#1716. Inhibition of c-Myc by APTO-253 as an Innovative Therapeutic Approach to Induce Cell Cycle Arrest and Apoptosis in Acute Myeloid Leukemia

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Abstract

c-Myc multifunctional transcription factor protein, a product on the *c-myc* proto-oncogene, contributes to the pathogenesis of many human cancers through multiple mechanisms of proliferation, apoptosis, cell cycle progression and senescence. c-Myc is frequently overexpressed in acute myeloid leukemia (AML), yet strategies to effectively and safely modulate c-Myc function do not exist. APTO-253 is a small molecule agent being developed clinically for the treatment of AML and high risk myelodysplastic syndromes (MDS), and we evaluated the effect of APTO-253 on *c-myc* gene expression. We first confirmed that c-Myc mRNA levels were significantly higher in AML cell lines as compared to peripheral blood mononuclear cells (PBMCs) isolated from healthy human donors. However, c-Myc expression in AML cells was inhibited by APTO-253 in dose-dependent and time-dependent manners at both the mRNA and protein levels, and c-Myc inhibition occurred as an early mechanistic event (6hr). Likewise, APTO-253 induced AML cell apoptosis in dose-dependent and time-dependent manners, as demonstrated by increases in Annexin-V staining and cleaved poly (ADP-ribose) polymerase (c-PARP). APTO-253 induced G1/G0 cell cycle arrest, increased p21 expression, decreased expression of cyclin D3 and cyclin-dependent kinases 4 (CDK4) in AML cells, and increased p53 levels in p53-positive MV4-11 and EOL1 cell lines. Collectively, these data suggest that inhibition of c-Myc by APTO-253 leads to global cell cycle arrest and apoptosis events in AML cells. Importantly, we demonstrated that APTO-253 selectively targeted tumor cells but not normal healthy cells, with MV4-11 AML cells and normal PBMCs having IC50s of 0.25±0.03μM and more than 100μM, respectively. Our previous studies (56th ASH abstract #4813) showed that APTO-253 induces the Krüppel-like Factor 4 (KLF4) transcription factor and was effective and well tolerated as a single agent in multiple AML xenograft models without causing bone marrow suppression. Taken together, our results indicate that nM levels of APTO-253 mechanistically inhibit c-Myc expression in AML cells and subsequently induce cell cycle arrest and apoptosis, and suggest that APTO-253 may serve as an effective and safe c-Myc inhibitor for AML.

APTO-253 Selectively Inhibits Proliferation of AML Cells But not Healthy Normal Cells

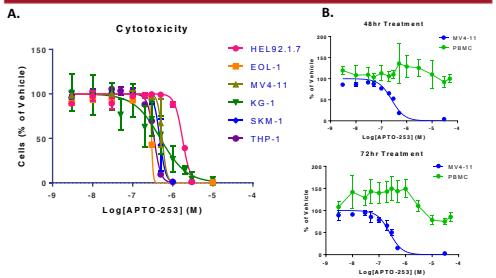

Figure 1. Cytotoxic effect of APTO-253 on AML cells and healthy normal PBMCs. Cells were plated and treated with and without APTO-253 at indicated concentrations in 96-well cell culture plates at 37°C. MTS based assay was performed at the end to quantify cell viability. Fifty percent cell growth inhibition (IC_{50}) was calculated with GraphPad Prism 7. ***A).*** AML cells treated for 120 hours. Data from 3~7 independently experiments for different cell lines. ***B).*** PBMCs isolated from whole blood of 5 healthy human donors were treated for 48 and 72 hours in parallel with MV4-11 cells. MV4-11 cells and normal PBMCs had IC50s of 0.25±0.03μM and >100μM, respectively, at both time points.

Disclosures: Zhang: Aptose Biosciences Inc.: Employment. Local: Aptose Biosciences Inc.: Employment. Benbatoul: Aptose Biosciences Inc.: Employment. Folger: Aptose Biosciences Inc.: Employment. Sheng: Aptose Biosciences Inc.: Employment. Esquivies: Aptose Biosciences Inc.: Employment. Lightfoot: Aptose Biosciences Inc.: Employment. Vellanki: Aptose Biosciences Inc.: Employment. Rice: Aptose Biosciences Inc.: Employment.

