#4813. APTO-253 Induces KLF4 to Promote Potent in Vitro Pro-Apoptotic Activity in Hematologic Cancer Cell Lines and Antitumor Efficacy As a Single Agent and in Combination with Azacitidine in Animal Models of Acute Myelogenous Leukemia (AML)

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Abstract

APTO-253, a small molecule that mediates anticancer activity through induction of the Krüppel-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 I study in patients with solid tumors using a dosing schedule of days 1, 2, 15, 16 of a 28 day cycle (two rounds of D1,2 per 14 day dosing interval i.e. two rounds of 2x q14d during the 28 day cycle) but recent scientific observations guided APTO-253 toward AML and high risk MDS. Indeed, suppression of KLF4 was reported as a key driver in the leukemogenesis of AML and subsets of other hematologic diseases. The vast majority (~90%) of patients with AML aberrantly 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 consensus sequences within the KLF4 promoter, leading to suppression of KLF4 expression in HSPC (Faber 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 hematological cancers. APTO-253 showed potent antiproliferative activity in vitro against a panel of blood cancer cell lines, with ηM IC_{50}values in AML (6.9 - 305 ηM), acute lymphoblastic leukemia and chronic myeloid leukemia (39 – 250 ηM), non-Hodgkin's lymphoma (11 – 190 ηM) and multiple myeloma (72 – 180 ηM). 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 q14d schedule. The 2x q7d schedule was 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 = 0.028 and 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 approximately the same extent as 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 as a single agent using the 2x q7d schedule showed significant efficacy, similar to that of azacitidine, while the combination of APTO-253 and azacitidine demonstrated greatly improved antitumor effects relative to either drug alone. APTO-253 was effective and well tolerated as a single agent or in combination with azacitidine in multiple AML xenograft models, plus APTO-253 does not cause bone marrow suppression. Taken together, our results indicate that APTO-253 may serve as a targeted agent for single agent use and may provide enhanced efficacy to standard of care chemotherapeutics for AML and other hematological malignancies.

Disclosures: Rice: Aptose Biosciences Inc.: Employment. Vellanki: Aptose Biosciences Inc.: Employment. Lee: Aptose Biosciences Inc.: Employment. Lightfoot: Aptose Biosciences Inc.: Employment. Peralta: Aptose Biosciences Inc.: Employment. Jamerlan: Aptose Biosciences Inc.: Employment. Jin: Aptose Biosciences Inc.: Employment. Lum: Aptose Biosciences Inc.: Employment. Cheng: Aptose Biosciences Inc.: Employment.

APTO-253 Targets KLF4 Mechanism in AML

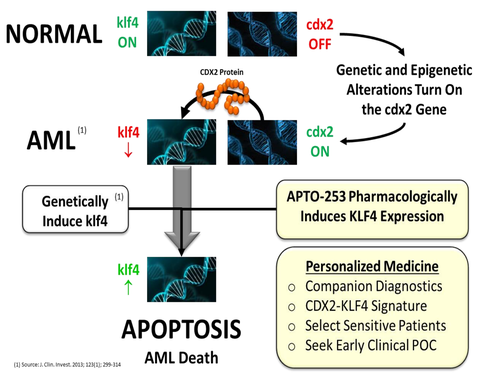

Figure 1. CDX2 is a homeobox transcription factor that functions in embryonic organogenesis and early hematopoietic development in vertebrates. CDX2 is not normally expressed in adult murine and human normal hematopoietic cells, but is reactivated in 90% of AML patients (Scholl et al., 2007). Aberrant expression of CDX2 in bone marrow stem and progenitor cells results in down-regulation of KLF4 expression as the putative leukemogenic event (Faber et al., 2013). APTO-253 pharmacologically induces expression of the suppressed KLF4 gene, leading to cell death by apoptosis.

Weekly Dosing of APTO-253 Delivers Superior Efficacy to Biweekly Schedule

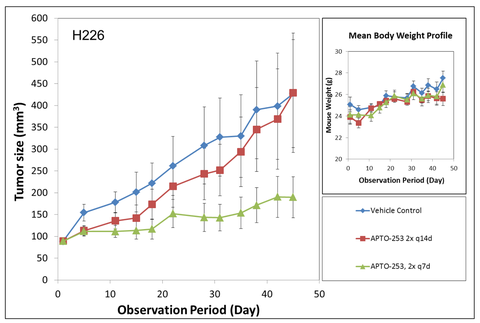

Figure 3. Antitumor activity of APTO-253 in an NCI-H226 non-small cell lung cancer xenograft model. Athymic nude mice (n = 8) were inoculated in the lower mid-back with $5x10^6$ human H226 cells. When tumors reached the desired size, or vehicle control or APTO-253 (10 mg/kg) was administered by IV injection once daily for two consecutive days (Days 1, 2), followed by a non-dosing period of either 12 days (2x q 14d) or 5 days (2x q7d) for four rounds of dosing. Mean tumor sizes ±SE are shown. Weekly dosing with APTO-253 resulted in markedly improved efficacy compared to biweekly dosing. No apparent toxicity was observed with APTO-253 based on clinical observations and body weight measurements (insert).

