hybridization (FISH) confirmed the presence of a homozygous deletion of *PTPN2* in 90% of the bone marrow cells (Fig. 1b). Quantitative PCR on genomic DNA isolated from diagnosis, remission and relapse, confirmed that the deletion was acquired at diagnosis and again present at relapse (Fig. 1c).

PTPN2 is highly expressed in thymocytes.[12] In order to identify additional cases with *PTPN2* deletion, we analyzed the gene expression profiles of 90 T-ALL cases,[13] which identified 5 cases with low PTPN2 expression (Fig. 1d). Analysis of genomic DNA confirmed the presence of acquired homozygous deletions restricted to the *PTPN2* locus specifically in the 5 cases with low expression levels (Fig. 1a, Supplementary Fig. 2 online). Strikingly, array CGH profiles suggested that in all cases with *PTPN2* deletion, the breakpoints were highly similar. The deletion breakpoints were subsequently mapped within two *Alu* repeat regions flanking *PTPN2* (Supplementary Fig. 3 online). SNP analysis excluded the presence of uniparental disomy at chromosome 18, providing evidence that deletion of the 2 different *PTPN2* alleles occurred as 2 independent events.

We next screened an additional set of T-ALL cases (n=106) for copy number alterations (n=97), expression (n=9) or mutations (n=70) of *PTPN2*. Using this strategy, we identified 2 additional individuals with bi-allelic deletion, and 5 individuals with mono-allelic deletion of *PTPN2* (Fig. 1a, Supplementary Fig. 4 online). Individuals with bi-allelic or mono-allelic deletion of *PTPN2* showed significantly lower mRNA expression levels as compared to individuals with normal *PTPN2* copy 3 number (Fig. 1e, Supplementary Fig. 5 online). One case with mono-allelic deletion of *PTPN2* harbored a nonsense mutation in the residual *PTPN2* allele (Table 1), but DNA methylation was not detected as a mechanism to silence *PTPN2* gene expression in T-ALL (Supplementary Fig. 6 online). Deletion of *PTPN2* was not detected in T-ALL cases with normal *PTPN2* expression levels, nor in AML cases (n=60, data not shown), nor in published SNP array profiles of B-ALL.[14] Strikingly, all individuals with homozygous deletion of *PTPN2* belonged to the *TLX1 (n=12) or TLX3 (n=1)* positive T-ALL subgroup (Fig. 1d, Table 1, Supplementary Table 1 online). *TLX1* positive cells accumulate at the $CD4^+CD8^+CD3^{-/low}$ differentiation stage,[6] where *PTPN2* expression is high (Supplementary Fig. 7 online). This observation suggests that $CD4^+CD8^+CD3^{-/low}$ thymocytes may be most sensitive to loss of PTPN2 function. Our data show that inactivation of *PTPN2* occurs in approximately 6 % of T-ALL cases, associated with intermediate age (range 4–49 years, median 24 years), and the *TLX1* positive subgroup (12 of 36 TLX1 positive cases, 33 %). Deletion of the entire PTPN2 gene is the most common inactivation mechanism.

PTPN2 was described as a phosphatase for both JAKs and STATs,[15–17] which are important signaling proteins downstream of cytokine receptors. To determine the effect of loss of PTPN2 on cytokine receptors implicated in T-cell development, we knocked down the expression of PTPN2 in human T-ALL cell lines and primary mouse T-ALL cells and measured the effect on IFNγ, IL2 and IL7 receptor signaling.[18] JURKAT cells were electroporated with PTPN2 targeting siRNA or non-targeting siRNA and stimulated with IFNγ for various time periods. Knockdown of PTPN2 resulted in a significant increase of both the strength and the time of JAK1 and STAT1 phosphorylation in this cell line (Fig. 2a). HPB-ALL cells, another human T-ALL cell line, showed expression of the IL7 receptor and were responsive to exogenous stimulation with IL7. Knockdown of PTPN2 in these cells resulted in a significant increase of JAK1 and STAT5 phosphorylation in response to IL7 stimulation (Fig. 2b).

