Regulation of oncogenic transcription and tumor growth in pediatric cancers by the CDK9 inhibitor KB-0742

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Background

Disruption of transcriptional regulatory networks that drive normal cellular differentiation and development can result in oncogenic transformation and transcriptional addiction. Many pediatric sarcomas are defined by/harbor oncogenic fusion proteins, resulting from chromosomal translocations such as the EWSR1 gene fused to an ETS family transcription factor (TF) gene (FLI1 or ERG) in Ewing sarcoma (ES), or PAX3/PAX7 and FOXO1 translocations in alveolar rhabdomyosarcoma (ARMS). In neuroblastoma, MYCN, a member of the MYC family of TFs, is often amplified and localizes to super enhancer regions, where it rewires lineage-specific transcriptional programs driving oncogenesis.

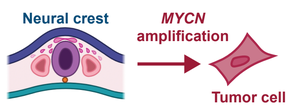
Oncogenic TFs have proven difficult to target directly; we and others have proposed targeting associated transcriptional co-regulators to inhibit their activity. CDK9 interacts with many oncogenic TFs and is essential for TF-mediated transcription elongation through phosphorylation of the C-terminal domain of RNA pol II. KB-0742 is a potent, selective, and orally bioavailable inhibitor of CDK9 currently in clinical development that shows antitumor activity in preclinical models of sarcoma and neuroblastoma.

Materials and methods

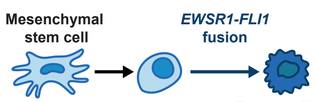
Cell lines and low passage patient-derived cells (PDCs) were tested for antiproliferative effects of KB-0742, using either Cell Titer Glo (Promega) or Alamar Blue cell viability reagent (Bio-Rad). Pharmacodynamic (PD) markers of KB-0742 treatment, including phospho-SER2 (pSER2) on RNA pol II, MYCN, MYC, and cleaved poly ADP ribose polymerase (PARP), were measured by Western blot. The antitumor activity of KB-0742 was evaluated using patient-derived xenograft (PDX) models of ES and ARMS *in vivo*. Tumor samples and plasma were collected to determine PD effects and drug concentrations, respectively. The transgenic TH-MYCN model of neuroblastoma was used to study antitumor effects of KB-0742. All *in vivo* models were performed according to IACUC guidelines.

Disrupted transcription regulatory networks in pediatric cancers

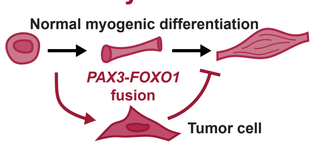
Neuroblastoma



Ewing's sarcoma

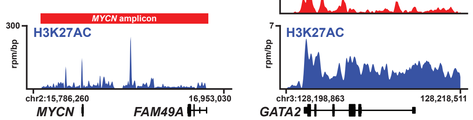


Rhabdomyosarcoma

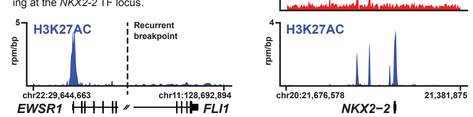


Genetic deregulation of a TF → **Rewiring of developmental gene expression programs**

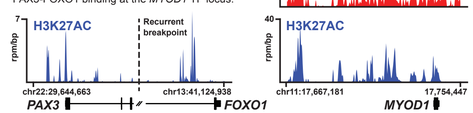
ChIP-seq in Kelly neuroblastoma cells from *Poon et al., 2020 JCI*. **Below:** H3K27AC at the *MYCN* amplicon. **Right:** MYCN binding to the *GATA2* TF locus.



ChIP-seq in SKMNC Ewing sarcoma cells from Riggi et al., Cancer Cell 2014. **Below:** H3K27AC at the characteristic *EWSR1-FLI1* translocation locus. **Right:** EWSR1-FLI1 binding at the *NKX2-2* TF locus.



