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CDK9 Inhibition via KB-0742 Is a Potential Strategy to Treat Transcriptionally Addicted Cancers

Melinda A. L. Day[1], Douglas C. Saffran[1], Tressa Hood[1], Nikolaus Obholzer[1], Akanksha Pandey[1], Akul Singhania[1], Charles Y. Lin[1], Pavan Kumar[1], Daniel M. Freed[2], Jorge DiMartino[1]

[1]Kronos Bio, Inc., San Mateo, CA; [2]Chordoma Foundation, Durham, NC

Abstract

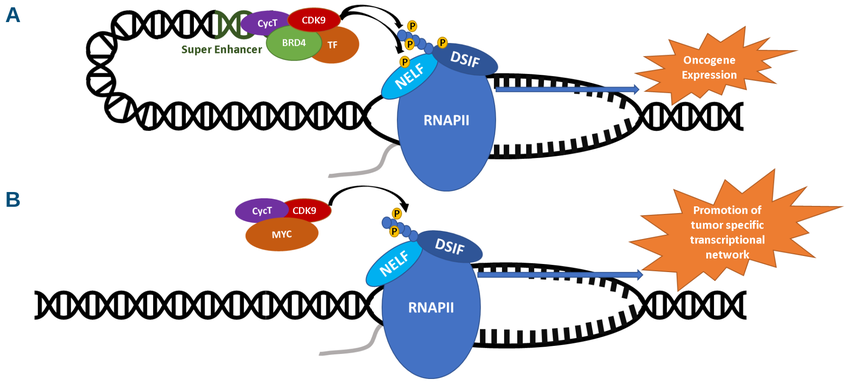
- Transcriptional addiction is defined as a state in which a tumor cell is critically dependent (more than normal cells) on highly efficient functioning of the transcriptional machinery for its growth and survival. This can be due to requirements for high rates of transcription of a critical oncogene such as *MYC*. Alternatively, certain tumors rely on dysregulated activity of a particular transcription factor to drive their malignant phenotype. These include the fusion gene *EWS-FLI1* in Ewing sarcoma, *PAX3/7-FOXO1* fusions in rhabdomyosarcoma, and *brachyury (T)* in chordoma. Cyclin-dependent kinase 9 (CDK9) controls progression through the elongation phase of the transcription cycle and represents a promising target in transcriptionally addicted tumors. We have developed a potent, selective, and orally bioavailable CDK9 inhibitor, KB-0742, which is currently in the dose-escalation stage of a phase 1/2 study (NCT04718675).
- Using the BROAD PRISM screen, we observed a trend in Ewing sarcoma cell lines, with lower half maximal inhibitory concentrations (IC_{50}S) in higher expressing *MYC* cell lines. We pulled out three cell lines and grew individually to assess sensitivity to KB-0742. All 3 Ewing sarcoma cell lines tested were sensitive to KB-0742, showing maximum inhibition rates of over 100%. We then evaluated the activity of KB-0742 in 5 patient-derived cell line (PDC) models, with all 5 showing a cytotoxic response to treatment as measured by negative growth rate (GR) efficacy (GR_{max}) values. KB-0742 was also found to be active in a single patient-derived organoid (PDO) model of adult rhabdomyosarcoma.
- The activity of KB-0742 was assessed in vivo using 2 patient-derived xenograft (PDX) models of chordoma. In model CF466, a dose-dependent response was observed as evidenced by increased tumor growth inhibition (TGI) activity and target engagement. We then evaluated KB-0742 as a single agent and in combination with afatinib (an EGFR inhibitor and preclinical gold standard compound for chordoma) in the CF539 model. KB-0742 as a single agent showed similar TGI activity as afatinib, whereas the combination showed an increased response.

CDK9 Is a Key Dependency in Tumor Transcriptional Reprogramming

As a transcriptional regulator, CDK9 is a key dependency in transcriptionally addicted tumors. CDK9 helps promote the tumor-associated transcriptional landscape through 2 mechanisms:

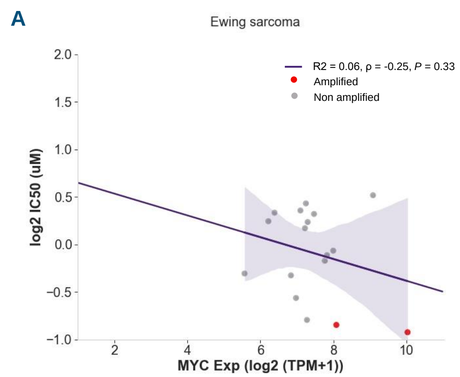
(A) Supporting expression of key oncogenes, and

(B) Working as a cofactor to oncogenic transcription factors such as MYC to promote high rates of transcription



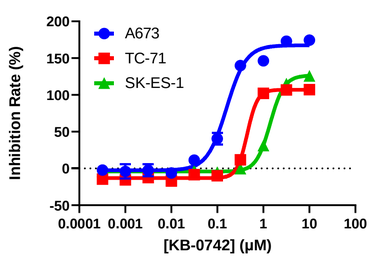
BRD4 = bromodomain protein 4; CycT = cyclin T; DSIF = 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole sensitivity-inducing factor; NELF = negative elongation factor; P = phosphate; RNAPII = RNA polymerase II; TF = transcription factor.