These data were further extended using *ex vivo* cultures of mouse primary T-cell leukemia cells, isolated from a spontaneous murine T-cell leukemia ($CD4^+CD8^+$), and expanded ex vivo

in the presence of IL2 and IL7. Analysis of the response of these cells to IL2 and IL7 by monitoring phospho-JAK1 and phospho-STAT5 levels, revealed that knockdown of Ptpn2 caused an increased JAK/STAT signaling downstream of the IL2 and IL7 receptors (Fig. 3a, b). In addition, also for IFNγ a similar effect as observed in JURKAT cells, was observed in the mouse T-cell leukemia cells (Fig. 3c). To test if the augmentation in cytokine responsiveness also provided a proliferative advantage to these murine T-cells, we deprived cells of cytokines for 24h and followed their proliferation after re-stimulation with either low concentration of IL7 alone or a combination of IL2 and IL7. Knockdown of Ptpn2 caused a significant increase in the proliferation compared to control cells (Fig. 3d). Taken together, the data obtained in human and mouse T-cell leukemias indicate that loss of PTPN2 sensitizes T-ALL cells to cytokine stimulation resulting in enhanced activation of cytokine receptor pathways, which may support T-ALL development and proliferation under limiting cytokine concentrations in vivo.

In addition to a general effect on cytokine receptor signaling, our genetic data indicated a possible link between *PTPN2* deletion and expression of the NUP214-ABL1 tyrosine kinase. Out of nine *NUP214-ABL1* positive T-ALLs analyzed in the described study, we have identified four *NUP214-ABL1* positive individuals with a concomitant deletion of *PTPN2*. Two individuals featured bi-allelic (individuals 2 and 5) and two individuals (individuals 9 and 20) mono-allelic loss of *PTPN2* (Table 1). The presence of *NUP214-ABL1* was initially detected by array CGH for all four individuals and later confirmed by FISH analysis for individuals 5, 9 and 20 (data not shown). In case of individual 5, an extrachromosomal (episomal) amplification of *ABL1* was detected in more than 80% of the bone marrow cells using the LSI BCR-ABL1 translocation probe. For individual 20, both FISH and array CGH data confirmed a local duplication of NUP214-ABL1. In conclusion, both deletion of *PTPN2* and *NUP214-ABL1* were present in the majority of cells and thus also occurred together within the same cells.

To determine if PTPN2 directly modulates NUP214-ABL1 signaling, we applied a retroviral system that allowed us to induce stable overexpression of either the wild type PTPN2 or a catalytic inactive mutant (PTPN2-D182A) in ALL-SIL cells.[19] ALL-SIL is a cell line derived from a young adult T-ALL case expressing the NUP214-ABL1 fusion that is sensitive to the ABL1 kinase inhibitor imatinib.[7] Overexpression of wild type PTPN2 in these cells resulted in a clear dephosphorylation of the NUP214-ABL1 protein, while this was not observed in cells expressing the catalytic inactive mutant (Fig. 4a). The PTPN2-D182A mutant is also known to form a complex with physiological substrates that is stable enough to allow purification upon interaction.[20,21] Immune precipitation of the PTPN2-D182A from ALL-SIL cells resulted in co-precipitation of the NUP214-ABL1 protein, demonstrating a direct interaction between the tyrosine kinase and the phosphatase (Fig. 4a). Finally, downregulation of PTPN2 in ALL-SIL cells resulted in a significant increase in proliferation of the cells under normal culture conditions (Fig. 4b), which was in part attributed to increased signaling of NUP214-ABL1 through downstream proteins STAT5 and STAT3 (Fig. 4c). In addition, other signaling proteins such as LCK, JAK3 and ERK1/2 were also found to be constitutively active in ALL-SIL cells and more activated upon PTPN2 knockdown, therefore likely to contribute to the observed growth advantage (Fig. 4c, d).

A similar relationship between NUP214-ABL1 and Ptpn2 was demonstrated in the IL3 dependent pro-B cell line Ba/F3. We have previously shown that NUP214-ABL1 can transform Ba/F3 to IL3 independent proliferation, but its oncogenic activity is less potent than the BCR-ABL1 kinase.[22] shRNA-mediated downregulation of Ptpn2 expression made Ba/F3 cells more sensitive to NUP214-ABL1 transformation (Fig. 4e). NUP214-ABL1 showed stronger autophosphorylation when expressed in Ba/F3 cells with decreased Ptpn2 expression, and vice

versa, induced overexpression of wild type PTPN2 decreased NUP214-ABL1 phosphorylation and downstream signaling (Fig. 4f, g).

