PARP1 and *POLD2* as prognostic biomarkers for multiple myeloma in autologous stem cell transplant

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Received: November 9, 2022.
Accepted: February 23, 2023.
Early view: March 2, 2023.

https://doi.org/10.3324/haematol.2022.282399

Abstract

Multiple Myeloma (MM) is an incurable plasma cell malignancy often treated by autologous stem cell transplant (ASCT). Clinical response to ASCT has been associated with DNA repair efficiency. Here we interrogated the role of the base excision DNA repair (BER) pathway in MM response to ASCT. Across 450 clinical samples and six disease stages, expression levels of genes in the BER pathway were found to be highly upregulated during the development of MM. In a separate cohort of 559 patients with MM treated with ASCT, expression of BER pathway members *MPG* and *PARP3* was positively associated with overall survival (OS) while expression of *PARP1*, *POLD1*, and *POLD2* was negatively associated with OS. In a validation cohort of 356 patients with MM treated with ASCT, *PARP1* and *POLD2* findings were replicated. In patients with MM who never received ASCT (n=319), *PARP1* and *POLD2* were not associated with OS, suggesting that the prognostic effect of these genes may be treatment-dependent. In preclinical models of MM, synergy was observed in anti-tumor activity when poly (ADP-ribose) polymerase (PARP) inhibitors (olaparib, talazoparib) were used in combination with melphalan. The negative prognosis associated with *PARP1* and *POLD2* expression along with the apparent melphalan-sensitizing effect of PARP inhibition may suggest this pathway as a potential biomarker in patients with MM in the setting of ASCT. Further understanding of the role of the BER pathway in MM is vital to improve therapeutic strategies related to ASCT.

Introduction

Multiple Myeloma (MM) is an incurable plasma cell malignancy that accounts for 18% of all hematologic malignancies.[1] Despite significant therapeutic advances for MM, autologous stem cell transplant (ASCT) following high-dose, single-agent melphalan conditioning remains a cornerstone of therapy.[1] Although ASCT provides significant benefits to some patients with MM, it is not curative and is associated with significant heterogeneity in clinical benefit.[2] The primary driver of response to ASCT is the depth of anti-myeloma activity of melphalan.[3] Patients achieving deeper remission receive the greatest duration of clinical benefit following ASCT.[4] While studies have historically noted an increased overall survival (OS) in patients with MM who receive ASCT, the recent DETERMINATION trial demonstrates only progression-free survival (PFS) benefit in patients with MM randomized to receive triplet therapy (lenalidomide, bortezomib, and dexamethasone followed by lenalidomide maintenance) with or without ASCT.[5] This data proves an urgent need to identify biomarkers of *de novo* response and resistance to ASCT in order to improve patient selection for this therapeutic modality.

Melphalan acts by alkylating DNA and causing single-strand DNA breaks as well as other DNA lesions, primarily repaired through the base excision repair (BER) pathway.[6] Poly (ADP-ribose) polymerases (PARP) are a family of enzymes that catalyze the transfer of ADP-ribose to target proteins (poly ADP-ribosylation) and are involved in nucleic acid metabolism, modulation of chromatin structure, DNA synthesis, and DNA repair.[7] PARP are a pivotal component of the BER complex, which consists of DNA ligase III, DNA polymerase β, and the XRCC1 proteins, and contributes to BER response to single-strand DNA breaks. Key proteins involved in the BER pathway, including APEX1/2, XRCC1, PARP1, POLD2, have been associated with chemoresistance across many cancer types.[8–11] The expression and activity of genes in the BER

pathway increase in response to the accumulation of DNA alkylating or damaging agents as well as radiation-induced DNA damage.[12,13] It is therefore unsurprising that the activity of DNA repair through the BER pathway in MM has been associated with response to melphalan.[14–16] While individual members of the BER pathway have been studied in the setting of MM and ASCT, comprehensive assessment of this pathway has yet to be conducted.

Because of the heterogeneity inherent to melphalan exposure and anti-myeloma activity, there remains a critical need to better understand biomarkers of melphalan resistance, both *de novo* and acquired, in patients with MM receiving ASCT.[17,18] In this study, we comprehensively assessed the expression of the BER pathway across MM. We leveraged large transcriptomic datasets containing MM tumors to quantify the role of genes in the BER pathway in MM. Our results indicated that the expression of *PARP1* and *POLD2* were significantly associated with OS in patients with MM treated with ASCT followed by high-dose melphalan treatment. We then used both *in vitro* and *in vivo* MM models to test the impact of BER pathway attenuation, through PARP inhibition, on sensitivity to melphalan. Together, our results demonstrate that *PARP1* and *POLD2* may represent an ASCT-specific biomarker with potential for optimizing the therapeutic modality of melphalan-conditioned ASCT in patients with MM.

Methods

Publicly available datasets

Publicly available data were collected from the Gene Expression Omnibus (GEO), the Multiple Myeloma Research Foundation (MMRF), and the genomics of drug sensitivity in cancer database as approved by the University of Florida Institutional Review Board (#IRB202101136).[18-23] Further details are available in the *Online Supplementary Appendix*.

Multiple myeloma cell lines, reagents and assays

MM cell lines MM1S and NCI-H929 were obtained from American Type Culture Collection (ATCC; Manassas, VA). These cell lines were regularly authenticated using short tandem repeat polymorphism (STRP) analysis as recommended by ATCC, were mycoplasma free, and used within 6 months of receipt from ATCC. All *in vitro* studies were conducted in at least triplicate and in at least three independent experiments. Therapeutic reagents included melphalan, olaparib, and talazoparib (Sigma-Aldrich). Cell viability was assayed using the MTT Cell Proliferation Assay Kit (Roche, Indianapolis, IN) following the manufacturer's directions. Cellular apoptosis were measured using the Attune NxT Flow Cytometer and the standard manufacture recommended protocol (ThermoFisher). Further details are available in the *Online Supplementary Appendix*.

