Resistance to APTO-253 caused by internal deletion and alternate promoter usage of the MYC gene in Raji B cells.

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INTRODUCTION

- APTO-253 is a novel small molecule that inhibits expression of the MYC oncogene, leading to DNA damage, cell cycle arrest and apoptosis in human-derived solid tumor and hematologic cancer cells.

- In a Phase 1 trial in patients with solid tumors, APTO-253 was well tolerated and produced evidence of antitumor activity but did not cause myelosuppression even at the maximum tested dose.

- A Phase 1a/b trial of APTO-253 in patients with relapsed/refractory high risk MDS and AML is currently underway (NCT02267863).

- The purpose of this project was to understand how APTO-253 regulates MYC and how MYC regulation escapes as resistance emerges in B-cell lines.

MATERIALS and METHODS

- Cytotoxicity study: Cell viability of primary patient cells and cultured cell lines was measured by MTS assay.

- Selection of Raji cells for resistance: The APTO-253-resistant Raji (Raji/253R) cell line was generated by exposure to progressively higher concentrations of APTO-253 over a period of 6 months.

- RT-qPCR and RNA-seq: Total cellular RNA was isolated using the RNeasy mini kit and cDNA was synthesized utilizing Transcriptor Universal cDNA master mix. Expression was calculated as fold change over control samples after normalizing to GAPDH (β-actin). RNA-seq was performed at the UCSF IGM Genomics Center on an Illumina Sequencer HiSeq4000.

- Statistical Analysis: All two-group comparisons utilized Student’s t-test with the assumption of unequal variance. Data are presented as mean ± SEM of a minimum of 3 independent experiments.

RESULTS

- APTO-253 potently kills hematologic malignant cell lines and primary samples from AML and CLL patients.

- APTO-253 binds/stabilizes MYC DNA G-Quadruplex motif leading to inhibition of MYC gene expression and cell apoptosis

CONCLUSIONS

- APTO-253 potently kills malignant cells in both cell lines and primary patient samples.

- APTO-253 targets G-quadruplex motif in the P1/P2 promoter region of MYC gene and inhibits MYC gene expression to induce apoptosis.

- MYC driven Raji cells become resistant to APTO-253 via multiple mechanisms:
  - Up-regulation of ACG2
  - Acquisition of a more stable MYC protein lacking the conserved core sequence of MYC Box III generated by deletion of internal region of MYC gene
  - Utilization of an alternate P3 promoter not inhibited by G4 binding and stabilization

- Cells required three years and multiple modifications in MYC gene to generate high level drug resistance.

- Confirms essential role of MYC in the mechanism of APTO-253

- APTO-253 may serve as a safe and effective non-myelosuppressive first-in-class c-Myc inhibitor for treatment of hematologic malignancies including AML and CLL.