Next, we tested the effect of PTPN2 knockdown on the survival and proliferation of ALL-SIL cells upon treatment with the ABL1 kinase inhibitor imatinib. Downregulation of PTPN2 expression resulted in prolonged survival of ALL-SIL cells after imatinib treatment as compared to cells electroporated with a non-targeting siRNA (Fig. 5a). The proliferation of the cells was inhibited by imatinib, but with a shift of the cellular IC50 value from 73 to 126 nM (Fig. 5b). These data indicate that loss of PTPN2 affects response to treatment, which is likely to be caused by the direct effect on NUP214-ABL1 activation and its downstream signaling, as observed by an increased activation state of its important downstream mediator STAT5 (Fig. 5c). This is further supported by the enhanced phosphorylation of additional protein kinases such as LCK and ERK1/2 which persisted upon inhibitor treatment (Fig. 4c). A similar effect was observed in HSB-2, a cell line derived from a childhood T-ALL that is dependent on LCK for its proliferation and survival.[23] Our results indicate a direct modulating effect of PTPN2 on LCK activation (Supplementary Fig. 8 online).

Recently, mutational analysis of *JAK1* resulted in the identification of activating point mutations in 10% of T-ALL cases,[24] directly implicating deregulated JAK1 signaling in the pathogenesis of leukemia. In this study we identify inactivation of PTPN2 in T-ALL cells as another mechanism that results in increased JAK/STAT signaling downstream of cytokine receptors. PTPN2 is an important negative regulator of a variety of tyrosine kinases as well as other signaling proteins, which indicates that its loss in T-cells may affect a diverse set of signaling proteins thereby sensitizing T-cells to growth factors and increasing the effects of oncogenic kinases such as NUP214-ABL1. Our data identify the NUP214-ABL1 oncogene as a novel substrate of PTPN2 and reveal a correlation between activation status of the NUP214-ABL1 pathway, treatment response to kinase inhibitor imatinib and PTPN2 expression levels. This study adds further complexity to the genetics of T-ALL and shows that in addition to tyrosine kinase activation also inactivation of tyrosine phosphatases may provide a proliferation and survival advantage to these leukemias. Mutation and expression analysis of *PTPN2* in various tumors is warranted.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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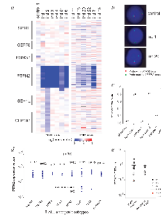
**Figure 1. Comprehensive analysis of T-ALL individuals featuring *PTPN2* deletion**

a.  Agilent array CGH and Affymetrix SNP array data for 13 T-ALL cases indicating bi-allelic deletion of PTPN2 in individuals 1 to 7, and mono-allelic deletion of *PTPN2* in individuals 18, 20 and 22. *blast cell content for individual 5 was only 50%.

b.  Interphase fluorescence *in situ* hybridization (FISH) confirmed the presence of *PTPN2* deletion in >90% of the cells for individuals 1 (bi-allelic deletion) and 20 (mono-allelic deletion).

c.  Quantitative PCR analysis on genomic DNA isolated from diagnosis, remission and relapse samples of individual 1 confirmed that the deletion was acquired at diagnosis, and again present at relapse. A primer set for *CDH2* (18q12) was used for normalization.

d.  PTPN2 expression level was analyzed in a T-ALL patient set (n=90) using Affymetrix gene expression arrays (probeset 213136_at) and categorized according to oncogenic T-ALL subtypes (x-axis). Y-axis displays PTPN2 expression levels as a logarithmic scale. N indicates number of patients analyzed.

