Supplemental Data:

A Novel Alkylating Agent Melflufen Induces Irreversible DNA Damage and Cytotoxicity in Multiple Myeloma Cells

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Running title: Alkylating drug melflufen as myeloma therapy

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Authors' contributions: AR designed research, performed the experiments, analyzed data, and wrote the manuscript; DSD and YS contributed in Western blot analysis and flow cytometry; JC synthesized melflufen; PGR provided clinical samples; DC designed research, analyzed data, and wrote the manuscript; and KCA analyzed data and wrote the manuscript.

Supplementary Figure Legends:

Figure S1: Flow analysis of the induction of γ-H2AX in different primary cells. MM tumor cells were isolated from the bone marrow of relpased/refractory patients (n=3), and cells were treated with melflufen (5 μM) or melphalan (10 μM) for 1h, washed with PBS to remove drugs, fixed and stained with Alexa Fluor 647 conjugated anti-H2AX (pS139) Ab followed by FACS analysis. Bar Graph: Quantification of γ-H2AX populations on MM tumor cells (mean ± SD; $p < 0.05$; n=3).

Total MNCs were isolated from the peripheral blood samples of normal healthy donors (3 normal PBMC samples). Cells were treated with melflufen or melphalan (10 μM) for 1h, washed with PBS to remove drugs, fixed and stained with Alexa Fluor 647 conjugated anti-H2AX (pS139) Ab followed by FACS analysis. Melflufen/Melphalan-treated normal MNCs were also analyzed for the induction of γ-H2AX on T or B cells. For this, the cells were subjected to 2-color staining; either CD3-FITC plus γ-H2AX-Alexa-647 (pan T cells), or CD20-FITC plus γ-H2AX-Alexa-647 (B cells). The FITC-expressing cells were first gated out based respective forward versus side scatter plots. The FITC-expressing populations were further analyzed for the induction of γ-H2AX based on signals in Alexa-Fluor-647/APC channel. Bar Graph: Quantification of γ-H2AX populations on total MNCs, CD3-T or CD20-B cells (mean ± SD; $p < 0.05$; n=3, each sample measured in quadruplicate).

Figure S2: Comparison of melflufen and melphalan-induced apoptosis in CD138+ve MM tumor cells isolated from the BM of relpased/refractory patients: Tumor cells were treated with melflufen (5 μM) or melphalan (10 μM) for 1h, washed with PBS to remove the drug, followed by culture in the absence of inhibitor and presence of recombinant human IL-6 (5 ng/ml) in (10%FCS + RPMI1640) medium. Cells were harvested after 48h and 72h and subjected to flow analysis for viable versus nonviable cells. Bar Graph: Quantification of dead cells in melflufen- and melphalan-treated populations (mean ± SD; $p < 0.05$; n=3).