

Title: *IKK-16 Inhibits Primary Tumor Growth and Metastatic Progression in Chondrosarcoma via NF-κB Pathway Suppression*

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**Background:** Chondrosarcoma (CSA) is an aggressive bone malignancy resistant to chemotherapy and radiation, with surgical resection as the primary treatment. Metastatic CSA carries a poor prognosis due to limited effective systemic therapies. This study aimed to identify and evaluate actionable therapeutic targets in metastatic CSA, specifically testing the efficacy of the NF-κB inhibitor IKK-16 in preclinical models.

**Methods:** High-throughput drug screens using CellTiter Glo were performed in four independent CSA cell lines. In vivo efficacy was tested in two immunocompromised mouse models: (1) subcutaneous patient-derived CSA cell implantation for primary tumor growth, and (2) tail vein injection for pulmonary metastasis. IKK-16 or vehicle was administered when primary tumors reached 5mm x 5mm or 24 hours post-injection in the metastasis model. Tumor progression was monitored by caliper measurement, IVIS imaging, and histology. Kaplan-Meier and t-tests assessed survival and tumor burden.

**Results:** High-throughput screening identified IKK-16 as selectively cytotoxic to metastatic CSA cells. IKK-16 significantly reduced primary tumor growth ( $p=0.0066$ ) and extended time to endpoint ( $p=0.0061$ ) versus controls. In the metastasis model, 5/6 control mice developed pulmonary metastases, while 0/5 IKK-16 treated mice exhibited tumor burden, confirmed by IVIS and histology. Treated primary tumors showed increased H&E necrosis. Mechanistically, IKK-16 significantly inhibited pro-metastasis markers ICAM1, LCP1, and MMP9.

**Conclusions:** IKK-16, via NF-κB inhibition, suppresses primary tumor progression and prevents metastatic colonization in preclinical CSA models. These findings support IKK-16 as a potential systemic therapy for metastatic and highlight NF-κB as a therapeutic target for future translational studies.

**Significance/Clinical Relevance:** This study addresses a critical unmet need for systemic therapy in metastatic chondrosarcoma (CSA), which is highly resistant to standard chemotherapy and radiation. The preclinical success of IKK-16 in both primary and metastatic models, confirmed by tumor growth inhibition and prevention of metastatic colonization, highlights the NF-κB pathway as a promising therapeutic target. These findings provide a strong rationale for advancing IKK-16, or similar NF-κB inhibitors, toward future translational and clinical studies as a potential treatment for patients with this aggressive and currently incurable malignancy.

\*Authors have no disclosures or conflict of interests to declare\*

\*Figures available in supplementary document\*