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616. ACUTE MYELOID LEUKEMIA: NOVEL THERAPY, EXCLUDING TRANSPLANTATION: POSTER I | DECEMBER 03, 2015

Inhibition of the PI3K/AKT/mTOR Pathway Leads to Down-Regulation of c-Myc and Overcomes Resistance to ATRA in Acute Myeloid Leukemia

Ronan T. Swords , MDPhDFRCPI FRCPath,[1] Tino Schenk , PhD,[*,2] Sven Stengel , PhD,[*,2] Veronica Sylvina Gil , PhD,[*,2] Kevin R. Petrie , PhD,[2] Aymee Perez , PhD,[*,1] Rodriguez Ana ,[*,1] Justin M. Watts , MD,[*,1] Fernando Vargas , MD,[*,1] Roy Elias ,[*,1] Arthur Zelent , PhD[1]

[1]Sylvester Comprehensive Cancer Center, University of Miami, Miami,

[2]Division of Molecular Pathology, Institute of Cancer Research, London, United Kingdom

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Abstract

Acute Promyelocytic Leukemia (APL) accounts for 5% of all cases of acute myeloid leukemia (AML). This disease is highly curable with all-trans-retinoic acid (ATRA) based therapy. In non-APL AML, ATRA has limited activity, and little is known about mechanisms of ATRA resistance. The apparent selective efficacy of ATRA in PML/RARα-associated APL poses an important question as to whether the presence of this fusion protein renders APL uniquely susceptible. Two compelling arguments can be made to counter this view. First, experiments in vitro show that ATRA effectively differentiates HL-60 cell lines, which lack the PML/RARα fusion protein. Second, clinical studies with ATRA in previously untreated older AML patients (excluding APL) have reported clinical activity. These observations confirm the therapeutic potential of ATRA beyond APL. In this context, our group has previously identified the lysine demethylase LSD-1, as a therapeutic target to re-sensitize leukemic blasts to ATRA. A clinical investigation of ATRA combined with LSD-1 inhibition is currently underway (NCT02273102). It is likely that other defects leading to ATRA resistance will be similarly amenable to pharmacologic manipulation. Defects in the proto-oncogene c-Myc have been widely implicated in the initiation and maintenance of AML. Over-expression of c-Myc in leukemic blasts enhances clonogenic survival and blocks ATRA induced differentiation. We hypothesized that down-regulation of c-Myc might increase the anti-leukemic effects of ATRA in AML. To date, c-Myc

has been an evasive target for direct pharmacologic inhibition however, inhibitors of the PI3K/AKT/mTOR pathway have been shown to indirectly lower levels of c-Myc in leukemic blasts.

In the current study, we show that the pro-differentiation effects of ATRA are markedly potentiated when combined with agents that target PI3K/AKT/mTOR signalling. In AML cell lines and primary patient samples, we observed additive pro-differentiation effects when ATRA was combined with inhibitors of PI3K (ZSTK474) and mTOR complex proteins (Torin-1, WYE-125132). However, when combined with the bromodomain inhibitor NVP-BEZ235, a dual inhibitor of PI3K and mTOR, we observed synergistic induction of CD11b by FACS analysis. Combination studies revealed loss of cell viability, cell cycle arrest in G1 phase, and impaired clonogenic survival, which was more prominent for ATRA combination treatments than with any agent used alone (Figure 1). To assess the role of c-Myc in mediating these effects, we measured c-Myc protein levels and PI3K/AKt/mTOR pathway markers at different time-points following treatment with ATRA alone and in combination with the inhibitors described above (Figure 2). Our findings suggest that ATRA alone quickly down-regulates c-Myc (within 6 hours) through transcriptional repression. Disruption of the PI3K/AKT/mTOR pathway further down-regulates c-Myc (within 3 hours) through destabilization and enhanced degradation. ATRA combined with NVP-BEZ235 produced maximal c-Myc suppression, and led to more cell kill than any other combination tested. Detailed analysis of changes in the transcriptome in MV-411 cells following treatment with ATRA and NVP-BEZ235 revealed that both agents act jointly on the regulation of the same biological pathways and processes, but regulate different sets of genes within these pathways. Updated mechanism based studies will be presented.

In conclusion, suppression of c-Myc levels through disruption of PI3K/AKT/mTOR signalling augments the anti-leukemic effects of ATRA. These data support the clinical investigation of ATRA combined with rapalogs or bromodomain inhibitors.

Figure 1. **Combination treatment with PI3K/mTORC inhibitors and ATRA decreases cell viability in AML cells.** MV4-11 cells were treated as indicated with combinations of BEZ (1μM), WYE (1μM) or ZSTK (2.5μM) and ATRA (0.1 μM). Number of cells was determined by CellTiter-Glo¨ luminescent cell viability

assay (Promega). Data were analyzed by one-way ANOVA ($P < 0.0001$) followed by TukeyÕs post-hoc test. * P< 0.05, ** $P < 0.01$, *** P < 0.001,**** P < 0.0001.

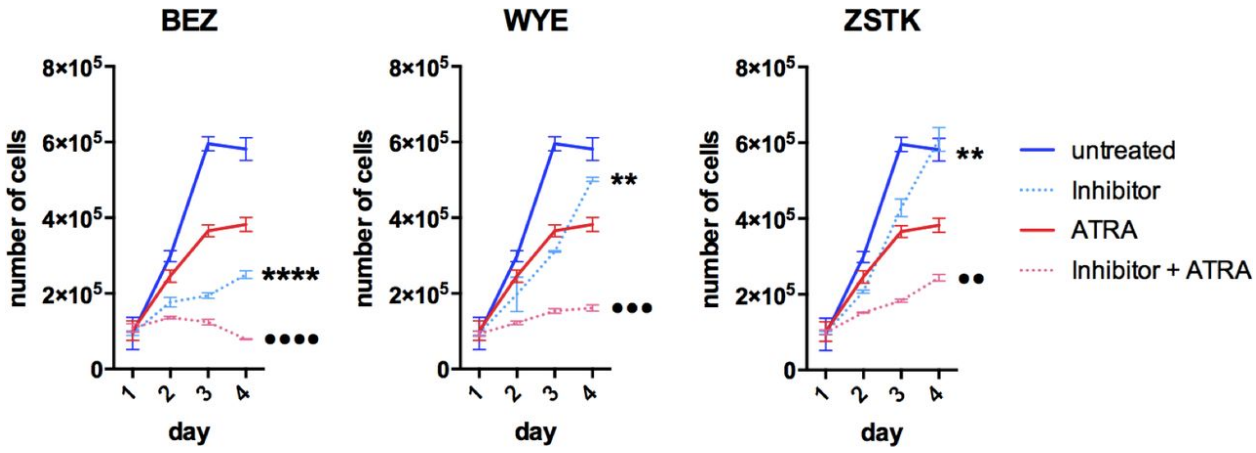

Figure 2. **Reduced expression of MYC protein by inhibition of the PI3K/AKT/mTORC pathways. Immunoblotting**/quantification of MYC protein levels in MV4-11 cells following treatment with combinations of WYE (1μM), BEZ (1μM), ZSTK (2.5μM) and ATRA (0.1 μM).

Disclosures

No relevant conflicts of interest to declare.

Author notes

*Asterisk with author names denotes non-ASH members.