PDGFRβ promotes oncogenic progression via STAT3/STAT5 hyperactivation in anaplastic large cell lymphoma

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Abstract

Background: Anaplastic large cell lymphoma (ALCL) is an aggressive non-Hodgkin T cell lymphoma commonly driven by NPM-ALK. AP-1 transcription factors, cJUN and JUNb, act as downstream effectors of NPM-ALK and transcriptionally regulate PDGFRβ. Blocking PDGFRβ kinase activity with imatinib effectively reduces tumor burden and prolongs survival, although the downstream molecular mechanisms remain elusive.

Methods and results: In a transgenic mouse model that mimics PDGFRβ-driven human ALCL in vivo, we identify PDGFRβ as a driver of aggressive tumor growth. Mechanistically, PDGFRβ induces the pro-survival factor Bcl-xL and the growth-enhancing cytokine IL-10 via STAT5 activation. CRISPR/Cas9 deletion of both STAT5 gene products, STAT5A and STAT5B, results in the significant impairment of cell viability compared to deletion of STAT5A, STAT5B or STAT3 alone. Moreover, combined blockade of STAT3/5 activity with a selective SH2 domain inhibitor, AC-4-130, effectively obstructs tumor development in vivo.

Conclusions: We therefore propose PDGFRβ as a novel biomarker and introduce PDGFRβ-STAT3/5 signaling as an important axis in aggressive ALCL. Furthermore, we suggest that inhibition of PDGFRβ or STAT3/5 improve existing therapies for both previously untreated and relapsed/refractory ALK+ ALCL patients.

Keywords: ALCL, PDGFRβ, STAT3, STAT5A, STAT5B, NPM-ALK, Apoptosis

Key points

- PDGFRβ-STAT5 activity, acting in parallel to the NPM-ALK-STAT3 signaling axis, correlates with an inferior Event Free Survival (EFS) and Cumulative Incidence of Relapse (CI-R) in ALCL.
- PDGFRβ induced STAT5 activity, increases proliferation by stimulating IL-10 secretion and blocking apoptosis by upregulating Bcl-xL, characterizing PDGFRβ as a novel biomarker.
• Combined loss of STAT5A and STAT5B is lethal in ALK+ ALCL, suggesting STAT5 as a valid therapeutic target that can be successfully inhibited with the STAT inhibitor AC-4-130.

Introduction

Anaplastic Large Cell Lymphoma (ALCL), characterized by expression of CD30, refers to a family of Non-Hodgkin T cell lymphomas divided into four entities: systemic Anaplastic Lymphoma Kinase (ALK) positive (ALK+ ALCL), systemic ALK negative (ALK− ALCL), primary cutaneous ALCL (pc-ALCL) and breast-implant associated (BIA-ALCL) [1]. In 70% of systemic ALK+ ALCL cases, patients present with the t(2;5)(p23;35) translocation, resulting in abundant cytoplasmic and nuclear expression of the Nucleophosmin-Anaplastic Lymphoma Kinase (NPM-ALK) chimeric protein [2]. The expression of this ligand-independent oncogene is mediated via the NPM1 promoter but drives disease pathogenesis via the ALK kinase domain [3]. NPM-ALK stimulates a plethora of oncogenic signal transduction pathways including JAK/STAT, RAS/RAF/ERK, PI3K/AKT/mTOR and JNK/p38/AP-1, but the key vulnerable nodes for targeting remain illusive [4]. In the context of ALCL, STAT3 has been identified as a crucial signaling modulator downstream of NPM-ALK responsible for disease maintenance [5–7]. Additional members of the STAT family, such as STAT1 [8] and STAT5A/B [9], have also been reported in ALCL however their function is not extensively studied and remains enigmatic.

Event Free Survival (EFS) and Overall Survival (OS) rates for ALK+ ALCL patients treated with standard CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) chemotherapy are relatively successful [10–13]. However, current relapse rates range from 20 to 40%, highlighting the urgency to develop alternative therapy approaches for relapsed patients [14]. The restricted expression of ALK in neuronal cells during development make it an ideal drug target with potential to overcome relapse. First-generation ALK tyrosine kinase inhibitor (TKI) crizotinib (Xalkori), initially approved by the Federal Drug Administration (FDA) for Non-Small Cell Lung Cancer (NSCLC) patients harboring ALK fusions, has recently been authorized for pediatric patients with relapsed or refractory systemic ALK+ ALCL (NCT00939770). Second [15–19], third [20], and fourth-generations [21] of ALK TKIs have since been developed and are in clinical use for ALK+ NSCLC [22, 23]. Mounting evidence suggests that patients with ALK-expressing malignancies eventually develop point mutations in ALK [14], rendering them insensitive to ALK inhibition and provoking recurrence of a more aggressive disease [6, 24]. Thus, there is a clear need for more effective therapies targeting alternative core signaling pathways.

We identified the AP-1 TFs, cJUN and JUNB, as downstream effectors of NPM-ALK [25], transcriptionally regulating the expression of Platelet Derived Growth Factor Receptor Beta (Pdgfrb) [26]. Strikingly, inhibition of PDGFRβ by the TKI imatinib (Gleevec) induced a complete and sustained remission in a late-stage relapsed ALK+ ALCL patient [26]. Furthermore, our findings resulted in the initiation of a clinical study evaluating imatinib efficacy according to PDGFR status [27]. Imatinib treatment can thus be considered as a promising therapeutic strategy for relapsed ALK+ ALCL. However, the mechanism of action and the extent of PDGFRβ involvement in ALCL pathogenesis still remains elusive.

In this study, we determine the influence of PDGFRβ on ALCL by developing a genetic ALK+ ALCL mouse model lacking PDGFRβ expression in neoplastic CD4+ T cells. Strikingly, genetic loss of Pdgfrb results in a significant increase in survival in line with the observed benefits of PDGFRβ kinase activity blockade via imatinib in patients. Mechanistically, we identify STAT5 as a novel downstream target of PDGFRβ in ALCL. Blockade of STAT5 results in a significant dampening of viability by mediating the pro-survival factor Bcl-xL and the growth-enhancing cytokine IL-10. Additionally, we report the small molecule inhibitor AC-4-130 inhibits both STAT5 and STAT3 activation in our experimental setting, significantly inhibiting tumor development in vivo and highlighting the added benefit of a dual STAT3/5 inhibition. Overall, our findings suggest expression of PDGFRβ in ALCL stimulates a malignant boost, providing an attractive alternative/additive pathway for pharmacologic inhibition.

Materials and methods

Additional materials and resources can be found in Suppl. Materials and Methods.

Transgenic mouse strains

All animal experiments were performed in agreement with the ethical guidelines of the Medical University of Vienna and the Austrian Federal Ministry of Science and Research (Project number: BMWFFW-66.009/0183-WF/V/3b/2017; BMWFFW-66.009/0057-V/3b/2018; BMWFFW-66.009/0401-V/3b/2018; 2020–0.103.412). Mice were kept in a pathogen-free environment under standard conditions. Cd4-NPM-ALK transgenic mice [28] were crossed with Cd4-Cre mice [29] and a second strain carryingloxP-flanked Pdgfrb (Exons 4–7) [30] in a C57BL/6 x BALB/c mixed background resulting in the desired genotypes: Cd4-NPM-ALKTg/+ Cd4-CreTg/+ PdgfrbΔ/Δ (fl/fl Pdgfrb) and Cd4-NPM-ALKTg/+ Cd4-CreTg/+ PdgfrbΔ/Δ (Δ/Δ Pdgfrb).
mice. Mice were genotyped using the primers listed in Suppl. Table 1.

**Human tissue microarrays**
All human samples were obtained with informed written consent and in accordance with the Declaration of Helsinki.

**Adult TMAs**
Formalin-fixed paraffin-embedded (FFPE) tissue specimens from both male and female adult patients, diagnosed with systemic ALK+ (n = 25) or ALK− (n = 25) ALCL, were obtained and reviewed by the local ethic boards of the Medical University of Vienna, Austria (no.1437/2016 and 1221/2019) and the University Hospital Brno, Czech Republic (no. 4–306/13/1). Diagnoses were assigned according to the WHO classification of lymphoid neoplasms.

**Pediatric TMA**
FFPE tissues specimens from 98 pediatric patients diagnosed with ALK+ ALCL included in the studies NHL-BFM90, NHL-BFM95 or enrolled in the European intergroup trial ALCL99 (NCT00006455) between 1992 and 2006, were obtained. All patients were treated with comparable Berlin-Frankfurt-Münster (BFM)-type chemotherapy, as previously described [12]. Eligibility was determined by detection of oncogenic NPM-ALK: either via NPM-ALK PCR, two color fluorescence in situ hybridization for the translocation (t(2;5)(p23;35) or nuclear/cytoplasmic IHC staining for ALK. Staining procedures included bone marrow aspiration cytology and a spinal tap. Bone marrow involvement was defined by cytologically detectable ALCL cells, irrespective of cellular quantity. The studies were approved by the institutional ethics committee of the primary investigator of the NHL-BFM study group.