e.  Quantitative real-time PCR analysis of T-ALL patients detects lower expression levels for T-ALL cases with loss of *PTPN2* compared to T-ALL patients with no deletion. Expression values were normalized for HPRT gene expression and blotted at the y-axis in logarithmic scale. Samples derived from bone marrow or peripheral blood were analyzed separately. Closed rectangles: no deletion (n=8); open triangles: mono-allelic deletion (n=5); open circle: bi-allelic deletion (ind 1). The individual with an additional mutation in *PTPN2* is shown in red.
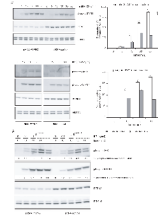
**Figure 2. Knockdown of PTPN2 causes increased sensitivity of T-cell lines to cytokine stimulation**

a.  Western blot analysis of WCL showing increased sensitivity of JURKAT cells to cytokine stimulation (IFNγ) due to decreased PTPN2 expression. Stimulated cells expressing reduced levels of PTPN2 displayed an increased and prolonged phosphorylation of STAT1 as well as JAK1 (right panel). Results of two independent experiments are shown. ERK1/2 was used as loading control. Open bars: siRNA PTPN2; closed bars: siRNA control; bars show average ± s.e.m., n=3; error bars represent s.e.m. *p<0.05, **p<0.005

b.  HPB-ALL cells expressing reduced (siRNA PTPN2) or normal levels (siRNA control) of PTPN2 were stimulated with different concentrations of IL7 for indicated time points and WCL were analyzed for activation of STAT5 and JAK1 by western blot. Quantification of western blot experiments showed that knockdown of PTPN2 resulted in stronger activation of JAK1 and STAT5. Normalized quantification values calculated as fold- change compared to unstimulated cells are shown below respective immunoblots. ERK1/2 was used as loading control.
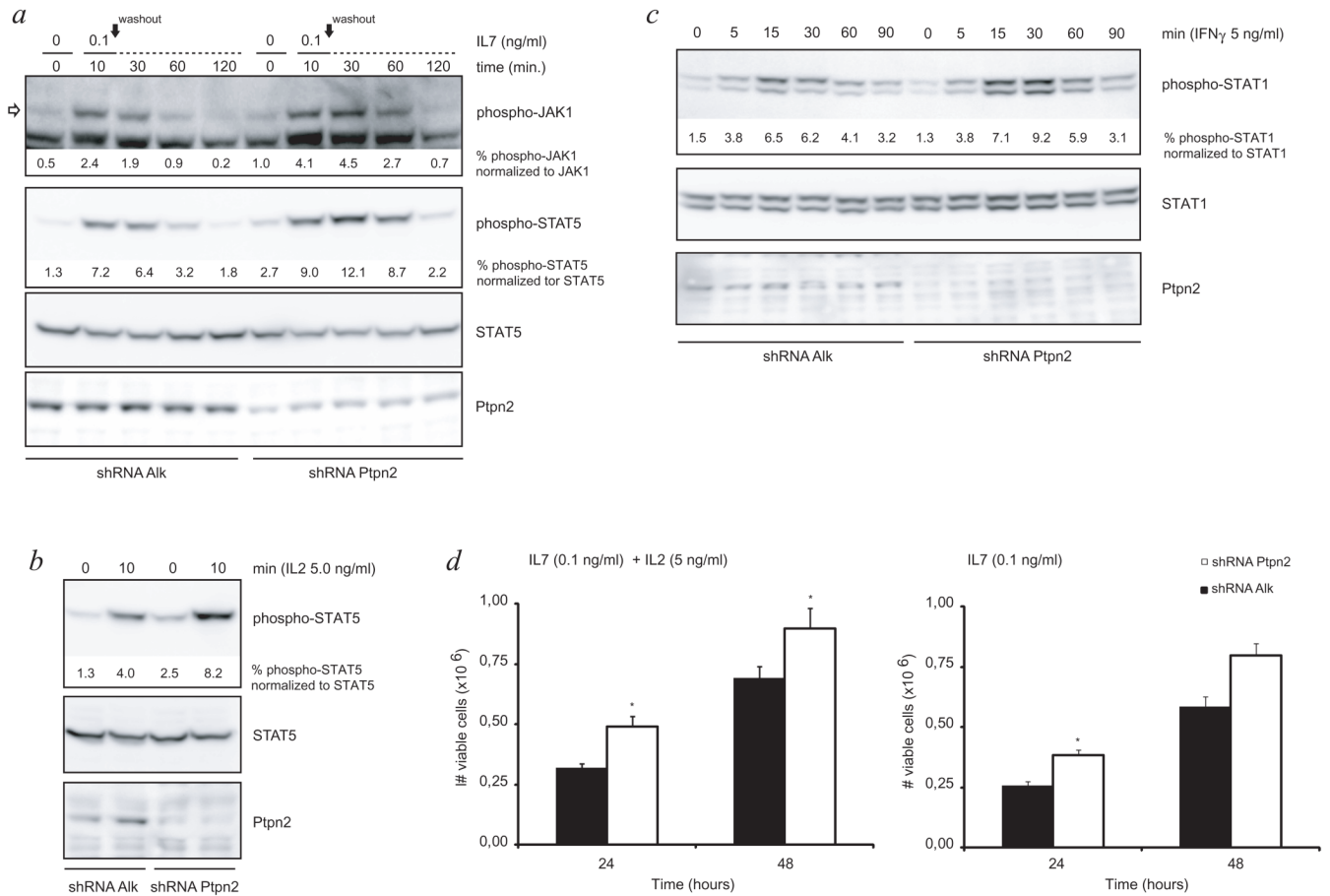
**Figure 3. Mouse T-ALL cells display altered cytokine receptor signaling in correlation with Ptpn2 expression levels**