APTO-253 In Vivo Efficacy in KG-1 AML Tumor Xenograft Model Treatment

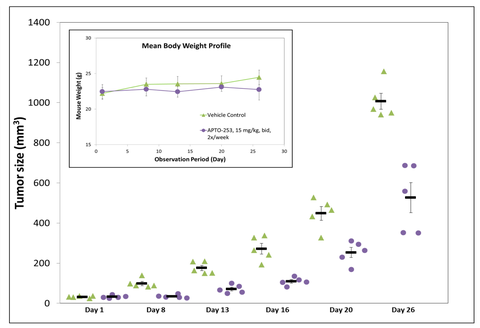

Figure 5. Antitumor activity of APTO-253 in a KG-1 AML xenograft model. Athymic nude mice (n = 5) were inoculated in the lower mid-back with $1x10^7$ human KG-1 cells. APTO-253 (15 mg/kg) or vehicle control was administered by IV injection twice per day (bid) for two consecutive days (Days 1, 2) per week for the duration of the study. Mean tumor sizes ±SE are shown. APTO-253 treatment resulted in a significant decrease in tumor growth compared to control mice (P=0.0004; Student's t-test at day 26). APTO-253 showed minimal toxicity based on mouse body weight compared to vehicle control (mean ±SE shown in insert).

APTO-253 In Vivo Efficacy in THP-1 Model: Single Agent and Combination Treatment

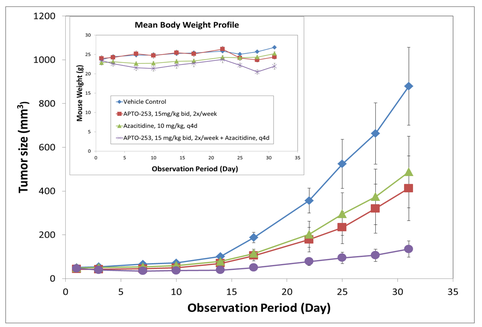

Figure 7. A. Line graphs of antitumor activity of APTO-253 alone and in combination with azacitidine in a THP-1 AML xenograft model in athymic nude mice (n = 12). Mice were treated with vehicle control or APTO-253 (15 mg/kg) by IV injection 2X per day for two consecutive days per week (Days 1,2) with or without azacitidine (SC, q4d), or azacitidine alone. Mean tumor sizes ±SE are shown. APTO-253 showed equal efficacy compared to azacitidine, while dosing with APTO-253 in combination with azacitidine resulted in significant efficacy compared to vehicle control-treated mice (P=0.0004; Student's t-test at d31). Combination treatment showed minimal toxicity based on mouse body weight (mean ±SE shown in insert).

In Vitro Activity of APTO-253 Correlates with CDX2 Expression in Heme Cancers

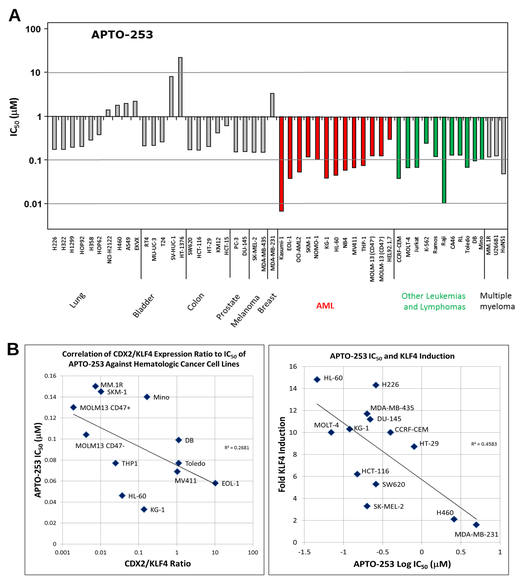

Figure 2. A. Antiproliferative activity of APTO-253 in solid tumor and heme cancer cell lines. Cells were seeded in 96-well cell culture plates and incubated overnight at 37°C, followed by the addition of medium containing APTO-253 or 0.1% DMSO. After incubation at 37°C for 5 days, cell viability was quantitated with the use of an XTT colorimetric assay. Values shown are mean concentrations of APTO-253 that inhibit cell growth by 50% (IC50). **B.** IC50s for cancer cell lines were plotted against ratios of basal CDX2/KLF4 mRNA levels (left graph) and fold KLF4 induction (right graph) following treatment with 1 μM APTO-253 for 16 h. KLF4 and CDX2 levels were measured by quantitative RT-PCR and normalized to GAPDH.