Sarcoma Cell Lines Are Sensitive to KB-0742



B

Cell Line	IC_{50} (μM)	Maximum % Inhibition
A673	0.11	174.4
TC-71	0.46	107.1
SK-ES-1	1.25	125.8



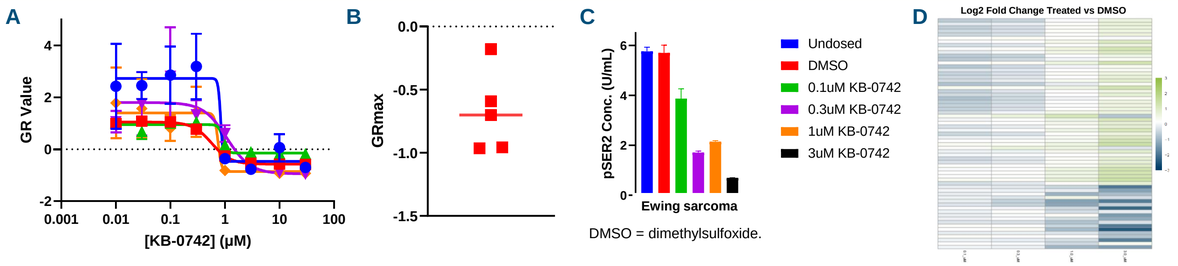
TPM = transcripts per kilobase million.

Immortalized cell lines were screened for sensitivity to KB-0742. All the cell lines were treated with a range of concentrations of KB-0742, and the IC_{50} was calculated. **(A)** In the Broad PRISM screen, a trend was observed in Ewing sarcoma with cells expressing higher levels of the oncogenic transcription factor *MYC*, having lower IC_{50}s to treatment with KB-0742. Additionally, the 2 *MYC*-amplified cell lines had the lowest IC_{50}s of the Ewing sarcoma lines tested. **(B)** Three Ewing Sarcoma cell lines were tested individually for response to KB-0742. All 3 cell lines showed maximum inhibition rates of over 100% and 2 of the 3 cell lines had IC_{50}s below 500 nM. **(C)** Dose-response curves of the 3 Ewing sarcoma cell lines.

Acknowledgements: The authors would like to thank K2 Oncology (Beijing, China) for patient-derived organoid cultures; Imagen Therapeutics (Manchester, UK) for patient-derived cell line studies; Broad Institute of MIT and Harvard (Cambridge, MA) for immortalized cell line studies; WuXi AppTec (Shanghai, China) for immortalized cell line studies; Qiagen (Germantown, MD) for sequencing services.

Presented at the American Association for Cancer Research Annual Meeting; April 8-13, 2022; New Orleans, LA.

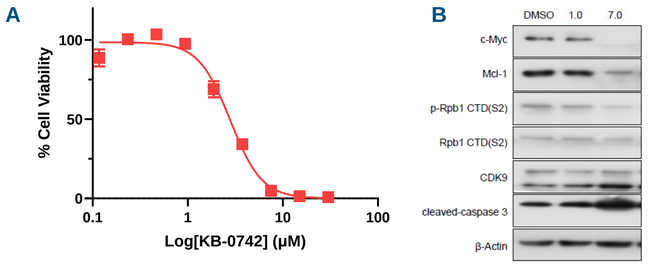
KB-0742 Is Cytotoxic in PDC Models of Sarcoma



DMSO = dimethylsulfoxide.

Five models of sarcoma, including 3 Ewing sarcoma models, were treated with concentrations of KB-0742 ranging from 30 μM down to 10 nM and were incubated for 72 hours. **(A)** GR values were calculated for each concentration of KB-0742 by comparing to a time 0 cell count number and graphed as a dose-response curve. **(B)** KB-0742 was cytotoxic in all 5 models as determined by negative values for GR_{max}. **(C)** Target engagement was assessed in 1 model of Ewing sarcoma. Cells were treated with the noted concentrations of KB-0742 for 6 hours before being collected and lysed for protein analysis. RNAPII pSER2 levels were measured using a Meso Scale Discovery assay. KB-0742 treatment reduces pSER2 protein in a dose-dependent manner. **(D)** RNA-sequencing analysis of the same model as (C) showed reduction of gene expression with KB-0742 treatment, indicating transcriptional repression.