**Immunohistochemistry and whole tissue scans**
IHC staining was performed with tissue fixed for 24 hours in formalin before embedding in paraffin blocks, as described [26]. Antibodies used for IHC can be found in Suppl. Table 2. A Panoramic MIDI slide scanner (3DHistech Ltd., Budapest, Hungary) with 40x optics was used to digitalize the tissue sections. Quantitation was performed using Definiens® TM Tissue Studio histomorphometry software (Definiens AG, Munich, Germany). Images were taken with a Zeiss AxioImager Z1 microscope.

**Multiplex immunobead cytokine assay**
Murine fl/fl Pdgfrb and Δ/Δ Pdgfrb cells were cultured at a density 1 x 10^5 cells in a 12-well plate and treated with AC-4-130 or DMSO as vehicle control. Post 72 hours of treatment, supernatants were collected and analyzed using ProcartaPlex antibody-based, magnetic bead reagent assay panels for multiplex protein quantitation using the Luminex xMAP technology and instrument platform. Concentrations of cytokines in the supernatants were determined simultaneously with the customized 27-plex immunoassay kit (ProcartaPlex Mouse 27-plex, Thermofisher Scientific) using magnetic beads. Undiluted frozen samples (50 μL) were processed in 96-well plates according to the manufacturer’s instructions. Standard curves for each analyte were generated by measuring individual standards in duplicate and using the reference concentration supplied by the manufacturer. Measurement was performed on a calibrated Bio-Plex 200 system (Bio-Rad) in combination with Bio-Plex Manager software, version 6.1 (Bio-Rad). The concentrations were calculated from the standard curve using five-parameter logistic (5PL) regression curve fitting.

**ChIP-seq and initial processing**
ChIP-seq was performed as previously described [31]. The antibodies used for each experiment are listed in Suppl. Table 3. For each ChIP, 5 μg of antibody coupled to 2 μg of magnetic Dynabeads (Life Technologies) was added to 3 ml of sonicated nuclear extract from formaldehyde-fixed cells. Chromatin was immunoprecipitated overnight, cross-links were reversed, and DNA was purified by precipitation with phenol:chloroform:isoamyl alcohol. DNA pellets were resuspended in 25 μl of TE buffer. Illumina sequencing, library construction, and ChIP-seq analysis methods were previously described [31]. Reads were aligned to the human reference genome (hg19) using bowtie v1.2.2 with parameters –k 2 –m 2 –best and –l set to the read length. For visualization, WIG files were created from aligned read positions using MACS v1.4 with parameters –w –S –space=50 –nomodel –shiftsize=200 to artificially extend reads to 200bp and to calculate their density in 50-bp bins. Read counts in 50-bp bins were normalized to the millions of mapped reads, giving RPM values. WIG files were visualized in the IGV browser version 2.7.2.

**CUT&RUN sequencing and initial processing**
CUT&RUN coupled with high-throughput DNA sequencing was performed using antibodies listed in Suppl. Table 3 and Cutana pA/G-MNase (EpiCypher) according to the manufacturer’s protocol. Briefly, cells were washed and incubated with activated concanavalin A beads for 10 min at room temperature. Cells were then resuspended in antibody buffer containing 0.01% digitonin, 1 ml of each antibody (Suppl. Table 3) was added to individual cell aliquots, and tubes were rotated at 4°C overnight. The
following day, targeted chromatin digestion and release were performed with 2.5 ml of Cutana pA/G-MNase and 100 mM CaCl₂. Retrieved genomic DNA was purified with a MinElute PCR purification kit and eluted in 10 ml of buffer EB. Sequencing libraries were prepared with an automated Swift 2S system, followed by 100-bp paired-end sequencing with NovaSeq 6000. Reads were aligned to the human reference genome (hg19) using bowtie v1.2.2 in single-end mode with parameters –k 2 –m 2 –best and –l set to the read length. For visualization, WIG files were created from aligned read positions using MACS v1.4 with parameters –w –S –space=50 –nomodel –shiftsize=200 to artificially extend reads to 200bp and to calculate their density in 50-bp bins. Read counts in 50-bp bins were then normalized to the millions of mapped reads, giving reads per million (RPM) values. WIG files were visualized in the Integrative Genomics Viewer (IGV) browser version 2.7.2. The antibodies used for CUT&RUN are listed in Suppl. Table 3.

Data and code availability
Raw and processed data files were deposited to the NCBI GEO server. Code written in R/python to perform analysis of ChIP-seq and CUT&RUN are available upon request.

Statistical analysis
Log-rank (Mantel-Cox) test, Student’s t test (Wilcoxon-Mann-Whitney correction), and half maximal inhibitory concentration (IC₅₀) statistical analyzes were performed using GraphPad Prism® Software version 8. P-values were defined as indicated in the figure legends: ns = p > 0.05; * = p < 0.05; ** = p < 0.01; *** = p < 0.001; **** = p < 0.0001.

Results
PDGFRβ is a biomarker for aggressive ALK⁺ ALC
Imatinib treatment results in tumor regression in Cd4⁺ NPM-ALK transgenic mice [26]. However, the mechanisms of action and whether this effect is dependent on NPM-ALK activity remained to be determined. This is particularly important as inhibition of alternative tumor-promoting pathways in combination with ALK inhibition might prevent the development of drug resistance [6]. We evaluated a set of ALC biopsies, all taken from children treated with an identical BFM-based chemotherapy in three separate clinical trials, for PDGFRβ expression and correlated expression levels with clinical variables. Patients with PDGFRβ expression on tumor cells (n = 11) had a significantly lower five-year EFS compared to patients lacking (n = 87) membrane-bound PDGFRβ (Fig. 1A). We next analyzed an independent lymphoma patient dataset using the Oncomine database. In the six ALC patient samples [32], we observed a significant upregulation of PDGFRβ expression when compared to healthy donor Cd4⁺ and Cd8⁺ T cells. In contrast PDGFRα was not significantly upregulated (Fig. 1B). Having correlated PDGFRβ expression in patients with poorer EFS, we set out to map the molecular signatures activated by this receptor. We conditionally deleted Pdgfrb in Cd4⁺ T cells in transgenic mice expressing the human NPM-ALK fusion oncogene under control of the murine Cd4 promoter and enhancer (NPM-ALKΔT) [28], resulting in littersmates either expressing wild type Pdgfrb (fl/fl Pdgfrb) or lacking Pdgfrb in Cd4⁺ T cells (Δ/Δ Pdgfrb) (Fig. 1C). Regardless of sex, NPM-ALKΔT mice, develop T cell lymphomas with a high penetrance [28]. We assessed and confirmed deletion of Pdgfrb in T cells by genotyping (Fig. S1A) and immunohistochemistry (Fig. 1D). NPM-ALK protein (Fig. S1B) and mRNA (Fig. S1C) were expressed at similar levels in fl/fl Pdgfrb and Δ/Δ Pdgfrb mice, suggesting that PDGFRβ does not influence NPM-ALK expression per se. Similarly, STAT3, an important downstream modulator of NPM-ALK, was strongly activated as demonstrated by tyrosine phosphorylation in 8 week-old thymi, confirming that NPM-ALK activity is independent of PDGFRβ expression (Fig. S1D) [33].