APTO-253 In Vivo Efficacy in Kasumi-1 AML Tumor Xenograft Model

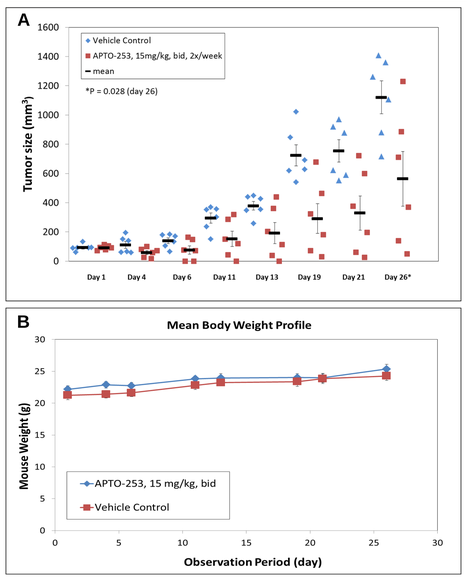

Figure 4. A. Antitumor activity of APTO-253 in a Kasumi-1 AML xenograft model. Athymic nude mice (n = 6) were inoculated in the lower mid-back with $1x10^7$ human Kasumi-1 cells. When tumors reached the desired size, APTO-253 (15 mg/kg) or vehicle control was administered by IV injection twice per day (bid) for two consecutive days per week (Days 1, 2). Scatter plots with mean tumor sizes ± SE are shown. APTO-253 treatment resulted in a significant decrease in tumor growth compared to control vehicle (*P=0.028; Student's t-test at day 26). **B.** Mice treated with APTO-253 showed no body weight loss compared to vehicle control (mean BW ± SE shown).

APTO-253 In Vivo Efficacy in HL-60 Model: Single Agent and Combination Treatment

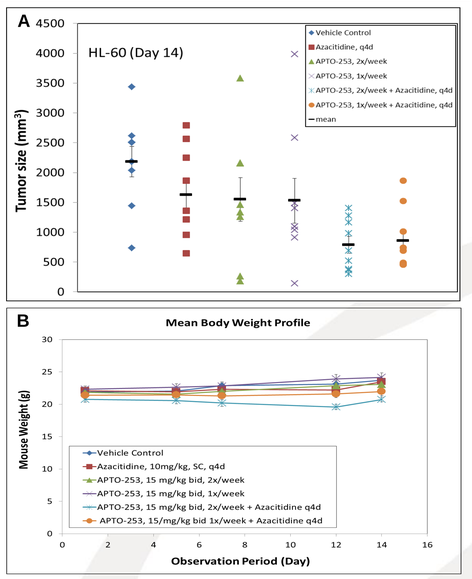

Figure 6. A. Antitumor activity of APTO-253 alone and in combination with azacitidine in an HL-60 AML xenograft model in athymic nude mice (n = 9). Mice were treated with vehicle control or APTO-253 (15 mg/kg) by IV injection twice per day either 1X or 2X per week (Days 1,2 for 2X) with or without azacitidine every fourth day (q4d), or azacitidine alone. Mean tumor sizes ±SE are shown. Non-toxic doses of APTO-253 showed equal efficacy compared to azacitidine. Once or twice weekly dosing with APTO-253 in combination with azacitidine resulted in significant efficacy (P<0.001; Student's t-test at day 14) that was well tolerated based on body weights (**B.**).

Summary

- Approximately **90% of patients with AML aberrantly express the CDX2** gene in bone marrow stem and progenitor cells, resulting in **down-regulation of KLF4** expression and contributing to **development of leukemia**
- **APTO-253** is a novel anticancer small molecule and the only **clinical-stage** agent under development as an **inducer of the KLF4 gene**
- APTO-253 showed **potent antiproliferative activity in a panel of AML** and other heme cancer cell lines, with IC_{50} values ranging from 0.007 - 0.3 μM, correlating with ratios of basal CDX2/KLF4 mRNA levels
- In animal models, APTO-253 demonstrated **significant antitumor activity** both as a single agent and in combination with azacitidine
- APTO-253 showed **in vivo activity in multiple AML models, with lack of toxicity and no evidence of myelosuppression**
- **Reactivation of KLF4 expression by APTO-253** in hematological cancers may occur by overcoming CDX2-mediated repression and other mechanisms of KLF4 silencing
- Collectively, these preclinical data strongly support further **development of APTO-253 as a targeted AML therapy**

References:

1. Scholl C et al., J Clin Invest. 2007. 117(4):1037-48.

2. Faber K et al., J Clin Invest. 2013. 123(1):299-314.