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Preclinical Activity of KB-0742, An Oral, Highly Selective, CDK9 Inhibitor, in Cell Lines and in MYC-High Expressing, Patient-Derived Models of Multiple Breast Cancer Subtypes

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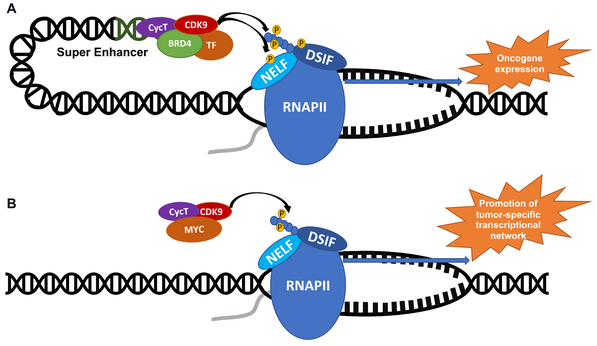
Introduction

- Phosphorylation of the C-terminal domain of RNA polymerase II (RNAPII) at serine 2 (pSER2) by cyclin-dependent kinase 9 (CDK9) is a necessary step to proceed from transcription initiation to elongation.[1]
- The oncogenic transcription factor MYC requires high rates of transcription and is therefore critically dependent on CDK9 for its expression and function as a cancer driver.[2]
- Thus, inhibition of CDK9 could have anti-tumor effects in tumors that express high levels of *MYC*, including triple-negative breast cancer (TNBC).
- KB-0742 is an orally available, clinical stage CDK9 inhibitor that is potent and highly selective for CDK9 over cell-cycle regulatory CDKs (eg, CDK4, 6).
- KB-0742 treatment decreased viability of primary, metastatic, and TNBC cell lines and patient-derived cell and organoid cultures, and inhibited growth of *MYC*-amplified, patient-derived xenograft (PDX) models.
- Inhibition of tumor growth was associated with a decrease in pSER2 in RNAPII, decreased MYC protein levels, and a modulated set of gene transcripts associated with CDK9 inhibition.
- These data demonstrate the efficacy of KB-0742 in preclinical models of breast cancer and support future clinical testing in *MYC*-amplified TNBC patients.

CDK9 Is a Key Dependency in Tumor Transcriptional Reprogramming

Figure 1. 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. By supporting expression of key oncogenes, and
B. By working as a cofactor to oncogenic transcription factors such as MYC to promote high rates of transcription.



DSIF = 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole sensitivity-inducing factor; NELF = negative elongation factor; P = phosphate; TF = transcription factor.

TNBC Expresses Significantly Higher Levels of *MYC* Than Either HER2+ or ER/PR+ Patient Tumors

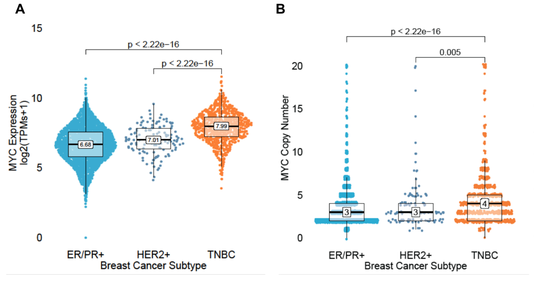


Figure 2. *MYC* expression across breast cancer subtypes. Evaluation of *MYC* expression and copy number was performed on 2651 ER/PR+ patients, 865 TNBC patients, and 129 HER2+ patients in the Tempus database. **(A)** A box plot of *MYC* TPMs by breast hormone subtype shows that TNBC has significantly higher expression of *MYC* than either HER2+ or ER/PR+ subtypes by Wilcoxon analysis. **(B)** A box plot of *MYC* copy number by subtype shows that TNBC has a significantly higher median *MYC* copy number than either HER2+ or ER/PR+ subtypes by Wilcoxon analysis.

ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2; PR = progesterone receptor; TPM = transcripts per kilobase million.

References

1. Peterlin BM, et al. *Mol Cell*. 2006;23(3):297-305. 2. Lin CY, et al. *Cell*. 2012 ;151(1):56-67.

KB-0742 Is a Potent and Selective CDK9 Inhibitor

A

	KB-0742
CDK9	IC_{50} = 6 nM
CDK13	62x
* CDK2	66x
CDK12	98x
CDK18	>200x
CDK3	>200x
* CDK7	>200x
CDK16	>200x
CDK5	>200x
CDK17	>200x
* CDK1	>200x
* CDK4	>200x
* CDK6	>200x
CDK14	>200x
CDK8	>200x
CDK19	>200x

*Denotes cell-cycle kinases.
IC_{50} = half maximal inhibitory concentration.