We next compared tumor development and survival rates of fl/fl Pdgfrb and Δ/Δ Pdgfrb mice. Strikingly, log-rank analysis of Kaplan-Meier survival curves indicated a 1.7-fold increase in survival for the Pdgfrb knockout mice (fl/fl Pdgfrb median survival: 19.3 weeks; Δ/Δ Pdgfrb median survival: 31.9 weeks) (Fig. 1E). Of note, both animal cohorts presented with identical thymic tumor phenotypes at the experimental end point (Fig. S1E), despite the total loss of Pdgfrb in tumor cells (Fig. 1F). These data suggest that PDGFRβ expression in tumor cells shortens the time to tumor development but does not prevent NPM-ALK driven lymphomagenesis. In addition to increased survival, Δ/Δ Pdgfrb mice had a decreased thymic tumor-to-body weight ratio (Fig. S1F), but spleen-to-body weight ratios were similar among the genotypes (Fig. S1G), consistent with an absence of splenic tumor development regardless of PDGFRβ status. Histopathological analysis of various organ parenchymas showed decreased dissemination of tumor cells to the kidney and liver in Δ/Δ Pdgfrb mice, whereas no significant differences were observed when comparing dissemination into the heart and lungs (Fig. 1G, S1H). Altogether, these results demonstrate that PDGFRβ expression facilitates ALK⁺ tumor formation and dissemination, and that our generated genetic mouse model is a valid tool to study the effects of PDGFRβ in ALK⁺ ALC pathogenesis.
Fig. 1 PDGFRβ is a biomarker for aggressive ALCL. A Tissue microarrays from 98 NPM-ALK+ ALCL patients enrolled to NHL-BFM 90 and 95 studies and the ALCL99 clinical trial, were stained for total PDGFRβ by immunohistochemistry (IHC) and expression levels were correlated with event-free survival (EFS). Staining was quantified according to high (n = 11; orange line) versus low (n = 87; grey line) PDGFRβ expression. B PDGFRB and PDGFRA mRNA transcript levels (log2 median-centered intensity) comparison between (n = 6) ALCL patients and (n = 10, T cells; n = 5, CD4+; and n = 5, CD8+) healthy donors of the Piccaluga dataset [32] extrapolated from the Oncomine database. C Schematic representation of the breeding strategy to obtain a genetic knockout of Pdgfrb in CD4+ T cells harboring the human NPM-ALK oncogenic fusion under the control of the Cd4 enhancer. NPM-ALKTg littermates either expressing wild type Pdgfrb (fl/fl Pdgfrb; grey mouse) or lacking Pdgfrb in CD4+ T cells (ΔΔ Pdgfrb; orange mouse) were generated using Cre-mediated recombination driven by the Cd4 promoter. D Representative pictures of PDGFRβ IHC analysis of fl/fl Pdgfrb and ΔΔ Pdgfrb mouse thymomas at the experimental end point. Black squares in the left picture represent the area chosen for the magnification depicted on the right. Scale bars: 50 μm. Staining of (n = 6) end point thymic tumors per genotype were scanned and whole-slide quantification was performed using Definiens™ software. E Kaplan Meier cumulative survival analysis of (n = 3) wild type (black line), (n = 19) fl/fl Pdgfrb (grey line) and (n = 24) ΔΔ Pdgfrb (orange line) mice in biological replicates. Values next to the dotted lines on the x-axis indicate median life expectancy for each genotype. F Quantitative RT-qPCR of Pdgfrb mRNA transcripts from (n = 4) fl/fl Pdgfrb (grey) and (n = 4) ΔΔ Pdgfrb (orange) primary tumor cell lines. Pdgfrb levels were normalized to 18S ribosomal RNA expression and depicted as fold-change over one fl/fl Pdgfrb biological replicate set to 1. G Hematoxilin and Eosin (H&E)-stained sections of tumor dissemination into the liver of two fl/fl Pdgfrb (grey) and ΔΔ Pdgfrb (orange) mice at the experimental end point. The black dashed line represents malignant infiltration into secondary organs. Scale bars: 50 μm. A and E p values were determined by the log-rank (Mantel-Cox) test. B, D, F and G Data are shown as the means ± SD and p values were determined by the unpaired two-tailed Student’s t-test (ns = p > 0.05; * = p < 0.05; ** = p < 0.01; *** = p < 0.001; **** = p < 0.0001).
**PDGFRβ promotes autocrine growth of ALK+ ALCL cells via IL-10 secretion**

We next set out to address the mechanisms behind the observed prolongation in survival upon loss of PDGFRβ. Analysis of tumors at the experimental end point did not reveal any differences in the percentage of proliferating cells (Fig. S2A). Hence, we investigated proliferation capacity over time, as analysis of established tumors is a static observation and as such not representative of the process of lymphomagenesis. To do so, we performed in vitro proliferation assays seeding primary tumor cells at a low cell density (1×10^5 cells). In this restricted growth environment, we observed decreased proliferation for Δ/Δ Pdgfrb primary tumor cells (Fig. 2A), consistent with delayed tumor development in Δ/Δ Pdgfrb mice observed in vivo (Fig. 1E). Comparable to primary tumor cell lines, CRISPR/Cas9 knock-out of PDGFRβ in ALK+ cells (Fig. S2B) also led to a decrease in proliferation when seeded at a low cell density (Fig. 2B). We next inoculated immunodeficient (NOD.Cg-Prkdacaked, Il2rg<sup>tm1Wjl</sup>/Szl) mice with fl/fl Pdgfrb and Δ/Δ Pdgfrb primary tumor cells at low (1×10^5) and ten-fold higher (1×10^6) densities and monitored tumor initiation and development over time. Animals inoculated with a high density of fl/fl Pdgfrb cells reached the maximum tumor volume cut-off (2000 mm<sup>3</sup>) around 23 days post inoculation whereas animals inoculated with Δ/Δ Pdgfrb cells were sacrificed around 26 days post inoculation (Fig. S2C). We performed longitudinal analyzes of the tumor growth curves over the entire duration of the experiment and observed no difference in tumor volume between NSG mice inoculated with high density fl/fl Pdgfrb and Δ/Δ Pdgfrb cells (Fig. S2D). However, when NSG animals were inoculated with a low density of Δ/Δ Pdgfrb cells, we observed a significant delay in tumor growth (Fig. 2C) resulting in an average of 38 days to reach the experimental end point, compared to 29 days for the PDGFRβ expressing tumors (Fig. 2D).

Considering the role cytokines play in maintaining homeostatic T cell survival and proliferation, we hypothesized a change in the cytokine profile may be responsible for the observed effects. ProcartaPlex cytokine ELISA analysis indeed revealed that IL-10 was significantly reduced in the supernatant of Δ/Δ Pdgfrb tumor cells cultured in vitro (Fig. 2E, S2E), as were transcript levels of IL-10 (Fig. 2F). In contrast, IL-19, a cytokine of the IL-10 super-family, was detected at higher levels in the supernatant of Δ/Δ Pdgfrb tumor cells, perhaps compensating for the lack of IL-10 (Fig. 2E, S2F). Interestingly, the six ALCL patients with upregulated PDGFRβ expression [32] (Fig. 1B) also showed a downregulation of IL-10 and an upregulation of IL-10 mRNA levels in tumor cells compared to healthy control cells (Fig. 2G). Finally, using genome-wide DNA methylation data [35], we identified IL-10 hypomethylation in five ALK+ ALCL patients when compared to peripheral blood-derived activated CD3+ T cells from five healthy donors (Fig. S2G). In contrast, human control cell lines showed DNA hypermethylation of the IL-10 locus. In summary, our data suggests PDGFRβ orchestrates autocrine signaling in ALK+ ALCL cells that is sensitive to cellular density and IL-10 secretion.

**PDGFRβ activates STAT5 in ALK+ ALCL**

It has recently been shown that resistance to ALK inhibition via crizotinib is mediated by aberrant upregulation of IL-10RA rewiring the STAT3 signaling pathway in ALCL. STAT3 consecutively binds to the promoters of IL-10, IL-10RA and IL-10RB, maintaining oncogenic signaling regardless of NPM-ALK phosphorylation [6]. As we detected elevated IL-10 levels in primary tumor cells expressing PDGFRβ, we hypothesized that overexpressed PDGFRβ might additionally fuel STAT3 activity in ALCL. Supporting this hypothesis, end stage tumors developing in Δ/Δ Pdgfrb animals indeed exhibited a decrease in both active STAT3 and STAT5 levels when compared to those from fl/fl Pdgfrb mice (Fig. 3A). Because both PDGFRβ and NPM-ALK are potent tyrosine kinases, we produced kinase dead (KD) versions of NPM-ALK (K210R or D309A) or PDGFRβ (K634A or D826A) via Site Directed Mutagenesis to further understand the phosphorylation cascade (Fig. S3A). PDGFRβ and NPM-ALK in either wild type (WT) or KD forms were expressed in HEK293FT cells and as expected, neither of the KD versions were phosphorylated on the indicated tyrosine residues normally associated with activity (Fig. S3B). As anticipated, WT NPM-ALK induced STAT3 phosphorylation [36] (Fig. 3B, S3C). In contrast, PDGFRβ selectively induced STAT5 phosphorylation (Fig. 3B, S3D), suggesting that NPM-ALK and PDGFRβ have preferential STAT3 or STAT5 tyrosine kinase substrates. This was confirmed by imatinib inhibition of PDGFRβ recombinant protein activity, which prevented STAT5 phosphorylation in an in vitro kinase assay (Fig. 3C). In addition, the fl/fl Pdgfrb cell lines subjected to CRISPR/Cas9 deletion of PDGFRβ similarly showed a dampening of phosphorylated and total STAT5 (Fig. 3D). Finally, co-staining for PDGFRβ and STAT5 indicated both factors are expressed in tumor cells and do not originate from different cell populations (Fig. S3E).