  **a.** Knockdown of Ptpn2 enhanced responsiveness of the IL7 receptor pathway to ligand stimulation as displayed by increased strength and duration of the phosphorylation status of JAK1 and downstream protein STAT5 when compared to control cells. Open arrow points at the protein band corresponding to JAK1. Black arrow indicates time point of cytokine removal. STAT5 was used as loading control.

  **b.** Western blot analysis of WCL revealed increased sensitivity of primary mouse T-ALL cells to IL2 stimulation due to decreased Ptpn2 expression as analyzed by phosphorylation level of STAT5. STAT5 is shown as loading control.

  **c.** Cytokine depleted cells were exposed to INF-γ for indicated time periods and subsequently analyzed for activation of STAT1 by western blot. Quantification of western blot experiments showed that reduction of Ptpn2 protein resulted in stronger activation of STAT1. STAT1 was assayed to ensure equal protein loading.

  **d.** Primary T-ALL cells were deprived from cytokines for 24 hours before re-stimulation with either IL7 alone or a combination of IL2 and IL7. Under both conditions, knockdown of Ptpn2 provided cells with a significant proliferative advantage in response to cytokine re-addition. Open bars: shRNA Ptpn2; closed bars: shRNA Alk (control); bars show average ± s.e.m. Error bars represent s.e.m. *p<0.05