B

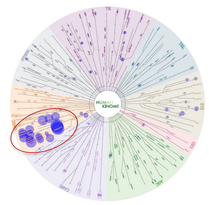
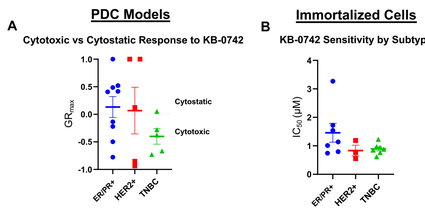


Figure 3. KB-0742 is a potent and selective CDK9 inhibitor. (A) Heat map of the biochemical IC_{50}s of KB-0742 against different CDKs. KB-0742 is selectively active against CDK9 with a single-digit nanomolar IC_{50}. **(B)** KB-0742 was tested at a single concentration of 10 μM against a large panel of 631 kinases using the Reaction Biology Corporation HotSpot® assay platform. Fifty percent or greater enzyme inhibition was only observed for a subset of CDK kinases.

Enzyme % Inhibition Ranges	Kinases
>99%	CDK9
85% to 99%	None
50% to 85%	CDK1, 2, 3, 4, 6, 7, 16, 17, 18

KB-0742 Inhibits Growth of Patient-Derived Cell Lines (PDC) and Immortalized Breast Cancer Cell Lines



GR_{max} = maximum growth rate.

Figure 4. TNBC cells are preferentially sensitive to CDK9 inhibition. (A) Twenty-three PDC models representing different breast cancer subtypes were tested for sensitivity to KB-0742. Each model was treated with KB-0742 across a range of doses and analyzed for cell number, cell death, and cell growth. Growth rate inhibition analysis showed that the TNBC subtype was especially sensitive to KB-0742 with 4 out of 5 models showing cytotoxic responses as measured by GR_{max} (values <0 indicates cytotoxicity). **(B)** Seventeen immortalized breast cancer cell lines were screened for sensitivity to KB-0742. Each model was treated with a titration curve of KB-0742 and analyzed for cell death. The IC_{50} values for the TNBC and HER2+ subtypes were lower than for the ER/PR+ subtypes.

KB-0742 Inhibits Growth of TNBC Patient-Derived Organoid Cultures (PDO) That Are Resistant to Treatment With Standard of Care (SOC) Agents

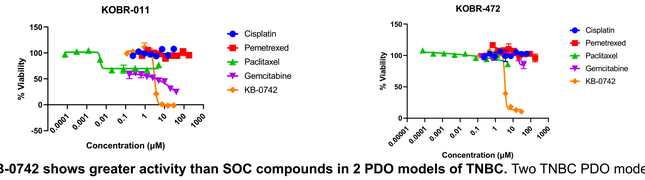
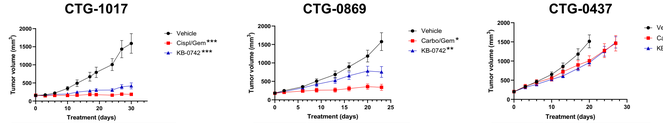


Figure 5. KB-0742 shows greater activity than SOC compounds in 2 PDO models of TNBC. Two TNBC PDO models with different treatment histories were used to compare the activity of KB-0742 to 4 SOC compounds. KB-0742 showed greater activity in both models than all 4 SOC compounds.

Anti-tumor Activity of KB-0742 in *MYC*-Amplified TNBC PDX models



MYC CN	MYC Exp (log_2 RPKM)
7	6.965

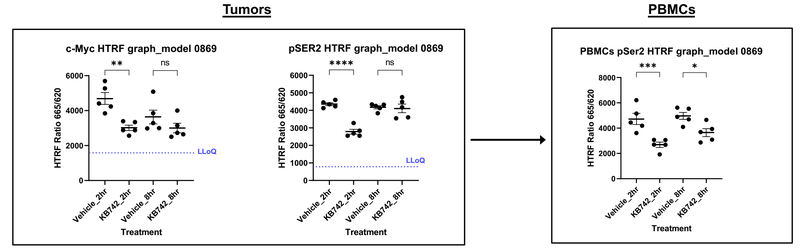
MYC CN	MYC Exp (log_2 RPKM)
5	7.327

MYC CN	MYC Exp (log_2 RPKM)
4	7.968

N = 10 per group for each model. *P<0.05; **P<0.005; ***P<0.0005 vs vehicle; SOC vs KB-0742 was not significant.
Carbo = carboplatin; Cispl = cisplatin; CN = copy number; Gem = gemcitabine; IP = intraperitoneally, IV = intravenously; Pacl = paclitaxel; PO = by mouth; Q7Dx3 = 1 injection every 7 days times 3 injections; Q14Dx2 = 1 injection every 14 days times 2 injections; RPKM = reads per kilobase million.