We next correlated PDGFRβ and STAT5 expression in ALCL patient biopsies by dividing samples into two cohorts according to PDGFRβ high and low IHC-scores (Fig. 3E). Interestingly, patients with a high PDGFRβ IHC-score also had high STAT5 expression levels (Fig. 3F). We analyzed a second cohort of 16 ALCL...
PDGFRβ promotes autocrine growth in ALCL. **A** Live cell counts of \((n = 4)\) \(\text{fl/fl} \text{Pdgfrb}\) (grey) and \((n = 4)\) \(\Delta/\Delta \text{Pdgfrb}\) (orange) primary mouse tumor cell lines seeded in 6-well plates at low confluency \((1 \times 10^5 \text{ cells})\). Cells were stained with Trypan blue and live cell counts were recorded on days two, four and six. 

**B** Representative pictures of a \(\text{fl/fl} \text{Pdgfrb}\) cell line (\(\text{PdgfrbEV}; \text{empty vector transduced}\)) subjected to CRISPR/Cas9 deletion of \(\text{Pdgfrb}\) (\(\text{PdgfrbACRISP}\)) and seeded in a 96-well plate for a limiting dilution assay. Right graph: arbitrary fluorescence units measured using a resazurin assay were measured and correlated to the matched empty vector control \((n = 5)\).

**C** Longitudinal analysis of tumor volume increase of 6-week old immunodeficient \((\text{NOD.Cg}-\text{Prkdc}^{\text{scid}}\text{/Il2rg}^{\text{tm1Wjl/kg}}/\text{SzJ})\) female mice inoculated with either \((n = 8)\) \(\text{fl/fl Pdgfrb}\) (grey) or \((n = 8)\) \(\Delta/\Delta \text{Pdgfrb}\) (orange) primary mouse tumor cell lines at a low concentration \((1 \times 10^5 \text{ cells/flank})\).

**D** Kaplan Meier cumulative survival analysis of 6-week old NSG female mice inoculated with either \((n = 8)\) \(\text{fl/fl Pdgfrb}\) (grey) or \((n = 8)\) \(\Delta/\Delta \text{Pdgfrb}\) (orange) primary mouse tumor cell lines at a low concentration \((1 \times 10^5 \text{ cells/flank})\). Values next to the dotted lines on the x-axis indicate median life expectancy before tumor size reaches the 2000 mm\(^3\), the set threshold volume.

**E** Heatmap depicting cytokine concentrations \((\text{pg/ml})\) in the supernatants of \((n = 3)\) \(\text{fl/fl Pdgfrb}\) and \((n = 3)\) \(\Delta/\Delta \text{Pdgfrb}\) primary mouse tumor cell lines cultivated in vitro at a low density \((1 \times 10^5 \text{ cells})\). 

**F** Quantitative RT-qPCR of \(\text{Il-10}\) mRNA transcripts from \((n = 8)\) \(\text{fl/fl Pdgfrb}\) (grey) and \((n = 6)\) \(\Delta/\Delta \text{Pdgfrb}\) (orange) primary mouse tumor cell lines. Data was normalized to 18S ribosomal RNA expression.

**G** Gene comparison between \((n = 6)\) ALCL patients and healthy donors of the Piccaluga dataset \([32]\) extrapolated from the Oncomine database \([34]\). Heatmap colors represent z-scores normalized to depict relative values within rows, where blue represents least expressed and red represents most expressed.

**Notes:** Data are shown as means \pm SD, and \(p\) values were determined by the unpaired two-tailed Student’s t-test. **D** \(p\) value was determined by log-rank (Mantel-Cox) test \((ns = p > 0.05; ^{*} = p < 0.05; ^{**} = p < 0.01; ^{***} = p < 0.001; ^{****} = p < 0.0001)\).
patient samples to validate our findings and found a significant positive correlation between PDGFRβ expression and active levels of STAT5 (Fig. 3G), supporting our in vitro findings.

Inhibiting the PDGFRβ-STAT5 axis induces apoptosis of ALK+ ALC cells

STAT5 plays an essential role in maintaining self-renewal capacities of hematopoietic cells via the tight...
regulation of downstream targets such as GRANZYM B, PERFORIN, OSM, HIF2A, and BCL-2 family members [37, 38]. As STAT5 has been reported to have anti-apoptotic functions in hematopoietic cells [39], we next assessed apoptosis in the murine tumors. IHC analysis for Cleaved Caspase 3 (CC3) revealed an increase in clusters of apoptotic cells in Δ/Δ Pdgfrb tumors compared to fl/fl Pdgfrb controls (Fig. 4A). This increase in apoptotic clusters correlated with a decrease in levels of STAT5 (Fig. S4A) and its anti-apoptotic target Bcl-xL (Fig. 4B). The ALCL patient biopsies, previously identified to have high PDGFRβ IHC-scores, also had elevated Bcl-xL levels (Fig. 4C, S4B), indicating that our murine model mirrors the molecular signatures observed in human patients.

To confirm the importance of Bcl-xL in ALCL, we made use of the Piccaluga Lymphoma dataset [32] and observed significant upregulation of BCL2L1 (Bcl-xL) expression when compared to healthy donor T cells, whereas BCL2 and MCL1 were not significantly upregulated (Fig. 4D). These data suggest that STAT5 drives cell survival by activation of pro-survival pathways preventing apoptosis. Indeed, by re-analyzing several publicly available ChIP-Seq datasets derived from human and murine cell lines or ex-vivo models, we identified binding of both active STAT3 and STAT5 to the promoter of Bcl-xL (Fig. 4E, S4C and D). As a biological read-out, we performed a 7AAD/Annexin V FACs co-staining and identified higher levels of apoptosis in our primary tumor cells (Fig. 4F, S4F), whereas the cell cycle was not significantly affected (Fig. S4F). Western blot analysis revealed that inhibition of STAT activity using the SH2 domain small molecular weight inhibitor AC-4-130, successfully induces apoptosis via CC3 (Fig. 4G, S4G). Our data suggest that genetic deletion or inhibition of the PDGFRβ-STAT5 axis induces a CC3-mediated apoptotic phenotype in ALK+ ALCL.

STAT5A and STAT5B are essential for unrestricted cell proliferation
To determine whether the two STAT5 gene products mediate apoptosis to the same extent on a PDGFRβ+ background, we proceeded to delete either STAT5A, STAT5B or both genes simultaneously (Fig. S5A). CRISPR/Cas9 deletion of either Stat5a or Stat5b resulted in complete gene knock-out within the bulk population. On the contrary, the guide RNA designed to target both Stat5a/b only resulted in a knockdown of either gene product within the bulk population (Fig. 5A). Knockdown of Stat5a/b resulted in a decrease in proliferation similar to that achieved following single Stat5a or Stat5b knock-out (Fig. 5B), suggesting at least one of the two gene products is necessary for survival. To confirm this hypothesis, we next attempted to isolate single clones from Stat5a/bACRISPR bulk populations. Sanger sequencing coupled with Western blot analysis suggested simultaneous deletion of Stat5a/b is lethal for ALK+ ALCL cells as we never obtained a clone with a complete deletion (Fig. S5B).

To track the effects of the double deletion of Stat5a/b over time we next performed a CRISPR/Cas9-based competition assay (Fig. 5C). We firstly transduced our primary tumor cell line derived from a fl/fl Pdgfrb mouse with a vector expressing SpCas9 and GFP. Following validation, we transduced the stably SpCas9-GFP-expressing clones with a vector expressing our sgRNAs of interest and mCherry. A competition assay was used to monitor competing growth kinetics of sgRNA-expressing mCherry and SpCas9-GFP positive (mCherry+) cells versus non-targeting SpCas9-GFP (GFP+) cells. Deletion of either Stat5a, Stat5b or Stat3 did not result in a significant decrease in viability indicated by the elevated expression of mCherry+ cells 39 days post transduction (Fig. 5C, S5D). On the contrary, the double deletion of Stat5a/b resulted in a decrease in mCherry+ vs GFP+ cells, suggesting the
loss of both gene products impacts viability (Fig. 5D). Normalizing the percentage of Stat5a/b<sup>Δ</sup>CRISPR mCherry<sup>+</sup> cells to either Stat5a<sup>Δ</sup>CRISPR or Stat5b<sup>Δ</sup>CRISPR indicated no selective preference for either gene product over time (Fig. 5E, F). These data suggests that loss of Stat5a/b is lethal for PDGFRβ<sup>+</sup> ALK<sup>+</sup> ALCL cells, providing a rational for the use of STAT inhibitors.