KB-0742 Is Active in a PDO Model of Adult Rhabdomyosarcoma

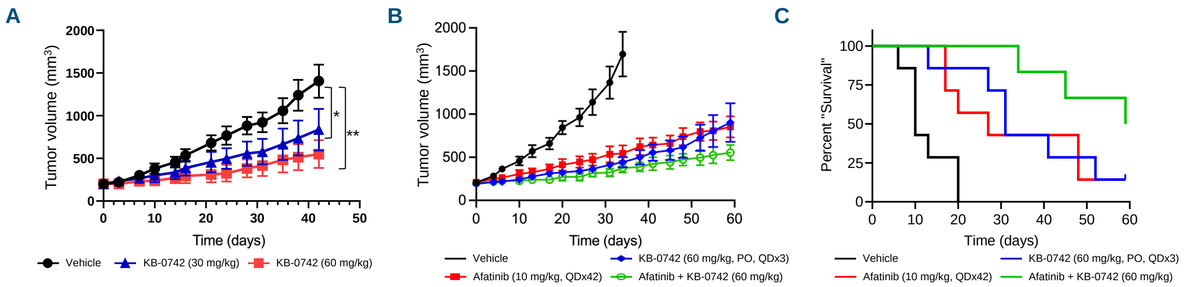


(A) A PDO model of treatment-naive adult rhabdomyosarcoma was treated with a titration curve of KB-0742. Treatment with KB-0742 resulted in an IC_{50} of 2.75 μM and a max inhibition rate of 98.61%.

(B) The same model was treated with DMSO and KB-0742 at 1 μM and 7 μM concentrations for 4 hours. Protein lysates were analyzed by western blot. KB-0742 treatment resulted in reductions in MYC, MCL-1, and pSER2 protein levels and an increase in cleaved-caspase 3 in a dose-dependent manner.

CTD = C-terminal domain; p-Rpb1 = Phospho-RNAPII subunit B1.

KB-0742 Shows Antitumor Activity in PDX Models of Chordoma



*P<0.05; **P<0.01

PDX models of chordoma were tested for sensitivity to KB-0742. **(A)** Model CF466 was treated with vehicle or KB-0742 at 30 mg/kg or 60 mg/kg (oral administration [PO], 3 days on/4 days off), and tumor volume was followed for 42 days. KB-0742 showed significant TGI activity in a dose-dependent manner (48% and 55%, respectively). Of the 7 mice treated in the KB-0742 60 mg/kg group, 2 had complete responses and were considered tumor-free survivors. **(B)** Model CF539 was treated with vehicle, KB-0742 (60 mg/kg, PO, 3 days on/4 days off), afatinib (10 mg/kg, PO, once daily [QD]), or the combination of KB-0742 plus afatinib for up to 60 days. Tumor volume GR curves were plotted over time, showing antitumor activity in all 3 treatment arms with the combination having the greatest reduction in growth. %TGI for each treatment arm was 74% (P<0.0001 vs control) for 60 mg/kg KB-0742, 77% (P<0.0001 vs control) for afatinib, and 88% (P<0.0001 vs control, P=0.0951 vs afatinib) for the combination. **(C)** Time to the tumors reaching 500 mm^3 was plotted using a Kaplan–Meier survival curve. The median time to 500 mm^3 was 10 days (vehicle), 27 days (afatinib), 31 days (KB-0742), and 59 days (KB-0742 with afatinib).

Conclusions

- Immortalized cell lines of sarcoma were sensitive to KB-0742.
- Cytotoxic responses to KB-0742 were observed in PDC models of sarcoma and were associated with a dose-dependent reduction in pSER2 protein levels.
- KB-0742 was shown to reduce c-MYC, MCL-1, and pSER2 protein levels in a PDO model of rhabdomyosarcoma that was associated with an increase in the cell-death marker cleaved-caspase 3.
- In models of chordoma, KB-0742 had antitumor activity that was dose dependent and showed combinatorial activity with the preclinical gold standard compound, afatinib.

Based on these data across multiple translation platforms, an expansion cohort in the ongoing phase 1/2 clinical trial of KB-0742 (NCT04718675) will evaluate the antitumor activity of KB-0742 at the recommended phase 2 dose in patients with relapsed or refractory sarcoma, chordoma, and other transcriptionally addicted solid tumors.