APTO-253, a small molecule that mediates anticancer activity through induction of the Krippel-like factor 4 (KLF4) tumor suppressor, is being developed clinically for the treatment of acute myelogenous (myeloid) leukemia (AML) and high risk myelodysplastic syndromes (MDS). APTO-253 was well tolerated in a Phase 1 study in patients with solid tumors using a dosing schedule of 1, 15, 16 of a 28-day cycle (two rounds of D1 per 14 day dosing interval i.e. two rounds of 2x q144 during the 28 day cycle) but recent scientific publications indicating APTO-253 toward AML and high risk MDS. Indeed, suppression of FLK4 was reported as a key driver in the leukemogenesis of AML and subsets of other hematologic diseases. The vast majority of patients with AML abnormally express the transcription factor CDX2 in human bone marrow stem and progenitor cells (HSPC) (Scholl et al., J Clin Invest. 2007, 117(4):1037-48). The CDX2 protein binds to CDX2 containing promoters within the FLK4 promoter, leading to suppression of FLK4 expression in HSPC (Fabre et al., J Clin Invest. 2013, 123(1):299-314). Based on these observations, the anticancer activity of APTO-253 was examined in AML and other hematopoietic cancers. APTO-253 showed potent antiproliferative activity in vitro against a panel of blood cancer cell lines, with IC50 values of 5.9 - 305 nM. AML acute lymphoblastic leukemia and chronic myeloid leukemia (39 - 250 nM), non-Hodgkin’s lymphoma (11 – 190 nM) and multiple myeloma (72 – 180 nM). To explore in vivo efficacy, dose scheduling studies were initially conducted in the H226 xenograft model in mice. In the H226 model, APTO-253 showed improved antitumor activity when administered for two consecutive days followed by a five day break from dosing (2x q7d) each week compared to the 2x q7d schedule used to evaluate antitumor activity of APTO-253 in several AML xenograft models in mice. In Kasumi 1 AML and KG-1 AML xenograft models, APTO-253 showed significant antitumor activity (p = 0.0004, respectively) as a single agent when administered using the 2x q7d schedule each week for four weeks compared to control animals. Mice treated with APTO-253 had no overt toxicity based on clinical observations and body weight measurements. Mice bearing HL-60 AML xenograft tumors were treated with APTO-253 for one day or two consecutive days per week for three weeks, either as a single agent or combined with azacitidine, or with azacitidine alone twice per week (on days 1, 4, 8, 11, 15 and 18). APTO-253 as a single agent inhibited growth of HL-60 tumors to an extent similar to azacitidine. Furthermore, both once weekly and twice weekly dosing of APTO-253 in combination with azacitidine resulted in significantly enhanced antitumor activity relative to either single agent alone (p = 0.0002 and p = 0.0006 for 1X and 2X weekly APTO-253 treatment, respectively, compared to control). Likewise, using a THP-1 AML xenograft model, APTO-253 administered by a 2x q7d schedule significantly inhibited the growth of AML cells, demonstrating a clinically relevant antitumor activity. To further investigate the potential of APTO-253 in combination with azacitidine, we treated these AML xenograft-bearing mice with APTO-253 in combination with azacitidine and examined the effects on tumor growth, overall survival, and toxicity. Our results indicate that APTO-253 in combination with azacitidine is a promising therapeutic approach for the treatment of AML.

**References:**