**Targeting STAT3/5 in vivo is therapeutically relevant in ALK<sup>+</sup> ALCL**

Having identified the PDGFRβ-STAT5 axis as an important oncogenic driver in ALCL, we next assessed the efficacy of the STAT inhibitor AC-4-130 in vivo. The original publication that identified AC-4-130 as a selective STAT5 inhibitor, postulated AC-4-130 might also
influence STAT1 and STAT3 activity [40]. Due to the dependency of the JAK/STAT signaling pathway in ALCL we hypothesized AC-4-130 would be an attractive therapeutic strategy in our PDGFRβ
$^+$ ALCL model. We engrafted $^{fl/fl}$ Pdgfrb and $^{Δ/Δ}$ Pdgfrb tumor cells subcutaneously into 6-week old NSG mice. Once tumors reached palpable dimensions, mice were treated daily with either vehicle (10% DMSO, 5% Cremophore in PBS) or single agent AC-4-130 (25 mg/kg) (Fig. S6A). AC-4-130 treatment administered via intraperitoneal injection was well tolerated with no significant decrease in animal weight nor toxicity (Fig. S6B, C and [40]). Longitudinal analysis of tumor volume over time indicated that $^{Δ/Δ}$ Pdgfrb tumors grew at a significantly slower rate than $^{fl/fl}$ Pdgfrb tumors, mirroring the phenotype observed in the transgenic mouse model (Fig. 1E). Strikingly, single agent AC-4-130 treatment led to a delay in tumor growth in both experimental cohorts relative to vehicle controls (Fig. 6A). All experimental animals were sacrificed when the $^{fl/fl}$ Pdgfrb vehicle control tumors reached 2000 mm$^3$ in size. At the experimental end point, AC-4-130 treated mice, in both cohorts, showed a significant decrease in tumor-to-body weight ratio (Fig. 6B). This experiment was repeated with a second set (biological replicates) of $^{fl/fl}$ Pdgfrb and $^{Δ/Δ}$ Pdgfrb tumor cells. Consistently, treatment with AC-4-130 resulted in a significant

![Graph showing protein levels and viability](image-url)
Residual masses following AC-4-130 treatment were harvested and morphologically analyzed via IHC. Macroscopically, xenografted tumors recapitulated the histological features of ALK+ tumors derived from Cdk4-NPM-ALK transgenic mice. H&E staining revealed small, round monomorphous cells with characteristically necrotic/apoptotic regions in the treated tumors (Fig. 6C). Consistent with treatment efficacy, immunostaining for Ki67 revealed a significant decrease in proliferating lymphoma cells upon AC-4-130 treatment (Fig. 6D). As previously reported, AC-4-130 treatment also resulted in a decrease in PDGFRβ expression (Fig. 6D and [40]). Western blot analysis of excised tumors indicated that AC-4-130 treatment decreases the activity of STAT5 and STAT3 in vivo (Fig. 6E and S6F), potentially due to the interwoven signaling network between NPM-ALK/STAT3 and PDGFRβ/STAT5.

Discussion
Imatinib has previously been identified as an effective treatment for PDGFRβ+ ALK+ ALC relapse patients, however the mechanisms of action and the factual contribution of PDGFRβ remained unanswered [26]. We show here that T cell-specific deletion of PDGFRβ in a genetically engineered mouse model mimicking human ALC in vivo, leads to delayed tumor growth and prolonged survival. To decipher the central signaling executor amongst the large repertoire of downstream targets activated by PDGFRβ, we used a tool kit of in vitro and in vivo models and identified that PDGFRβ signals preferentially via STAT5. The activation of the PDGFRβ/STAT5 axis in tumor cells results in a malignant boost by increasing autocrine-dependent proliferation and evading apoptosis. Treatment with the STAT5 SH2-domain inhibitor AC-4-130, results in a reduction in tumor growth in ALK+ ALC, irrespective of PDGFRβ expression. Interestingly, we observed a decrease in both STAT3 and STAT5 following in vivo AC-4-130 treatment. This effect on STAT3 and STAT5 was already observed by Wingelhofer et al., who discussed that AC-4-130 targets first cellular STAT5 at pharmacologically relevant concentrations while having smaller effects on STAT3 and STAT1 [40]. We therefore suggest these “smaller effects” are accentuated in diseases such as ALC that centrally rely on STAT3 activity to maintain malignancy. Our findings suggest that STAT5 plays a crucial role in ALC oncogenesis and that inhibition of the PDGFRβ/STAT5 axis, but also of the NPM-ALK-STAT3 axis, both upstream via imatinib/ALK inhibitors and downstream with AC-4-130, is therapeutically relevant in ALK+ ALC.

Murine thymic lymphoma cells lacking PDGFRβ proliferate at slower rates both in vitro (Fig. 2A) and when implanted subcutaneously into NSG mice (Fig. 2C). In contrast to ALK+ T cells lacking Tyk2 [8], deletion of PDGFRβ resulted in reduced proliferation suggesting that PDGFRβ acts as a malignant boost. Cytokine screening indicated that IL-10 is significantly downregulated in Δ/Δ Pdgfrb primary tumor cell lines providing an explanation for the observed density-dependent proliferation defect. IL-10 is one of the most abundant cytokines in peripheral blood of children diagnosed with ALC [41] and it is readily detected in both systemic and cutaneous ALC [42]. Our data echoes previous findings which demonstrated that IL-10 is central for TYK2-mediated STAT1 and STAT3 activity in ALC [8]. More recently, the IL-10R was found to directly activate STAT3, bypassing NPM-ALK, to bind to the promoters of IL-10, IL-10RA and IL-10RB [6]. Our findings, which are supported by two independent lymphoma patient datasets (Fig. 1B), propose PDGFRβ is yet another potent kinase in the list of regulators of IL-10 expression in ALC and that targeting the PDGFRβ/STAT5/IL-10 axis is an attractive therapeutic strategy. In parallel to the observed decrease of IL-10, we detected an increase in secreted IL-19 upon loss of PDGFRβ (Fig. 2E). IL-19 is a member of the IL-10 family and participates in the resolution of inflammation in the lung by recruiting macrophages and DCs [43]. While we observed a reduction in PDGFRβ expression and consequent decrease in IL-10 expression, the mechanism by which IL-19 expression is increased needs to be further elucidated. In the future, we aim to use ALC patient datasets to study the expression levels and the interplay of the PDGFRβ/STAT5 axis with other signaling pathways in ALC relapse patients.
cytokine superfamily and forms part of a gene cluster alongside IL-10 and IL-20 located on chromosome 1q32 [43, 44]. Interestingly IL-19, which has been reported to play both pro- and anti-inflammatory roles, induces STAT1 and STAT3 activation via binding to IL-20Rα and IL20-Rβ [44, 45]. It is thus attractive to speculate that in the absence of PDGFRβ, alternative pathways capable of mediating STAT phosphorylation, such as IL-19/IL-20Rα/β-STAT3 node, could compensate for decreased STAT activity.
To date STAT3 [46] dominates the spotlight of ALCL research although recent findings have begun to unravel the important involvement of STAT1 [8] and STAT5 [47]. In particular, STAT5A/B exhibit important functions in the pathogenesis of hematological neoplasias due to their involvement in lymphoid and myeloid cell differentiation [38, 48–50]. In human ALK+ ALCL cell lines, the two different STAT5 gene products were identified to play divergent functions [9]. Nevertheless, our data indicate both STAT5A and STAT5B are equally expressed in PDGFRβ ALK+ ALCL. Deletion of either Stat3, Stat5a or Stat5b did not result in cell death nor a detectable upregulation of other Stat family members, advocating for a defined Stat function during ALK+ ALCL development. However, we did not achieve a complete knockout of both Stat5a and Stat5b gene products simultaneously, suggesting that at least one of these remained active and was sufficient to maintain cellular homeostasis (Fig. 5D, S5B). In contrast to peripheral T cell leukemia/lymphoma (PTCL) [48, 51, 52], we did not observe a selective preference for maintenance of either Stat5a or Stat5b in malignant ALK+ cells expressing PDGFRβ. Kinetic tracking of viability, using mCherry expression, indicated knockdown of both Stat5a/b resulted in a significant decrease in cell viability, however this population was not completely lost over time, hinting towards sequential activation of STAT3/5 activity in ALK+ ALCL.