APTO-253 Dose- and Time- Dependently Inhibits c-Myc in AML Cells

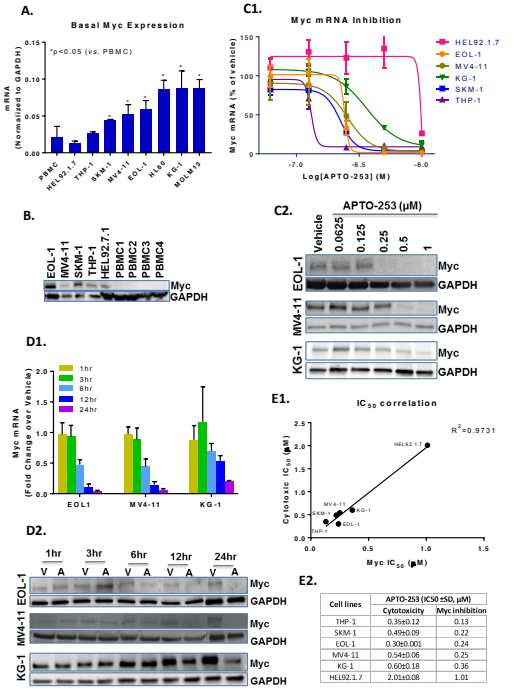

Figure 2. c-Myc inhibition by APTO-253 in AML cell lines. ***A).*** Basal Myc mRNA of AML cell lines and healthy normal PBMCs. RNA was extracted from non-treated cells and cDNA prepared from RNA. MYC expression was assayed by RT-qPCR and then normalized to GAPDH expression for each sample. Duplicate samples for HL60, HEL92.1.7, MOLM13, SKM1, and THP1, four replicates for EOL1 and KG1, six replicates for MV4-11 and nine replicates for healthy PBMCs. ***B).*** Basal Myc protein of AML cell lines and healthy normal PBMCs. The whole cell extract of non-treated cells was Western Blotted. ***C).*** Myc decreased by APTO-253 dose-dependently. Cells were treated with APTO-253 at indicated concentrations for 24 hours at 37°C . RNA (*C1*) and protein (*C2*, representative blots of at least 3 independent experiments) were extract and quantified by RT-qPCR and Western Blot, respectively. Fifty percent Myc mRNA inhibition (IC_{50}) was calculated with GraphPad Prism 7. ***D).*** Myc decreased by APTO-253 time-dependently. Cells were treated with APTO-253 at 0.5 μM for indicated time points at 37°C . RNA (*D1*) and protein (*D2*, representative blots of at least 3 independent experiments; v stands for vehicle treatment; A stands for APTO-253 treatment) were extract and quantified by RT-qPCR and Western Blot, respectively. ***E).*** APTO-253 cytotoxic effect correlates with its Myc inhibitory effect. The correlation of IC50s of cytotoxicity (as described in Figure 1. A.) and Myc inhibition (as described in Figure 2. C.) was analyzed by GraphPad Prism 7.

APTO-253 Deregulates Myc Transcription

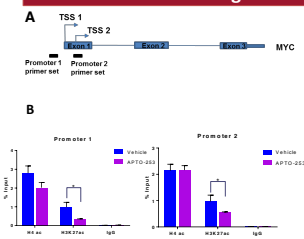

Figure 3. c-Myc inhibition by APTO-253 in AML cell lines by decreasing acetylated H3K27 at its promoters. MV4-11 cells treated for 24 hrs with vehicle (DMSO 0.1%) or 1 μM APTO-253. Cells crosslinked in 1% formaldehyde and soluble chromatin prepared by sonication. Chromatin immunoprecipitation was carried out with the antibodies listed and associated DNA was assayed by 2 separate MYC primer pairs located at promoters 1 and 2 (***A***). Graphed (***B***) as % input, averages of 2-4 replicates (* p-value <0.05).

APTO-253 Induces Apoptosis in AML Cells

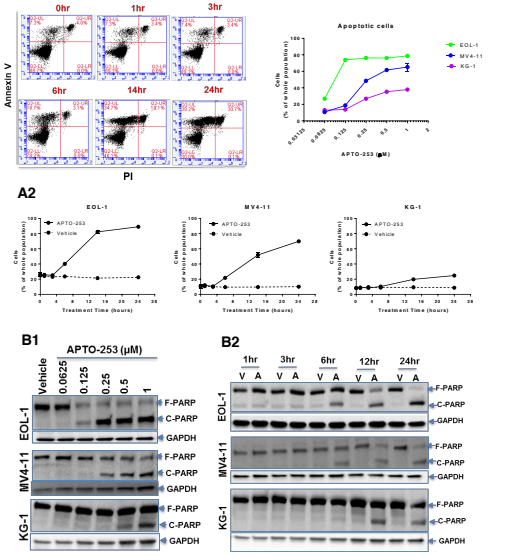

Figure 4. APTO-253 dose-dependently and time-dependently induces apoptosis in AML cells. AML cells EOL-1, MV4-11 and KG-1 were treated with vehicle DMSO (v) and APTO-253 (A) and subjected to apoptosis analysis by flow cytometry (***A1, A2 and B3***; ***A1*** as representative graphs of three independent experiments of three cell lines) and to apoptotic biomarker analysis by Western Blotting (***B1 and B2***, representative blots of three independent experiments for each cell line). For time course studies, cells were treated with 0.5μM APTO-253 for indicated times; for dose response studies, cells were treated with indicated concentrations for 24 hours. For the apoptosis analysis by flow cytometry, cells were stained with Annexin V and propidium iodide (PI) to distinguish live (Annexin V -/PI-), early apoptotic (Annexin V+/PI-), late apoptotic (Annexin V+/PI+) and dead (Annexin V-/PI+) cells. As indicated in ***A2*** and ***A3***, total apoptotic cells (Annexin V+/PI- plus Annexin V+/PI+) were induced by APTO-253 in dose- and time- dependent manners, which agreed with PARP cleavage.