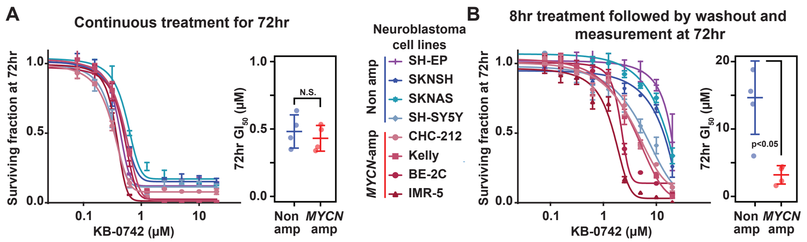
ChIP-seq in SCMC Rhabdomyosarcoma cells from Gryder et al., Cancer Discovery 2017. **Below:** H3K27AC at the characteristic *PAX3-FOXO1* translocation locus. **Right:** PAX3-FOXO1 binding at the *MYOD1* TF locus.



Overall results

KB-0742 decreased the viability of immortalized and low passage PDCs from ES, ARMS, and neuroblastoma. In neuroblastoma, cell lines with *MYCN* amplification were more sensitive to KB-0742 treatment. KB-0742–treated neuroblastoma cells had decreased pSER2, loss of expression of MYCN and MYC, and an induction of cleaved PARP. KB-0742 treatment of a TH-MYCN transgenic mouse model resulted in regression of established tumors. In PDX models of ES and ARMS, KB-0742 treatment inhibited tumor growth. Analysis of tumor samples revealed decreases in pSER2 and expression and function of the oncogenic TFs.

KB-0742 inhibits growth of *MYCN*-amplified neuroblastoma



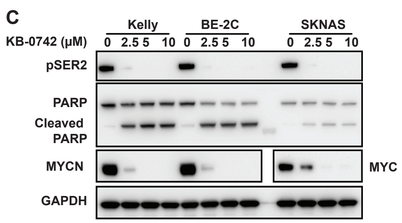


Figure legend: A,B) KB-0742 effects on cell viability across neuroblastoma cell lines plated for 24 hours and then treated with KB-0742. **A)** Continuous treatment. **B)** 8hr treatment followed by washout. Cell viability measured as surviving fraction of cells 72 hours post treatment. GI_{50} concentrations shown as dot plots next to each graph. Blue: non amplified lines. Red: *MYCN*-amplified lines. C) Western blots were performed on *MYCN*-amplified (Kelly and BE-2C) and non amplified (SKNAS) neuroblastoma cells at 8hr post treatment with measurement of pharmacodynamic biomarkers of CDK9 inhibition (pSER2), induction of apoptosis (full length and cleaved PARP), loss of MYCN or MYC. GAPDH is provided as a negative control.

KB-0742 causes tumor regression in MYCN-driven neuroblastoma genetically engineered mouse model

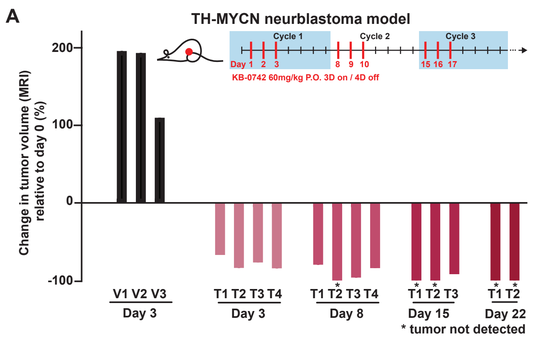


Figure legend: A) The TH-MYCN genetically engineered mouse model was used to assess KB-0742 efficacy in a MYCN-driven model of neuroblastoma. In this model, MYCN is expressed from the tyrosine hydroxylase promoter. Mice which developed evident tumors were either treated with vehicle (saline, n=3) or 60 mg/kg KB-0742 (n=4) on a schedule of 3-days on/4-days off per weekly cycle. Tumor volume was assessed by MRI at days 3, 8, 15 and 22.