The elevated levels of STAT3/5 in ALK+ patient samples, renders them attractive therapeutic targets as blocking would preferentially affect malignant lymphocytes while leaving homeostatic lymphopoiesis unaffected. In vivo and in vitro AC-4-130 treatments resulted in an induction of apoptosis mediated via CC3 and Bcl-xL in ALK+ lymphocytes. Interestingly, we found that Stat3 binds to super-enhancers that regulate high expression of other Stat family members, advocating for a defined Stat function during ALK+ ALCL development. However, we did not achieve a complete knockout of both Stat5a and Stat5b gene products simultaneously, suggesting that at least one of these remained active and was sufficient to maintain cellular homeostasis (Fig. 5D, S5B). In contrast to peripheral T cell leukemia/lymphoma (PTCL) [48, 51, 52], we did not observe a selective preference for maintenance of either Stat5a or Stat5b in malignant ALK+ cells expressing PDGFRβ. Kinetic tracking of viability, using mCherry expression, indicated knockdown of both Stat5a/b resulted in a significant decrease in cell viability, however this population was not completely lost over time, hinting towards sequential activation of STAT3/5 activity in ALK+ ALCL.

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In conclusion, we identify the PDGFRβ/STAT5 axis acts as a booster of malignancy, operating in parallel to the oncogenic NPM-ALK-STAT3 signaling cascade, resulting in a more aggressive ALCL disease entity. Our results highlight the importance of both STAT5A and STAT5B in ALK+ ALCL and suggest that blockade of STAT3/5 activity represents a new therapeutic strategy for the treatment of ALCL refractory patients. However, the use of STAT inhibitors will need to be rigorously assessed for their clinical applicability in the future. Thus, targeting activators upstream of the STATs, such as PDGFRβ and NPM-ALK, remains a highly relevant approach.

Abbreviations
ALCL: Anaplastic Large Cell Lymphoma; ALK: Anaplastic Lymphoma Kinase; AML: Acute Myeloid Leukemia; AP-1: Activator Protein-1 Transcription Factor Superfamily; ATP: Adenosine Tri-phosphate; BCL2: B-cell Lymphoma 2; Bcl-xL: B-cell Lymphoma Extra-Large; BCR-ABL: Breakpoint Cluster Region-Abelson Murine Leukemia Viral Oncogene; BIA-ALCL: Breast-Implant Associated ALCL; BFM: Berlin-Frankfurt-Münster Chemotherapy regimen; bZIP: Basic Leucine-Zipper Domain; ChIP: Chromatin ImmunoPrecipitation; ChOP: Polychemotherapy: Cyclophosphamide, Doxorubicin, Vincretin and Prednisone; CML: Chronic Myelogenous Leukemia; CRE: cAMP Response Element; CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats; DNA: Deoxyribonucleic Acid; EFS: Event Free Survival; ERK: Extra Cellular Signaling-Regulated Kinase; FDA: U.S Food and Drug Administration; FFPE: Fresh Frozen Paraffin Embedded; HEK293: Human Embryonic Kidney Cells; HL: Hodgkin Lymphoma; IL: Interleukin; JAK: Janus Kinase; MF: Mouse Embryonic Fibroblasts; mTOR: Mammalian Target of Rapamyacin; MYB: Myeloblastosis Viral Oncogene Homolog; NHL: Non-Hodgkin Lymphoma; NPM: Nucleophosmin1; NSCLC: Non Small Lung Cancer; NGF: NOD-SCID IId2™™. OS: Overall Survival; PDGFR: Platelet Derived Growth Factor Receptor; P38K/AKT: Phosphatidylinositol-4,5-bisphosphate 3-kinase; PTCL: Peripheral T cell Lymphoma; SH2: Src Homology 2; SRC Homology 2; STAT: Signal Transducers and Activators of Transcription; TKI: Tyrosine Kinase Inhibitor; TYK2: Tyrosine Kinase 2; WHO: World Health Organization.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12943-022-01640-7.

Additional file 1: Supplementary Fig. 1 A). Genotyping PCR from purified genomic mouse tail DNA after Cre expression in fl/fl Pdgfrb and ∆/∆ Pdgfrb samples. Depicted are results for ALK, Cdx4-CRE and the Pdgfrb alleles (without loxp sites: wild type (wt), with loxp sites: fl/fl and the recombined (∆) Pdgfrb locus. Genomic mouse tail DNA from a PDGFRβ fl/+ animal was used as the PDGFRβ genotyping PCR positive control. Expected fragment band sizes: ALK (TG/+): 177 bp, Cdx4-CRE (TG/+): 316 bp and the Pdgfrb (WT: 498 bp and fl/fl: 562 bp). B). Representative pictures of ALK IHC analysis of fl/fl Pdgfrb and ∆/∆ Pdgfrb mouse thymuses at experimental end point. Scale bars: 50 μm. Black squares in the left picture represent the area chosen for the magnification depicted on the right. Staining of n = 9 tumor samples per genotype were scanned and whole-slide quantified using the Definiens™ software. C). Quantitative RT-qPCR based quantification of NPM-ALK mRNA transcripts from: (n = 4) control animals (black), (n = 4) Cdx4-CRE and ∆/∆ Pdgfrb mice (pink), (n = 7) fl/fl Pdgfrb 8-week-old developing thymus (dark pink).
grey), (n = 6) Δ/Δ PDGFRβ 8-week-old developing thymy (red); (n = 5) end point thymomas (light grey); and (n = 8) end point thymomas (orange). Data was normalized to 18S ribosomal RNA and depicted as a fold-change over one fl/fl PDGFRβ biological replicate set to 1. D). Western blot analysis showing protein levels of phospho (p) STAT3 and total STAT3 for 8-week-old pre-thymoma lyses: (n = 3) control; (n = 3) fl/fl PDGFRβ and (n = 3) Δ/Δ PDGFRβ. GAPDH serves as loading control. The molecular weight of analyzed proteins is shown on the left. E). Western blot showing protein levels of PDGFRβ, phospho (p) STAT3 and total STAT3 in HEK293FT transfected cell lysates. GAPDH serves as loading control. The molecular weight of analyzed proteins in kiloDaltons (KDAs) is indicated. D). Western blot showing protein levels of PDGFRβ, phospho (p) STAT5 and total STAT5 in HEK293FT transfected cell lysates. GAPDH serves as loading control. The molecular weight of analyzed proteins in kiloDaltons (KDAs) is shown on the left. Phospho (p) STAT5 levels over total STAT5 are depicted as relative protein expression in % (B). Double immunofluorescence staining of fl/fl PDGFRβ and Δ/Δ PDGFRβ primary tumor cells derived from our transgenic Cd4-NPM-ALK Tg/+ mouse. Cells were fixed and stained with antibodies against PDGFRβ (green) and STAT3 (red). Cells were counterstained with DAPI (blue). Pictures were acquired with identical pixel density, image resolution, and exposure time using a confocal LSM Observer Z1. Image Scale; bars: 5 μm. A). Representative pictures of total STAT3 IHC analysis of (n = 8) fl/fl PDGFRβ and (n = 7) Δ/Δ PDGFRβ primary tumor cells at experimental end point. B). Representative pictures of CD11c+ IHC analysis and the PDGFRβ grading scheme used to quantify staining of tissue microarrays from human ALCL tumor samples. C). Normalized ChiP-seq alignment track for STAT3 in MAC1 cells, and normalized CUT&RUN alignment tracks for STAT3 in NCI60, HPC7, and JB6 cells, overlaid with H3K27ac ChIP-seq, shown at the gene loci for BCL2L1 (Bcl-xL). D). Western blot showing protein levels of PDGFRβ, phospho (p) STAT3 and total STAT3 in HEK293FT transfected cell lysates. GAPDH serves as loading control. The molecular weight of analyzed proteins in kiloDaltons (KDAs) is shown on the left. C). Western blot showing protein levels of total PDGFRβ, total STAT3 and total STAT5 at experimental end point thymomas (grey) and (n = 17) Δ/Δ PDGFRβ (orange) mice normalized to whole body weight at experimental end point. D). Western blot showing protein levels of PDGFRβ in three fl/fl PDGFRβ primary tumor cell lines subjected to CRISPR/Cas9 deletion of PDGFRβ (PDGFRβKO) and empty vector transduced control (PDGFRβ). GAPDH serves as loading control. The molecular weight of analyzed proteins in kiloDaltons (KDa) is shown on the left. A). Schematic representation of individual CRISPR guide RNAs designed to target the genomic murine PDGFRβ locus. The sequence of the guide RNA is depicted in blue and the protospacer adjacent motif (PAM) in red. B). Western blot showing protein levels of PDGFRβ in three fl/fl PDGFRβ primary tumor cell lines subjected to CRISPR/Cas9 deletion of PDGFRβ (PDGFRβKO) and empty vector transduced control (PDGFRβ). GAPDH serves as loading control. The molecular weight of analyzed proteins in kiloDaltons (KDa) is indicated.
at 10 days post inoculation with either \((n = 3)\) fl/fl Pdgfrb and \((n = 3)\) Δ/Δ Pdgfrb cell lines at experimental end point following vehicle or AC-4-130 treatment in two replicate experiments. **. Tumor volume (mm\(^3\)) increase of \((n = 3)\) fl/fl Pdgfrb and \((n = 3)\) Δ/Δ Pdgfrb inoculated NSG 6-week-old female mice treated with either vehicle or AC-4-130. **. Tumor to body weight ratio in % at experimental end point of NSG mice treated with either \((n = 3)\) vehicle or \((n = 3)\) AC-4-130. **. Western blot showing protein levels of PDGFRβ and Bcl-x\(_L\) in end point tumors excised from \((n = 3)\) fl/fl Pdgfrb and \((n = 3)\) Δ/Δ Pdgfrb inoculated NSG 6-week-old female mice treated with either vehicle or AC-4-130. GAPDH serves as loading control. The molecular weight of analyzed proteins in kiloDaltons (KDa) is shown on the left: PDGFRβ levels over GAPDH and Bcl-x\(_L\) levels over GAPDH are depicted as relative volume in %, B, C, E, and F Data are shown as mean±SD, and p values were determined by unpaired two-tailed Student's t-tests (multiple t-test application on GraphPad).