APTO-253 Upregulates p21 and p53

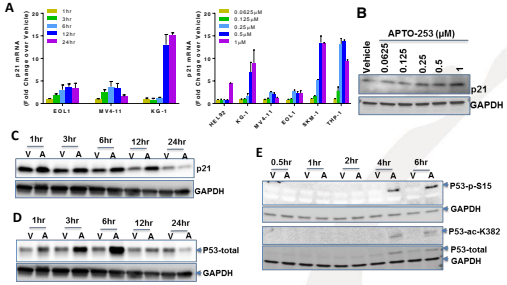

Figure 5. APTO-253 dose-dependently and time-dependently upregulates p21 and increases p53 activity in AML cells. AML cells were treated with vehicle DMSO (v) and APTO-253 (A) at indicated concentrations for indicated times. The time course studies were done with 0.5μM treatment, and dose response studies were treated for 24 hours. The p21 mRNA (***A***) was quantified by RT-qPCR of at least 3 independent experiments. The p21 (***B, C***) and p53 (***D, E***) Western Blots were shown for MV4-11 cells and represent three independent experiments. APTO-253 increases p21 expression at both mRNA and protein level. It increases total amount of p53 and its phosphorylation at Serine 15 and acetylation at Lysine 382 as early as 3-4 hour treatment

APTO-253 Induces G1/G0Cell Cycle Arrest in AML Cells

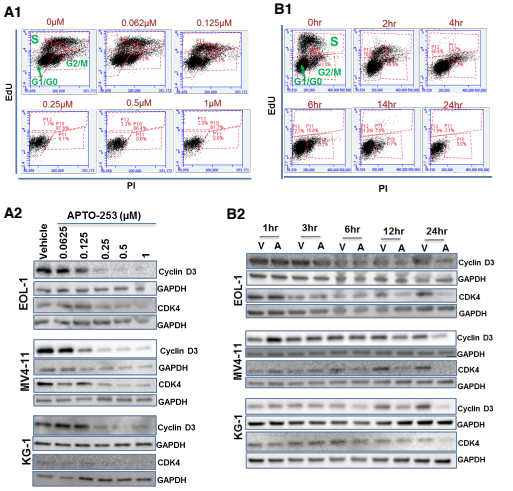

Figure 6. APTO-253 dose-dependently and time-dependently induces cell cycle arrest in AML cells. AML cells EOL-1, MV4-11 and KG-1 were treated with vehicle DMSO (v) and APTO-253 (A) and subjected to cell cycle analysis by flow cytometry (***A1 and B1***, representative graphs of three independent experiments of three cell lines) and to cell cycle biomarker analysis by Western Blotting (***A2 and B2***, representative blots of four independent experiments for each cell line). For time course studies, cells were treated with 0.5μM APTO-253 for indicated times; for dose response studies, cells were treated with indicated concentrations for 24 hours. For the cell cycle analysis by flow cytometry, cells were stained with 5-ethynyl-2 deoxyuridine (EdU) and propidium iodide (PI) to distinguish G1/G0, S and G2/M phases of cell cycle. Cell cycle biomarkers cyclin D1, cyclin D3, cyclin E, CDK4, CDK6, and CDK2 were Western Blotted. Only Cyclin D3 and CDK4 were consistently decreased by APTO-253 dose- and time-dependently in all three tested cell lines, which suggests cell cycle arrest at G1/G0 phase and is confirmed by flow cytometry analysis.

Summary

- APTO-253 selectively kills AML cells without affecting healthy normal cells.
- APTO-253 inhibits c-Myc expression at both mRNA and protein levels in dose- and time- dependent manners. Its inhibitory effect on c-Myc correlates closely with its cytotoxic effect on AML cells (R^2= 0.9731). Loss of acetylated H3K27 at Myc promoters suggests that APTO-253 transcriptionally deregulates c-Myc expression.
- APTO-253 dose- and time- dependently induces apoptosis and causes G1/G0 cell cycle arrest in AML cells.
- APTO-253 upregulates p21 and p53 at early time points (before 24 hour), which could contribute to apoptosis and cell cycle arrest.
- APTO-253 increases p53 stability and activity through inducing phosphorylation and acetylation, which could contribute to its mechanism of action in AML cells.
- Taken together, APTO-253 selectively targets tumor cells and effectively inhibits c-Myc expression in AML cells to induce cell cycle arrest and subsequent cell death.
- APTO-253 may serve as a safe and effective c-Myc inhibitor for AML chemotherapy that does not negatively impact the normal bone marrow cells.

References:

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