Efficient knockdown of Ptpn2 was confirmed using anti-Ptpn2 (3E2) antibody. Differences in phosphorylation signal intensity were quantified for JAK1 and STAT proteins and corresponding values are shown below blots.
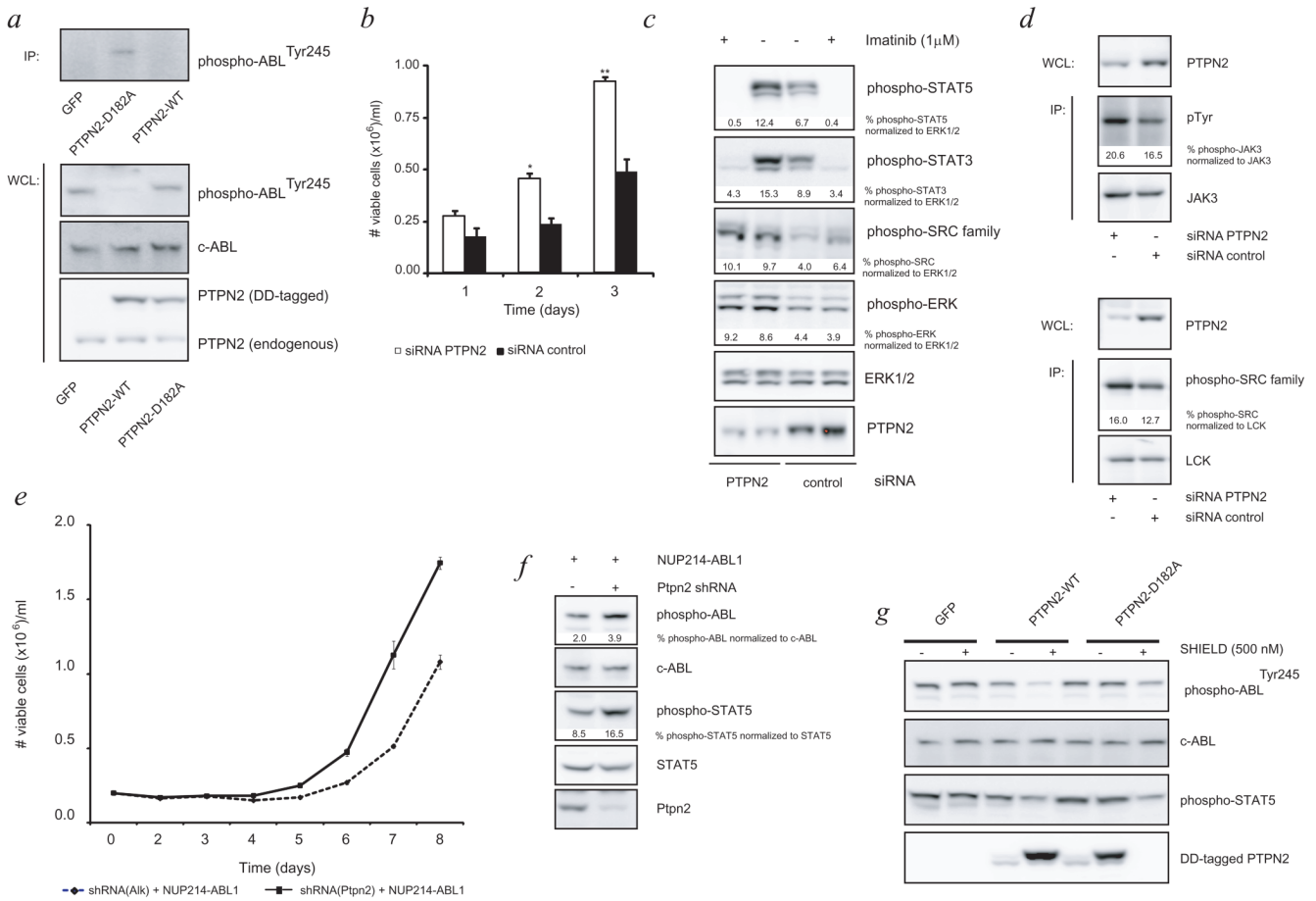
**Figure 4. Identification of NUP214-ABL1 oncogene as a novel substrate of PTPN2**

**a.** Desphosphorylation of NUP214-ABL1 was observed upon induced overexpression of wild type PTPN2 (PTPN2-WT). Immune precipitation (IP) of PTPN2-D182A resulted in co-precipitation of NUP214-ABL1 protein, showing a direct interaction between kinase and phosphatase.

**b.** Viable cell numbers were determined 3 days after electroporation with respective siRNA's. Decrease in PTPN2 protein levels resulted in a significant proliferative advantage of ALL-SIL cells.

**c.** Cells were exposed to imatinib at time point of maximal PTPN2 knockdown. Western blot analysis showed differential activation status of STAT family members downstream of NUP214-ABL1. ERK1/2 and a SRC-family kinase were detected to be constitutively active in ALL-SIL cells and knockdown of PTPN2 caused an augmented phosphorylation which stayed unchanged upon imatinib treatment. Loading control: ERK1/2

**d.** IP experiments of ALL-SIL cells after electroporation with siRNA's revealed an enhancing effect on JAK3 kinase activation (upper) and identified the differentially activated SRC-family kinase as LCK. Phosphorylation of JAK3 and LCK were detected with a phosphotyrosine (pTyr) or phospho-SRC family antibody, respectively. JAK3 or LCK were used to ensure equal protein loading.

   **e.** Ba/F3 cells carrying indicated constructs were grown in absence of IL-3 and mean growth ± s.e.m. was recorded in triplicate for 8 days. No s.e.m. is shown in case of values below 0.03.

   **f.** Western blot analysis of NUP214-ABL1 dependent Ba/F3 cells demonstrated stronger constitutive activation of NUP214-ABL1 and STAT5 in case of concomitant shRNA-mediated knockdown of Ptpn2. Loading control: c-ABL, STAT5

   **g.** Induced overexpression of PTPN2-WT in Ba/F3 cells carrying the NUP214-ABL fusion results in dephosphorylation of the oncogenic kinase and its downstream mediator STAT5. C-ABL is shown as loading control.