**p < 0.05; ***p < 0.001; ****p < 0.0001.

Additional file 2. Supplementary Materials and Methods.

Additional file 3: Supplementary Table 1. Genotyping primers. Supplementary Table 2. Antibody list. Supplementary Table 3. CUT&RUN and ChIP-Seq Antibodies. Supplementary Table 4. Guide RNAs. Supplementary Table 5. RT-qPCR primers.

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Conceptualization: IG, SL, LK. Funding acquisition: LK, SdT, OM. Formal analysis: IG, LZ, IW, PK, SE, CG, MZ, SK, GT, MK, HAN, SD, MS, BSS, TL, CP, OP, SH, ST, OM, AIS, CK, NP, MZ, BA, IA, LQM, SM, PW, DS, PBS, GE, WK, WW, TAL, PG, SdT, RM, SL, LK. Investigation: IG, LZ, IW, PK, MS, GT, SE, CG, MK, HAN, SD, BSS, SK, TL, MZ, M2I, NP, CP, OP, SH, ST, OM, AIS, CK, GE, LQM, WW, SdT, RM, SL, LK. Methodology: IG, PK, MS, GT, SE, OP, ST, SH, AIS, CK, TAL, BA, MZI. Project administration: IG, SL, LK. Resources: GT, HAN, SD, OP, SH, ST, OM, AIS, CK, PW, DS, PS, GE, JA, LQM, SM, WK, WW, ATL, PG, SdT, RM, SL, LK. Supervision: LK, SL. Validation and Visualization: IG. Writing – original draft: IG. Writing – review & editing: All. The author(s) read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during the study are included in the manuscript and supplementary information files. The following publicly available datasets were used: the Piccaluga Lymphoma dataset [32] using the Oncomine™ Research Premium Edition database (Thermo Fisher, Ann Arbor, MI) [34] and the human genomic DNA methylation dataset retrieved from Menotti et al., 2016 (data analyzed and tracked on UCSC genome browser), STAT3 human ALK+ (86) and ALK− (FE-PD and MAC2A) ChIP-Seq data originate from Menotti et al., 2019. Murine STAT3 and STAT5 ChIP-Seq data were obtained from Li et al., 2017 and Liao et al., 2011.

Declarations

Ethics approval and consent to participate

Institutional Review Board Statement: The use of clinical material was approved by Research Ethics Committee of the Medical University of Vienna, Austria (no.143/2016 and 1221/2019) and the University Hospital Brno, Czech Republic (no. 4–306/13/S). All human samples were obtained with informed written consent and in accordance with the Declaration of Helsinki. All animal experiments were carried out according to the animal license protocols (BMWF-66.009/0183-WF-V/3b/2017, BMWF-66.009/0057-V/3b/2018, BMWF-66.009/0401-V/3b/2018, 2020–0103.412) approved by the ethics committee of the Medical University of Vienna and the Austrian Federal Ministry of Science and Research.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127(20):2375–90. https://doi.org/10.1182/blood-2016-04-643569.
2. Stein H, Foss HD, Dürrkop H, et al. CD38+B+ anaplastic large cell lymphoma: a review of its histopathologic, genetic, and clinical features. Blood. 2000;96(12):3681–95. 11000048.
3. Morris SW, Kistene MN, Valentine MB, et al. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin’s lymphoma. Science. 1994;263(5151):1281–4.
4. Ducray SP, Natarajan K, Garland GD, Turner SD, Egger G. The transcriptional roles of ALK fusion proteins in tumorigenesis. Cancers (Basel). 2019;11(8):1–23. https://doi.org/10.3390/cancers11081074.
5. Marzec M, Zhang Q, Goradia A, et al. Oncogenic kinase NPM/ALK induces STAT3 expression of immunosuppressive protein CD274 (PD-L1). Proc Natl Acad Sci U S A. 2008;105(52):20852–7. https://doi.org/10.1073/pnas.0810958105.
6. Prokopf N, Probst NA, Lee LC, et al. IL10RA modulates crotontin sensitivity in NPM1-ALK+ anaplastic large cell lymphoma. Blood. 2020;136(14):1657–69. https://doi.org/10.1182/blood.2019003793.
7. Charle R, Simmons WJ, Cai H, et al. STAT3 is required for ALK-mediated lymphomagenesis and provides a possible therapeutic target. Nat Med. 2005;11(6):623–9. https://doi.org/10.1038/nm1249.
8. Prutsch N, Gunhofer E, Suske T, et al. Dependency on the TYK2/STAT1/MCL1 axis in anaplastic large cell lymphoma. Leukemia. 2019;33(3):696–709. https://doi.org/10.1038/s41375-018-0239-1.
9. Zhang Q, Wang HY, Liu X, Wasik MA. STAT3 is epigenetically silenced by the tyrosine kinase NPM1-ALK and acts as a tumor suppressor by reciprocally inhibiting NPM1-ALK expression. Nat Med. 2007. https://doi.org/10.1038/nm1659.
10. Brugieres MC, Le Deley H, Pacquement D, et al. CD30+ Anaplastic Large-Cell Lymphoma in Children: Analysis of 82 Patients Enrolled in Two Consecutive Studies of the French Society of Pediatric Oncology By; 1998. p. 3591–8.
11. Rosolen A, Pillon M, Garaventa A, et al. Anaplastic large cell lymphoma treated with a leukemia-like therapy: report of the Italian Association of Pediatric Hematology and Oncology (AIEOP) LNHI-92 protocol. Cancer. 2005;104(10):2133–40. https://doi.org/10.1002/cncr.21438.
12. Seidemann K, Tiemann M, Schrappe M, et al. Short-pulse B-non-Hodgkin lymphoma-type chemotherapy is efficacious treatment for pediatric anaplastic large cell lymphoma: a report of the Berlin-Frankfurt-Munster group trial NHL-BFM 90. Blood. 2001;97(12):3699–706. https://doi.org/10.1182/blood.v97.12.3699.
13. Laver JH, Kraveka JM, Hutchison RE, et al. Advanced-stage large-cell lymphoma in children and adolescents: results of a randomized trial incorporating intermediate-dose methotrexate and high-dose cytarabine in the maintenance phase of the APO regimen: a pediatric oncology group phase III trial. J Clin Oncol. 2005;23(3):541–7. https://doi.org/10.1200/JCO.2005.11.075.
14. Prokopf N, Larose H, Lim MS, Burke GAA, Turner SD. Treatment options for paediatric anaplastic large cell lymphoma (ALCL): current standard
and beyond. Cancers (Basel). 2018;10(4):1–18. https://doi.org/10.3390/cancers10040099.

15. Shaw AY, Kim TM, Cinó L, et al. Ceritinib versus chemotherapy in patients with ALK-rearranged non-small-cell lung cancer previously given chemotherapy and crizotinib (ASCEND-5): a randomised, controlled, open-label, phase 3 trial. Lancet Oncol. 2017;18(7):874–86. https://doi.org/10.1016/S1470-2045(17)30339-X.

16. Seto T, Nishio M, Hida T, et al. Final PFS analysis and safety data from the phase III J-ALEX study of alecibib (ALC) vs. crizotinib (CRZ) in ALK-inhibitor naive ALK-positive non-small cell lung cancer (ALK+/NSCLC). J Clin Oncol. 2019;37(Suppl 1):9002. https://doi.org/10.1200/JCO.2019.37.15_suppl.9002.