Figure 6. KB-0742 inhibits tumor growth in 3 *MYC*-amplified PDX models of TNBC. Animals bearing established subcutaneous TNBC PDX models were treated with either vehicle (saline), KB-0742, or SOC chemotherapeutics. MYC CN and expression are indicated for each model. Vehicle and KB-0742 at 60 mg/kg were administered PO in all models using an intermittent dosing schedule of 3-days on, 4-days off for up to 4 weekly cycles. Models CTG-1017 received cisplatin 5 mg/kg IP Q7Dx3 + gemcitabine 100 mg/kg IP Q7Dx3; CTG-0869 received carboplatin 40 mg/kg IP Q7Dx3 + gemcitabine 100 mg/kg IP Q7Dx3; and CTG-0437 received carboplatin 50 mg/kg IP Q14Dx2 + paclitaxel 10 mg/kg IV Q14Dx2. KB-0742 was well-tolerated in all 3 models.

Decreased Levels of MYC and pSER2 in Tumors and PBMCs After Treatment With KB-0742



*P<0.05; **P<0.005; ***P<0.0005; ****P<0.0001.
hr = hour; HTRF = homogeneous time resolved fluorescence; LLoQ = lower limit of quantification; ns = not significant; PBMCs = peripheral blood mononuclear cells.

Figure 7. Pharmacodynamic activity of KB-0742 in tumors and PBMCs from treated mice. Tumors and PBMCs from CTG-0869 tumor-bearing animals were collected at 2 and 8 hours post-terminal dose of either vehicle or 60 mg/kg KB-0742. Lysates were prepared and levels of MYC and pSER2 were determined using the respective HTRF assays. The plasma concentration of KB-0742 was 702 ng/mL (2.5 μM) and 176 ng/mL (0.6 μM) at 2 and 8 hours post-dose, respectively.

Gene Expression Profiling of TNBC PDX Tumors After Treatment With KB-0742

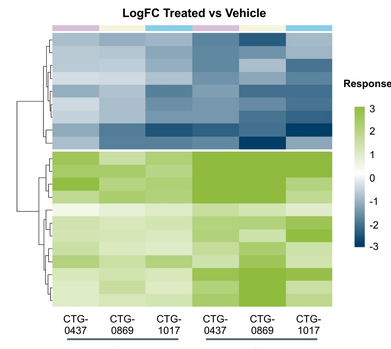


Figure 8. Treatment with KB-0742 modulates gene expression in PDX tumors. Gene expression among 3 PDX models treated with vehicle or KB-0742 60 mg/kg was assessed at 2 time points (2hr and 8hr) after dosing using RNAseq. Overlapping gene sets were extracted within models and across models, with an emphasis on genes that increased the magnitude log_2-fold change between 2 and 8 hours.

Conclusions

- KB-0742 is a highly selective and orally bioavailable CDK9 inhibitor.
- Treatment of a panel of breast cancer cell models with KB-0742, including HER2+, ER+, HER2+/ER+, and TNBC subtypes, resulted in decreased cell viability.
- Responses ranged from cytostatic to cytotoxic with effects on TNBC cells being preferentially cytotoxic.
- KB-0742 treatment resulted in tumor growth inhibition in 3 *MYC*-amplified TNBC PDX models.
- Pharmacodynamic assessments at the end of the study showed that treatment with KB-0742 decreased pSER2 and MYC levels in tumors and PBMCs in correlation with plasma concentrations of drug.
- Gene-expression profiling revealed a distinct pattern of transcription regulation associated with KB-0742 treatment that can be extended to clinical studies.
- These studies support evaluation of KB-0742 in patients with *MYC*-amplified triple-negative breast cancer.

A phase 1/2 clinical trial of KB-0742 (NCT04718675) is currently recruiting patients with relapsed or refractory solid tumors or non-Hodgkin lymphoma.

Acknowledgments

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