KB-0742 broadly inhibits growth of pediatric sarcoma cell lines

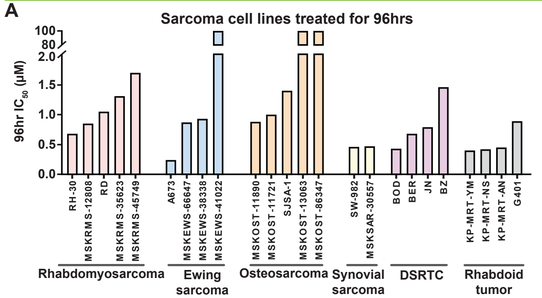


Figure legend: A) KB-0742, was screened against 24 cell lines across several pediatric sarcomas. Dose response assessments were performed by integrating 3 serial dose responses with peak values of 100μM, 10μM and 1μM in triplicate. The high controls (HC) contained 1% DMSO (v/v), the low controls (LC) 1μM 'killer mix' in 1% DMSO (v/v) that consists of a proprietary mixture of 6 cytotoxic compounds. The compounds were tested at 1% DMSO (v/v) for 96h. Dose–response curves were fitted using a logistic 4-parameter equation in GraphPad Prism 7 and IC_{50} values were determined.

KB-0742 inhibits growth of TF fusion positive sarcomas *in vivo*

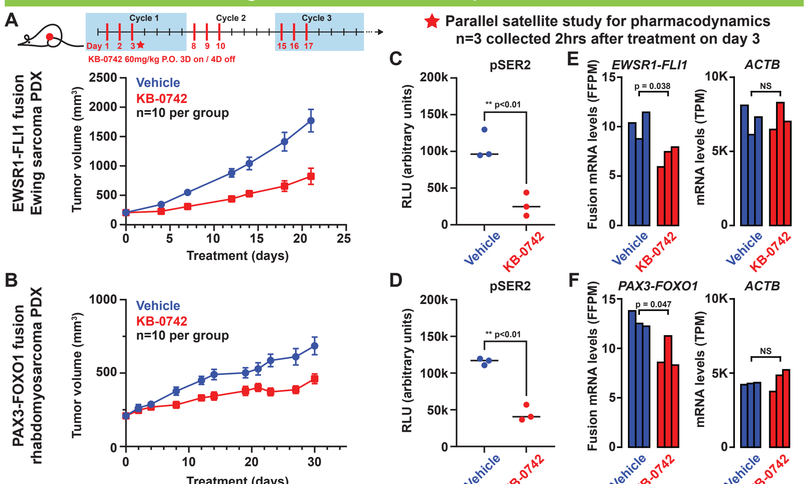


Figure legend: A,B) For patient derived xenografts (PDX), tumors were engrafted subcutaenously and mice were randomized (n=10 per group) upon tumors reaching >150mm^3 volume. PDX models were treated with vehicle (saline) or 60 mg/kg KB-0742 on a schedule of 3-days on/4-days off per weekly cycle. Tumor volumes and body weights were recorded twice weekly. **C-F)** A satellite study was conducted in parallel with tumors (n=3) collected 2hrs post treatment on day 3. **C,D)** Tumor lysates were prepared and RNA pol II pSER2 was measured using a Meso Scale Discovery (MSD) assay. Differences in pSER2 levels assessed using two-tailed *t*-test. **E,F)** Whole-transcriptome profiling using Tempus Labs hybrid capture IDT xGen Exome Research Panel v1.0. **Left)** RNA fusions detected using STAR-Fusion and Mojo and fusion TF mRNA levels are shown as fusion fragments per million reads (FFPM). **Right)** An unaffected control mRNA *ACTB* is shown in units of transcripts per million. Differences in mRNA levels assessed using two-tailed *t*-test.

Conclusions

CDK9 targeting by KB-0742 inhibits growth of multiple pediatric tumor types by modulating the expression and activity of key oncogenic TFs. KB-0742 is being evaluated in a phase I dose-escalation trial in patients with relapsed or refractory solid tumors or NHL (NCT04718675).

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