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

Hongyi Zhang, MD, PhD*, Andrea Local, PhD*, Khalid Benbatoul†, Peter Folger†, Susan Sheng, PhD†, Luis Esquivues, PhD*, Jeff Lightfoot, PhD‡, Avanish Vellanki§ and William G. Rice, PhD*.


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 that has been 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 donors. However, c-Myc expression in AML cell lines 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 (6h). Likewise, APTO-253 induced AML cell apoptosis in dose-dependent and time-dependent manners as demonstrated by increases in Annexin V staining and cleaved (apo) 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 MV-4;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 MV-4;11 AML cells and normal PBMCs having IC50s of 0.25 fold 0.5µM and more than 100µM, respectively. Our previous studies (56th ASH abstract #4813) showed that APTO-253 induces the Kripkine-like Factor (KLF) 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 when used in combination with other agents, APTO-253 may subsequently induce cell cycle arrest and apoptosis, and suggest that APTO-253 may serve as a selective and safe c-Myc inhibitor for AML.

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 control and APTO-253 treated cells and cDNA prepared from RNA. Myc expression was assayed by q-PCR and then normalized to GAPDH expression for each sample. Duplicate samples for H690, HL60, ML1.7, MOLM13, MLL/1, THP1, four for EOL1 and KG1, six replicates for MV-4;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. Green: treated with APTO-253 dose-dependently. Cells were treated with APTO-253 at indicated concentrations for 24 hours. C: RNAi (C3) and protein (C3) representative of at least 3 independent experiments were extract and quantified by RT-qPCR and Western Blot, respectively. Fifty percent Myc mRNA knockdown (IC50) was calculated with GraphPad Prism 7. D: Myc decreased by APTO-253 time-dependently. Cells were treated with APTO-253 at 0.5 µg/l for indicated time points at 37°C. RNA (D3) and protein (D3), representative of at least 3 independent experiments; for vehicle treatment, 1 hour to APTO-253 treatment were extract and quantified by RT-qPCR and Western Blot, respectively. E: APTO-253 cytostatic effect correlates with the IC50. The correlation of IC50s of cytostatic (as described in Figure 1. A) and Myc inhibition (as described in Figure 2. C.) was analyzed by GraphPad Prism 7.

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

Figure 3. 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 control and APTO-253 treated cells and cDNA prepared from RNA. Myc expression was assayed by q-PCR and then normalized to GAPDH expression for each sample. Duplicate samples for H690, HL60, ML1.7, MOLM13, MLL/1, THP1, four for EOL1 and KG1, six replicates for MV-4;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. Green: treated with APTO-253 dose-dependently. Cells were treated with APTO-253 at indicated concentrations for 24 hours. C: RNAi (C3) and protein (C3) representative of at least 3 independent experiments were extract and quantified by RT-qPCR and Western Blot, respectively. Fifty percent Myc mRNA knockdown (IC50) was calculated with GraphPad Prism 7. D: Myc decreased by APTO-253 time-dependently. Cells were treated with APTO-253 at 0.5 µg/l for indicated time points at 37°C. RNA (D3) and protein (D3), representative of at least 3 independent experiments; for vehicle treatment, 1 hour to APTO-253 treatment were extract and quantified by RT-qPCR and Western Blot, respectively. E: APTO-253 cytostatic effect correlates with the IC50. The correlation of IC50s of cytostatic (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 4. APTO-253 dose-dependently and time-dependently induces apoptosis in AML cells. AML cells EOL-1, MV-4;11 and KG-1 were treated with vehicle (DMSO) or APTO-253 (1µM) and subjected to cell cycle analysis by flow cytometry (A1 and A2) and subjected to apoptosis analysis by flow cytometry (A4 and A5). Representative graphs of three independent experiments of three cell lines. B: APTO-253 induced G1/G0 cell cycle arrest. C: APTO-253 induced apoptosis in AML cells. A4 and A5 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. D: 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 A4, total apoptotic cells (Annexin V-PI+) plus Annexin V+PI- cells were analyzed by APTO-253 in dose- and time-dependent manners, which agreed with PARP cleavage.

APTO-253 Induces G1/G0 Cell 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, MV-4;11 and KG-1 were treated with vehicle (DMSO) or APTO-253 (1µM) and subjected to cell cycle analysis by flow cytometry (A1 and A2). Representative graphs of three independent experiments of three cell lines. B: APTO-253 induces G1/G0 cell cycle arrest. D: APTO-253 time-dependently induces cell cycle arrest in AML cells. E: APTO-253 upregulates p21 and p53 at early time points (before 24 hour), which could contribute to apoptosis and cell cycle arrest. F: APTO-253 upregulates p53 stability and activity through inducing phosphorylation and acetylation, which could contribute to its mechanism of action in AML cells. G: Taken together, APTO-253 selectively targets tumor cells and profoundly decreases 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: