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PROTAC - A novel approach for Ewing Sarcoma therapy

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Ewing sarcoma (EWS) is a tumor in the bone that afflicts children and adolescents. The current standard of care (SOC) is chemotherapy with multiple drugs and local control with surgery and/or radiotherapy followed by a consolidation chemotherapy. The five-year survival rate of patients with localized disease is 50-70 %, which precipitously drops to 18-30 % in metastatic cases. These statistics warrant the development of novel therapeutics that are synergistic with current SOC. The present study explores a novel PROTAC (Proteolysis Targeting Chimera), 50-008, as a therapeutic option in EWS. PROTAC is a heterobifunctional compound, which can simultaneously bind to the protein of interest (POI) and an E3-ligase and uses the cellular ubiquitin proteasome system to degrade the POI. Unlike reversible inhibitors, the efficacy of PROTACs do not rely on the off-rate but on the number of times a ternary complex is formed between the POI: PROTAC: E3-ligase. A Profiling Relative Inhibition Simultaneously in Mixtures (PRISM) screen was used for rapid, high throughput multiplexed screening of 50-008 across a panel of 869 human cancer cell lines. Western blot analysis was used to elucidate the pathways targeted by 50-008. Synergism studies with 50-008 and the current SOC drugs (Cyclophosphamide, Doxorubicin, Etoposide, and Vincristine) was also conducted in EWS cell lines. Preliminary pharmacokinetic (PK) studies were carried out to determine the route of administration and bioavailability. The PRISM screen revealed a remarkable, 10/13 EWS cell lines were sensitive to 50-008 (IC50 < 1mM). Among them, SKES1 was the most sensitive with an IC50 of 15 nM. Analyses of the Western blot data showed that 50-008 perturbed the mitogen activated protein kinase pathway (MAPK) by altering the phosphorylation of key signaling molecules *viz.,* IKKb, JNK, p38 and ERK. Consistently, in A673 cells that harbor a BRAF mutation, 50-008 exhibited an activity comparable to Etoposide. Moreover, combination of 50-008 and etoposide exhibited synergistic growth inhibition in A673 cell line. Oral, intraperitoneal (I.P.) and intravenous (I.V.) administration of 50-008 to mice revealed that 50-008 is not orally available but has 63 % bioavailability upon I.P. administration. Our data suggests that the PROTAC 50-008 modulates the MAPK pathway and is effective in >75% of EWS cell lines tested. Also, the combination of 50-008 and Etoposide synergistically inhibited the growth of EWS cell lines. The preliminary cellular and PK results support advancing 50-008 to be evaluated in *in vivo* models of EWS.