Values shown below immunoblots represent normalized (for loading control) quantitative values. Open bars: siRNA PTPN2; closed bars: siRNA control; bars show average ± s.e.m. Error bars represent s.e.m. $*p<0.05$, $**p<0.005$.
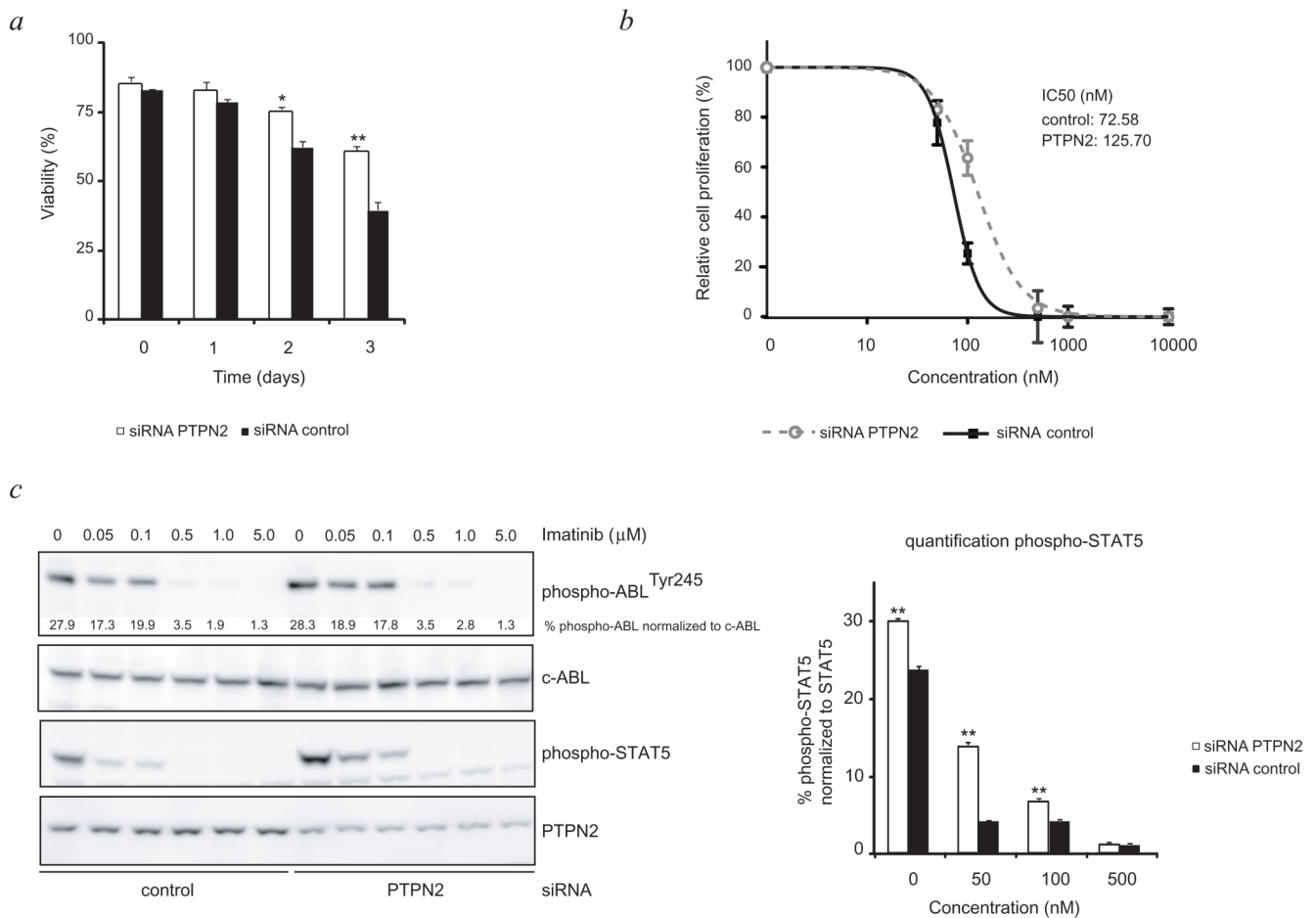
*a*

