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

Melinda A. L. Day1, Douglas C. Safran1, Tressa Hood1, Nikolaus Obholzer1, Akanksha Pandey1, Akul Singhania1, Charles Y. Lin1, Pavan Kumar1, Daniel M. Freed2, Jorge DiMartino1

1Kronos Bio, Inc., San Mateo, CA; 2Chordoma 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-FKHR 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 (IC50) 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) efficiency (GRmax) values. KB-0742 was also found to be active in a single patient-derived organoid (POD) model of adult rhabdomyosarcoma.

The activity of KB-0742 was assessed in vivo using 2 patient-derived xenograft (PDx) models of chordoma. In model CF539, 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) in Ewing sarcoma, 3 days on/4 days off), and tumor volume was followed for 42 days. KB-0742 was cytotoxic in all 5 models as determined by negative values for GRmax. 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 used for protein analysis. RNAipi pS315 levels were measured using a Wes BioScaleDiscovery. KB-0742 treatment reduces pS315 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.

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.

**Supporting Information:**

- **Table:** Sarcoma Cell Lines Are Sensitive to KB-0742
  - **A:** Ewing sarcoma cells were tested for sensitivity to KB-0742. All the cell lines were treated with a range of concentrations of KB-0742, and the IC50 was calculated. (A) In the Broad PRISM screen, a trend was observed in Ewing sarcoma cells with expressing higher levels of the oncogenic transcription factor MYC, having lower IC50 values compared to the MYC-competent cell lines (PDCs). KB-0742 treatment reduced MYC expression in all Ewing sarcoma cell lines tested. (B) Three Ewing sarcoma cell lines were tested individually for response to KB-0742. All cell lines showed maximum inhibition rates of over 100% and 2 of the 3 cell lines had IC50s below 500 nM. (C) Dose-response curves of the 3 Ewing sarcoma cell lines.

**Conclusions:**

- **Imortalized cell lines of sarcoma were sensitive to KB-0742.**
- **Cytostatic responses to KB-0742 were observed in PDX models of sarcoma and were associated with a dose-dependent reduction in pS315 protein levels.**
- **KB-0742 was shown to reduce c-MYC, MCL-1, and pS315 protein levels in a PDX model of rhabdomyosarcoma that was associated with an increase in the cell-death marker cleaved PARP.**
- **In models of chordoma, KB-0742 had antitumor activity that was dose-dependent and showed combinatorial activity with the preclinical gold standard compound, afatinib.**

**Figure:** KB-0742 Is Cytotoxic in PDX Models of Sarcoma

- **A:** Five models of sarcoma, including 3 Ewing sarcoma models, were treated with concentrations of KB-0742 ranging from 30 μM down to 10 μM. Responses were observed only to 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 GRmax. (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 used for protein analysis. RNAipi pS315 levels were measured using a Wes BioScale Discovery. KB-0742 treatment reduces pS315 protein in a dose-dependent manner. (D) RNA sequence analysis of the same model as (C) showed reduction of gene expression with KB-0742 treatment, indicating transcriptional repression.

**Figure:** KB-0742 Is Active in a PDX Model of Adult Rhabdomyosarcoma

- **A:** A PDX model of treatment-naive adult rhabdomyosarcoma was treated with a titration curve of KB-0742. Treatment with KB-0742 resulted in an IC50 of 2.75 μM and a maximum 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 pS315 protein levels and an increase in cleaved-caspase 3 in a dose-dependent manner.

**Figure:** KB-0742 Shows Antitumor Activity in PDX Models of Chordoma

- **A:** CD56, CD44, and CD10 were analyzed by flow cytometry at 48 hours post-treatment. CD56 and CD10 were reduced while CD10 was increased in chordoma models treated with KB-0742. (B) Time to the tumors reaching 500 mm3 was 10 days (vehicle), 27 days (afatinib), 31 days (KB-0742), and 69 days (KB-0742 with afatinib).