Supplemental Data

A Novel Agent SL-401 Induces Anti-Myeloma Activity by Targeting Plasmacytoid Dendritic Cells, Osteoclastogenesis, and Cancer Stem-like Cells

Short Title: Depletion of dysfunctional pDCs as myeloma therapy

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Supplementary Figure legends

Supplementary Figure S1: (**S1A) Effect of SL-401 on the viability of MM cell lines** MM.1S, MM.1R, RPMI-8226, Dox-40, and LR-5 cells were cultured in the presence or absence of SL-401 for 72h, followed by assessment of viability using MTT assay (mean ± SD, $p < 0.005$ for all cell lines) .

(S1B) Effect of SL-401 on pDC-induced growth of MM cell lines Different MM cells lines (5×10^4 cells/200 µl) and MM patient pDCs (1×10^4 cells/200 µl) were co-cultured (1:5 pDC/MM ratio) for 48h in the presence or absence of indicated concentrations of SL-401, and then analyzed for growth using WST assays (mean ± SD, $p < 0.005$; n=3). Data are presented as fold change in pDC-induced growth versus the respective cell lines alone.

Supplementary Figure S2 **Determination of the maximum tolerated dose and toxicity of SL-401** (A-D) SCID mice (4 mice/group) were intravenously injected with control vehicle or SL-401 [12 µg/kg (A), 16 µg/kg (B), 25 µg/kg (C), or 50 µg/kg (D)] for 5 consecutive days each week for 3 weeks. Mice were checked for any signs of acute toxicity assessed by agility, normalcy of kin coat, and weight changes. Bar graphs show average body weight changes in untreated- and SL-401-treated mice. Mice receiving 25 µg/kg of SL-401 showed decreased activity with 11.8% (trendline) drop in average weight versus control mice (C). Administration of 50 µg/kg of SL-401 led to a significant decrease (36.5% trendline drop) in body weight, and all mice in this cohort died.

Supplementary Figure S3 **Combination of SL-401 and bortezomib triggers synergistic anti-MM activity** Co-cultures of MM.1S cells and patient BM-pDCs at 1:5 (pDC/MM) ratio were treated with indicated concentrations of SL-401, bortezomib or SL-

401 plus bortezomib for 72h, and then analyzed for viability. Drug interactions at different combination concentrations were evaluated using Isobologram and Combination Index (CI) analyses. The graph (right) is derived from the values given in the table (left). Numbers 1–6 in graph represent combinations in Table. CI<1.0 indicates synergy.

Supplementary Figure S4 **Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN) derived Cal-1 cells induce MM cell growth** Cal-1 and MM.1S or MM.1R cells were cultured either alone or together at 1:5 (cal-1/MM) ratio for 48h, and then analyzed for growth using WST assays (mean ± SD, $p < 0.005$).

Supplementary Figure S5 **Synergistic anti-MM activity of SL-401 and pomalidomide *in vitro*** **(A)** Co-cultures of RPMI-8226 cells and patient BM-pDCs at 1:5 (pDC/MM) ratio were treated with indicated concentrations of SL-401, pomalidomide or SL-401 plus pomalidomide for 72h, and then analyzed for viability. Drug interactions at different combination concentrations were evaluated using Isobologram and Combination Index (CI) analyses. The graph (left) is derived from the values given in the table (right). (**B)** Numbers 1–9 in graph represent combinations in Table. CI<1.0 indicates synergy.

Supplementary Figure S6 **Comparative analysis of SL-401 activity against different clonogenic MM side population (MM-SPs) cells** (**A**) KMS-11, (**B**) OPM2, and (**C**) NCI-H929 were treated with increasing concentrations of SL-401 for 48h, followed by Hoechst 33342 staining (MM-SPs staining as described in methods; ~99% purity). Following staining, the cells were subjected to flow analysis. MM-SP cells were gated out and the effect of SL-401 on different side population cells was quantified. ***Left Panels****:* The bar graphs show the percentage of MM-SPs at different SL-401 concentrations (mean ± SD; n=3; $p < 0.005$). ***Right Panels:*** Respective MM cell lines and MM-SPs were analyzed for IL-3R expression using anti-IL3RA-PE Abs by flow cytometry (mean ± SD; n=5).

Supplementary Figure S7 **Effect of SL-401 Oct-4 transfection on CD123/IL-3R expression in RPMI-8226 cells** RPMI-8226 cells were transfected with Oct4-GFP cDNA, and stably expressing Oct4+ RPMI-8226 cells (RPMI-8226-Oct4) were selected with G418 (0.5 mg/ml). After selection, the cells were sorted for GFP/CD123 double positive populations to obtain RPMI-8226-Oct4. RPMI-8226 and RPMI-8226-Oct4 cells were then analyzed for CD123/IL-3RA expression by staining with anti-CD123/IL3RA-PE Abs. Bar graph shows the percent mean fluorescence intensity of IL-3RA/CD123^{hi} populations in RPMI-8226 and RPMI-8226-Oct4 cells.