*b*



*c*



**Figure 5. Reduction of PTPN2 expression levels affects treatment response of NUP214-ABL1 dependent cells to kinase inhibitor imatinib**

a.  Follow up of survival of ALL-SIL cells treated with imatinib (500 nM) demonstrated decreased sensitivity due to alteration of PTPN2 expression levels. Y-axis displays cell viability (%).Open bars: siRNA PTPN2; closed bars: control siRNA

b.  siRNA mediated knockdown of PTPN2 in ALL-SIL cells reduced activity of imatinib as depicted by an increased IC50 value (126 nM) compared to control cells (73 nM). Untreated cells were set to 100%. Graph displays relative cell growth (%) as a function of concentrations (nM, logarithmic scale). Open circles: siRNA PTPN2, closed rectangles: siRNA control.

c.  Western blot analysis of ALL-SIL cells exposed to increasing concentrations of imatinib revealed a stronger activation of NUP214-ABL downstream target STAT5 in knockdown cells (left panel). Bar graph displays calculated differences in STAT5 activation level due to alterations in PTPN2 expression levels (right panel). Loading control: c-ABL; open bars: siRNA PTPN2; closed bars: control siRNA

All values represent the average ± s.e.m. of three determinations. Significant differences compared to those of control siRNA are indicated. *p<0.05 and **p<0.005

**Table 1**

Cytogenetic and molecular findings in individuals with *PTPN2* deletion

| Ind | Sex | Age (years) | Karyotype^a | PTPN2 status^b | TLX1/TLX3 expression^c | NUP214-ABL1^d |
|---|---|---|---|---|---|---|
| 1 | F | 30 | 46, XXX, add(9)(p13), t(10;14)(q23;q11), del(12)(p12) / 46, XXX, idic(9)(p13), t(10;14)(q24;q11), del(12)(p12) | PTPN2 −/− | TLX1 | − |
| 2 | M | 24 | NA | PTPN2 −/− | TLX1 | + |
| 3 | F | 27 | 47,XX,del(6)(q21),t(7;10)(q35;q24),del(13)(q14), +mar | PTPN2 −/− | TLX1 | − |
| 4 | M | 21 | 46,XY | PTPN2 −/− | TLX1 | − |
| 5 | M | 45 | 46,XY,t(10;14)(q24;q11) | PTPN2 −/− | TLX1 | + |
| 6 | M | 8 | 46,XY | PTPN2 −/− | TLX3 | − |
| 7 | M | 43 | 46,XY,del(6)(q15),t(7;10)(q34;q24),del(9)(p21) | PTPN2 −/− | TLX1 | − |
| 8 | M | 20 | 46,Y,t(X;7)(p21;q36), del(6)(q23), t(10;14)(q24;q11), der(21)t(6;21)(q23;p11).ish del(9)(p21p21)x2 | PTPN2 −/− | TLX1 | − |
| 9 | F | 4 | 46,XX | PTPN2 +/− | TLX1 | + |
| 10 | M | 11 | 46,XY,t(10;14)(q24;q11) | PTPN2 +/− | TLX1 | − |
| 18 | F | 35 | 49,XX,+7,t(7;10)(q34;q24),+13, +20 | PTPN2 +/− | TLX1 | − |
| 20 | F | 9 | NA | PTPN2 +/− | TLX1 | + |
| 22 | F | 49 | 46,X,t(X;9)(q28;p22), del(5)(q23q33), t(7;10)(q34;q24),del(9)(p13) | PTPN2 +/− PTPN2 mutation (862 C>T, R288Stop) | TLX1 | − |

Summary of data on individuals with *PTPN2* deletion; additional data is provided in supplementary table 1 online.

^a Karyotype obtained from bone marrow culture at diagnosis.

^b Determined by FISH, Q-PCR or array-CGH.

^c Positivity for *TLX1* or *TLX3* expression or rearrangement.

^d Positivity for *NUP214-ABL1* expression or rearrangement (+, positive for NUP214-ABL1; −, negative for NUP214-ABL1). −/−, bi-allelic deletion; +/−, mono-allelic deletion; NA, not available; F, female; M, male.