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POSTER ABSTRACTS

617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

In Vivo Antileukemic Activity of Ost-01 in Acute Myeloid Leukemia (AML): A Novel Natural Product (NP) from *Baccharis Coridifolia*

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Baccharis is the largest genus in the Asteraceae and has been long used by phytomedicine practitioners in South America to treat a variety of medical conditions (i.e., wounds, infections, cancers). Herein, we report on the antileukemic activity of OST-01, a novel NP from *Baccharis coridifolia*. OST-01 was obtained through maceration of *Baccharis coridifolia* leaves in ethanol; the starting doses for testing the OST-01 activity against leukemic cells were derived empirically from routinely used dosages of similar plant extracts. OST-01 (0.25-2 μL/mL) inhibits cell growth and induces apoptosis of AML cell lines (MV-4-11, KG-1a, Kasumi-1, and HL-60) and primary CD34 $^{+}$ CD38 $^{-}$ AML blasts [enriched for leukemia stem cells (LSCs)] but did not affect normal human CD34 $^{+}$ CD38 $^{-}$ mononuclear cells [MNCs, enriched for hematopoietic stem cells (HSC)]. Compared to vehicle (V; 1 μl/g/BID PO x 21 days), OST-01 (1 μl/g/BID PO x 21 days) reduced leukemia burden (WBC: 59.4 vs 96.4x10 ^{3}/μL, p=0.01; spleen size: 0.38 vs 0.77 g, p<0.0001) and prolonged survival (median: 123 vs 92 days, p<0.0001) of *Mll* $^{PTD/WT}$/ *Flt3* $^{ITD/ITD}$ AML mice. In secondary transplant experiments, recipients of bone marrow (BM) MNCs from OST-01-treated donors, lived significantly longer than the recipients of BM MNCs from V-treated donors (median: 58 vs 26 days, p<0.0001), suggesting that OST-01 reduced LSC burden. We confirmed these results in a *Cbfb-MYH11* (*CM*) knock-in AML mouse model. OST-01-treated mice had a longer survival than V-treated controls (median: 70 vs 58 days, p<0.0001) and secondary recipients of BM MNCs from OST-01-treated donors lived also significantly longer than those of BM MNCs from V-treated donors (median: 63 vs 38 days, p<0.0001). Similar results were also observed in mice engrafted with luciferase-expressing FLT3-ITD+ MOLM-14 AML cells. Bioluminescence imaging indicated that OST-01 reduced leukemic burden compared to V treatment (@day 30: 2.3 vs 4.5x10 ^{6} photons/s/cm ^{2}/sr, p<0.0001); OST-01-treated mice lived significantly longer than V-treated controls (median: 46 vs 32 days, p<0.0001). Of note, no OST-01-related toxicity given within a dose range of 1-3 μl/g/BID, PO x 7, 28 or 56 days was observed in normal B6 mice (n=120 mice).

Compared with OST-01 alone or V, combination of OST-01 and the BCL-2 inhibitor, venetoclax (VEN) demonstrated a synergistic activity on AML cell lines and primary blasts (max synergy score: 28.16), and prolonged survival of *Mll* $^{PTD/WT}$/ *Flt3* $^{ITD/ITD}$ AML mice (VEN/OST vs OST and VEN, median: 156 vs 123 and 95 days, p=0.0003 and p<0.0001; secondary transplant median: 105 vs 58 and 31 days, p<0.0001 and p<0.0001, respectively) and *CM* AML mice (VEN/OST vs OST and VEN, median: 85 vs 70 and 58 days, p=0.0005 and p<0.0001; secondary transplant median: 95 vs 62.5 and 48 days, p=0.0003 and p<0.0001, respectively).

To gain insights into the mechanism of action of OST-01, we performed RNA-seq on OST-01- or V-treated HL-60 cells and CD34+CD38- AML blasts. By GSEA analysis, we identified 50 enriched Hallmark gene sets (26 upregulated and 24 downregulated) in OST-01- vs V-treated HL-60 cells and 49 (32 upregulated and 17 downregulated) in OST-01- vs V-treated CD34+CD38- AML blasts. The Hallmark gene sets comprising Myc targets were among the most downregulated in OST-01-treated cells. We confirmed these results showing an OST-01-dependent decrease in c-myc, inhibition of c-myc signaling, and deregulation of c-myc-dependent nucleolar structure and ribosome biogenesis in HL-60 cells and CD34+CD38- AML blasts. To this end, OST-01 enhanced ubiquitination and degradation of c-myc and nucleolar proteins (NPM1, NS, nucleolin) and in turn induced nucleolar disruption as shown by immunoblotting, immunoprecipitation, and electron microscope imaging in HL-60 cells and CD34+CD38- AML blasts. Of note, OST-01 also inhibited ErbB3 binding protein 1 (Ebp1)-enhanced ribosomal RNA (rRNA) synthesis through upregulation of expression and function of RNA Polymerase I (Pol I) and DEAD-box helicase 21 (DDX21).

In summary, we provide the first report of a significant *in vivo* antileukemic activity of a novel NP, OST-01. The mechanisms of action of OST-01 are mediated by inhibition of c-myc-dependent signaling, rRNA synthesis and ribosome biogenesis. To date, no preclinical toxicity has been observed. We concluded that OST-01 is a non-toxic, potentially new therapy for AML; translation from the bench to the bedside is underway.

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