17. Camidge DR, Dzidzouskzho R, Peters S, et al. Updated Efficacy and Safety Data and Impact of the EML4-ALK Fusion Variant on the Efficacy of Alectinib in Untreated ALK-Positive Advanced Non-Small Cell Lung Cancer in the Global Phase III ALEX Study. J Thorac Oncol. 2019;14(7):1233–43. https://doi.org/10.1016/j.jtho.2019.03.007.

18. Huber RM, Hansen KH, Paz-Ares Rodríguez L, et al. Brigatinib in Crizotinib-refractory ALK+/NSCLC: 2-year follow-up on systemic and intracranial outcomes in the phase 2 ALTA trial. J Thorac Oncol. 2020;15(3):404–15. https://doi.org/10.1016/j.jtho.2019.11.004.

19. Camidge DR, Kim HR, Ahn M-J, et al. Brigatinib Versus Crizotinib in Advanced ALK Inhibitor-Naive ALK-Positive Non-Small Cell Lung Cancer: Second Interim Analysis of the Phase III ALTA-1L Trial. J Clin Oncol. 2020;38(31):3592–603. https://doi.org/10.1200/JCO.20.00505.

20. Solomon BJ, Besse B, Bauer TM, et al. Lorlatinib in patients with ALK-positive non-small-cell lung cancer: results from a global phase 2 study. Lancet Oncol. 2018;19(12):1654–67. https://doi.org/10.1016/S1470-2045(18)30649-1.

21. Drilon A, Siena S, Ou S-H, et al. Safety and antitumor activity of the multi-targeted Pan-TRK, ROS1, and ALK inhibitor Entrectinib: combined results from two phase I trials (ALKA-320-001 and STARTTRK-1). Cancer Discov. 2017;7(4):400–9. https://doi.org/10.1158/2159-8290.CD-16-1237.

22. Sharma GG, Moa I, Molinogi L, Pat решуко E, Gambacorti-Passerini C, Charle R. Tumor resistance against ALK targeted therapy-where it comes from and where it goes. Cancers (Basel). 2018;10(3). https://doi.org/10.3390/cancers10030062.

23. Cristina V, La Mantia M, Iacono F, Galvanò A, Russo A, Bazan V. The emerging therapeutic landscape of ALK inhibitors in non-small cell lung cancer. Pharmaceuticals. 2020;13(12):474. https://doi.org/10.3390/ph13120474.

24. Gambacorti-Passerini C, Mussolin L, Bruegger L. Abrupt relapse of ALK-positive lymphoma after discontinuation of Crizotinib N Engl J Med. 2016;374(1):95–6. https://doi.org/10.1056/NEJMoa1511045.

25. Staber PB, Veesley P, Haq N, et al. The oncoprotein NPM-ALK of anaplastic large-cell lymphoma induces JUNB transcription via ERK1/2 and JunB phosphorylation. Blood. 2007;110(9):3374–83. https://doi.org/10.1182/blood-2007-02-071258.

26. Laimer D, Dolznig H, Kollmann K, et al. PDGFR blockade is a rational strategy and where it goes. Cancers (Basel). 2018;10(3). https://doi.org/10.3390/cancers10030062.

27. Staber PB, Knörr F, Damm-Welk C, Ruf S, et al. Blood cytokine concentrations in pediatric neuroblastomas and is activated through mechanisms including enhancer hijacking and focal enhancer amplification. Cancer Discov. 2020;10(5):320–35. https://doi.org/10.1158/2159-8290.CD-19-0993.

28. Piccaluga PP, Agostinelli C, Califano A, et al. Gene expression analysis of peripheral T cell lymphoma, unexplained, reveals distinct profiles and new potential therapeutic targets. J Clin Invest. 2007;117(3):823–34. https://doi.org/10.1172/JCI26833.

29. Redi E, Sheliabani-Tezrieri R, Cardona CDJ, et al. Requirement of DNMT1 to orchestrate epigenomic reprogramming for NPM-ALK-driven lymphomagenesis. Life Sci Alliance. 2021;4(2):1–22. https://doi.org/10.26508/lsa.202000794.

30. Rhodes DR, Yu J, Shanker K, et al. ONCOMINE: a Cancer microarray database and integrated data-mining platform. Neoplasia. 2004;6(1):1–6.

31. Reddy MR, Pulverer W, Lakshminarasimhan R, et al. Insights into the pathogenesis of anaplastic large-cell lymphoma through genome-wide DNA methylation profiling. Cell Rep. 2016;17(2):596–608. https://doi.org/10.1016/j.celrep.2016.09.018.

32. Zamo A, Charle R, Piva R, et al. Anaplastic lymphoma kinase (ALK) activ‑
ates Stat3 and protects hematopoietic cells from cell death. Oncogene. 2002;21(7):1038–47. https://doi.org/10.1038/sj.onc.1205152.

33. Redl E, Sheibani‑Tezerji R, Cardona C, et al. Requirement of DNMT1 for orchestration of epigenomic reprogramming for NPM‑ALK‑driven lymphomagenesis. Life Sci Alliance. 2021;4(2):1–22. https://doi.org/10.26508/lsa.202000794.

34. Rhodes DR, Yu J, Shanker K, et al. ONCOMINE: a Cancer microarray database and integrated data-mining platform. Neoplasia. 2004;6(1):1–6.

35. Hassler MR, Pulverer W, Lakshminarasimhan R, et al. Insights into the pathogenesis of anaplastic large-cell lymphoma through genome-wide DNA methylation profiling. Cell Rep. 2016;17(2):596–608. https://doi.org/10.1016/j.celrep.2016.09.018.

36. Zamo A, Charle R, Piva R, et al. Anaplastic lymphoma kinase (ALK) acti‑
ates Stat3 and protects hematopoietic cells from cell death. Oncogene. 2002;21(7):1038–47. https://doi.org/10.1038/sj.onc.1205152.
54. Li P, Mitra S, Spolski R, et al. STAT5-mediated chromatin interactions in superenhancers activate IL-2 highly inducible genes: functional dissection of the Il2ra gene locus. Proc Natl Acad Sci. 2017;114(46):12111–9. https://doi.org/10.1073/pnas.1714019114.

55. Park HJ, Li J, Hannah R, et al. Cytokine-induced megakaryocytic differentiation is regulated by genome-wide loss of a <scp>uSTAT</scp> transcriptional program. EMBO J. 2016;35(6):580–94. https://doi.org/10.15252/embj.201592383.

56. Jain RK, Laidenranta J, Fukumura D. Targeting PDGF signaling in carcinoma-associated fibroblasts controls cervical Cancer in mouse model. PLoS Med. 2008;5(1):e24. https://doi.org/10.1371/journal.pmed.0050024.

57. Mathew P, Thall PF, Bucana CD, et al. Platelet-derived growth factor receptor inhibition and chemotherapy for castration-resistant prostate Cancer with bone metastases. Clin Cancer Res. 2007;13(19):5816–24. https://doi.org/10.1158/1078-0432.CCR-07-1269.

58. Mohanty SK, Yagiz K, Pradhan D, et al. STAT3 and STAT5A are potential therapeutic targets in castration-resistant prostate cancer. Oncotarget. 2017;8(49):85967–6010. https://doi.org/10.18632/oncotarget.20844.

59. Morgan EL, Macdonald A. JAK2 inhibition impairs proliferation and Sensitises cervical Cancer cells to cisplatin-induced cell death. Cancers (Basel). 2019;11(12):1934. https://doi.org/10.3390/cancers11121934.

60. Liao W, Lin J-X, Wang L, Li P, Leonard WJ. Modulation of cytokine receptors by IL-2 broadly regulates differentiation into helper T cell lineages. Nat Immunol. 2011;12(6):551–9. https://doi.org/10.1038/ni.2030.

61. Sanjana NE, Shalem O, Zhang F. Improved vectors and genome-wide libraries for CRISPR screening. Nat Publ Gr. 2014;11. https://doi.org/10.1038/nmeth.3047.

62. Shah RR, Choleswa-Waclaw J, Davies FCJ, Paton KM, Chalighe R, Heard E, Abbott CM, Bird AP. Efficient and versatile CRISPR engineering of human neurons in culture to model neurological disorders. Wellcome Open Res. 2016;1:13. https://doi.org/10.12688/wellcomeopenres.10011.1.

63. Lagger S, Meunier D, Mikula M, Brunmeir R, Schiederer M, Artaker M, Pusch O, Egger G, Hagelkruys A, Mikulits W, Weitzen G, Mueller EW, Susani M, Kenner L, Seiser C. Crucial function of histone deacetylase 1 for differentiation of teratomas in mice and humans. EMBO J. 2010;29(23):3992–4007. https://doi.org/10.1038/emboj.2010.264.

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