Paper # 2003/Young Investigator Award Recipient

# BLOCKING THE BCL10-MALT1 INTERACTION IN DIFFUSE LARGE B-CELL LYMPHOMA

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**Background:** Activated B-cell diffuse large B-cell lymphoma (ABC-DLBCL) is characterized by oncogenic gain-of-function mutations that cause inappropriate activation of the three protein CARMA1-BCL10-MALT1 (CBM) signaling complex, which then drives lymphomagenesis. MALT1, the effector protein of this complex, possesses protease and scaffolding activities, both of which contribute to activation of the pro-survival NF-κB transcription factor. Existing MALT1 inhibitors target only MALT1 protease function and recent studies demonstrate that MALT1 protease inhibitors have efficacy in blocking MALT1-dependent lymphomagenesis. The clinical utility of isolated MALT1 protease inhibitors is thought to be limited by the inflammation that results from inhibiting only MALT1 protease function without inhibiting MALT1 scaffolding function.

**Objectives:** Here we seek to identify a pharmacologic approach to simultaneously block both MALT1 protease and scaffolding activities. We aimed to define the precise site of interaction between MALT1 and BCL10 and then evaluate whether a small molecule that blocks this interaction is able to inhibit both constitutive MALT1 protease and scaffolding activities in ABC-DLBCL lymphomagenesis.

**Design/Method:** Site directed mutagenesis and co-immunoprecipitation were used to identify a site of interaction between MALT1 and BCL10. An in silico drug-docking screen was used to identify M1i-124 as a compound predicted to disrupt this interaction. We tested the ability of M1i-124 to block the BCL10-MALT1 interaction using an ELISA assay. *In vitro* studies and *in vivo* xenograft experiments were performed to test the effect of M1i-124 on MALT1 oncogenic function in ABC-DLBCL tumor cells.

**Results:** Using mutagenesis and co-immunoprecipitation, we identified a region of MALT1 located between immunoglobulin-like domains 1 and 2 (Ig1-2) that is required for interaction with BCL10. Using an in silico drug screen targeting this MALT1 interface, we identified M1i-124 as a potential inhibitor of the BCL10-MALT1 interaction. We show that M1i-124 inhibits the binding of BCL10 and MALT1 in an ELISA assay. We find that M1i-124 inhibits both MALT1 protease and MALT1 scaffolding functions in ABC-DLBCL cells as well as tumor growth in an ABC-DLBCL xenograft mouse model.

**Conclusion:** A small molecule that is predicted to bind to the interface between MALT1 Ig1-2 domains is effective in inhibiting MALT1 protease and scaffolding functions in ABC-DLBCL cells and inhibiting ABC-DLBCL lymphomagenesis in a xenograft mouse model. These

results demonstrate that targeting the BCL10-MALT1 interaction is a valid strategy for inhibiting MALT1-dependent lymphomagenesis. This approach to targeting the MALT1 oncoprotein may have a therapeutic advantage over MALT1 protease inhibitors as it may avoid inflammation associated with selective MALT1 protease inhibition.

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## PTEFB-DEPENDENT TRANSCRIPTION REORGANIZATION UNDERPINS THE GLIOMA ADAPTIVE RESPONSE TO RADIOTHERAPY

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**Background:** Dynamic regulation of gene expression is fundamental for cellular adaptation to exogenous stressors. The functional endpoint of epigenetic signaling is the productive transcriptional elongation by RNA polymerase II (Pol II) at the appropriate genomic loci. PTEFb-mediated promoter proximal pause-release of Pol II is a conserved regulatory mechanism for synchronous transcriptional induction best described in response to heat shock, but this pro-survival role has not been examined in the applied context of cancer therapy.

**Objectives:** To characterize the scope and kinetics of transcriptional mechanisms underpinning the adaptive response to ionizing radiation (IR) in order to define specific regulators or cofactors amenable to therapeutic disruption.

**Design/Method:** In order to examine the dynamics of chromatin reorganization following radiotherapy, we performed a combination of ChIP-, ATAC-, and RNA-seq in model systems of diffuse intrinsic pontine glioma (DIPG) and other pediatric high-grade gliomas (pHGG) following IR exposure. We interrogated IR-induced gene expression in the presence or absence of PTEFb blockade, including both mechanistic and functional consequences of concurrent inhibition or genetic depletion. We utilized culture models with live cell imaging to assess the therapeutic synergy of PTEFb inhibition with IR, as well as the therapeutic index of this intervention relative to normal controls. Finally, we employed orthotopic models of pHGG treated with conformal radiotherapy and CNS-penetrant PTEFb inhibitors in order to assess tolerability and anti-tumor effect *in vivo*.

**Results:** Rapid genome-wide redistribution of active chromatin features and PTEFb facilitates Pol II pause-release to drive nascent transcriptional induction within hours of exposure to therapeutic ionizing radiation. Concurrent inhibition of PTEFb imparts a transcription elongation defect, abrogating canonical adaptive programs such as DNA damage repair and cell cycle regulation. This combination demonstrates a potent, synergistic therapeutic potential agnostic of glioma subtype, leading to a marked induction of tumor cell apoptosis and prolongation of

xenograft survival.

**Conclusion:** These studies reveal a central role for PTEFb underpinning the early adaptive response to radiotherapy, opening new avenues for combinatorial treatment in these lethal malignancies. In conjunction with our collaborators, this data will collectively form the basis for a phase 1, first-in-children trial of PTEFb inhibition in pediatric gliomas.