In vivo experiments

MM1S cells were injected in a phosphate-buffered saline/matrigel suspension of 100 μL in both right and left flanks of 60 nude mice (22 females and 38 males; The Jackson Laboratory, Bar Harbor, ME). Mice were monitored daily until tumors reached 200 mm^2 then randomized to receive vehicle control, talazoparib alone, melphalan alone, or melphalan in combination.[24,25] Mice were removed from the study if their weight was <80% of baseline for 2 days, or if total tumor size per mouse reached >1,600 mm^2. All animal studies were conducted following the Ohio State University Institutional Animal Care and Use Committee (IACUC) approval. Further details are available in the *Online Supplementary Appendix*.

Statistical methods

All data were analyzed in $R_{v.4.1.1}$ (The R Project for Statistical Computing, https://www.r-project.org) or Graphpad $Prism_{v.9.2.0}$ (GraphPad Software, San Diego, CA). Additional graphics were created with BioRender.com (BioRender, Toronto, Ontario). Members of the BER pathway were defined by the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database.[19] Single-sample gene set enrichment analysis (ssGSEA) was conducted using the $GSVA_{v1.40.1}$ package. Two-group analysis of continuous variables was conducted using the Mann Whitney Wilcoxon test while analysis of continuous variables across multiple groups was conducted using the Kruskal-Wallis test. Heatmaps were constructed using the $pheatmap_{v1.0.12}$ package. Uniform manifold approximation and projection for dimension reduction (UMAP) plots were generated using the package $umap_{v0.2.7.0}$. Survival analysis was tested using Cox proportional-hazards regression for continuous variables and log-rank tests for categorical variables. Stepwise Cox proportional-hazards regression was conducted using the package $My.stepwise.coxph_{v0.1.0}$. Survival graphs were created using the Kaplan–Meier estimator. Combination index was calculated using CompuSyn[20] (ComboSyn, Inc, Paramus, NJ) and Combenefit[21] (CRUK Cambridge Institute, Cambridge, UK) using the highest single-agent (HSA) model. Changes in tumor growth over time were tested by ANOVA with repeated measures. Unless otherwise stated, two-sided *P* values ≤0.05 were considered statistically significant. Adjustment of *P* values was conducted using false discovery rate. Further details are available in the *Online Supplementary Appendix*.

Results

Genes in the BER pathway across multiple myeloma developmental stages

In order to assess the expression levels of genes in the BER pathway across the developmental stages of MM, we collected data from four publicly available datasets:

GSE13591, GSE23113, GSE6477, GSE5900 (*Online Supplementary Table S1*). The data coved six developmental stages of MM including: normal plasma cells, monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma (sMM), MM, relapsed MM (rMM), and plasma cell leukemia (PCL). Studies were analyzed separately due to potential batch effect between studies. Using ssGSEA to measure pathway enrichment, the BER pathway gene set was consistently up-regulated across the development of MM (Figure 1A). In order to further test the difference in expression of genes in the BER pathway between MM and MGUS, we compared expression between disease stages for the three datasets with data available (Figure 1B-D). Genes in the BER pathway were generally more highly expressed in MM. Across all three datasets included in this analysis, *APEX1, FEN1, POLD2, POLD3*, and *UNG* were significantly up-regulated, while *APEX2, MBD4, PARP1, PARP2, PCNA, POLB,* and *TDG* were significantly up-regulated in MM in at least two datasets. The only gene to be consistently down-regulated in MM compared to MGUS was NEIL3. Using only the genes in the BER pathway, MM and MGUS clustered separately with MM demonstrating consistent deregulation expression of the BER pathway in MM (*Online Supplementary Figure S1*).

Expression of genes in the BER pathway correlates with overall survival in the training set

In order to determine the prognostic significance of genes in the BER pathway in MM, we collected gene expression and clinical outcomes data for 559 patients with newly diagnosed MM from the geo set GSE2658. All patients in the dataset received ASCT and either Total Therapy 2 (TT2) or Total Therapy 3 (TT3) treatment. Receipt of TT2 or TT3 in this dataset was not associated with OS in this dataset (P=0.95). Due to significant co-expression of genes in the BER pathway (*Online Supplementary Figure S2*), we used a stepwise Cox proportional-hazards regression to build a multivariable model for OS using genes in the BER pathway and a significance level for entry/stay of 0.1 (*Online Supplementary Table S2*). The resulting multivariable model included five genes that met our significance threshold (P<0.1): *MPG, PARP1, PARP3, POLD1,* and *POLD2*. Using median expression to categorize high and low expression samples, high expression of *PARP1* (hazard ratio [HR]=1.76; 95% confidence interval [95%CI]: 1.19-2.61; P=0.005) and *POLD2* (HR=1.47; 95% CI: 0.99-2.17; P=0.06) were associated with reduced OS (Figure 2A). High expression of *PARP3* (HR=0.45; 95% CI: 0.30-0.67; P<0.0001) and MPG (HR=0.67; 95% CI: 0.45-0.99: P=0.04) were associated with improved OS. *POLD1* was not associated with OS (HR=1.30; 95% CI: 0.88-1.93; P=0.19). In a multivariable Cox proportional-hazards regression model of *PARP1, POLD2, PARP3, MPG*, and *POLD1* as categorical variables, using a median gene expression cut-off, PARP1, *POLD2, PARP3*, and *MPG* remained significantly associated with OS (Figure 2B). The multivariate model was not changed when the total therapy cohort was included (P=0.95).

PARP1 and *POLD2* gene expression negatively correlate with overall survival in the validation set

In order to validate the findings from GSE2658, we collected gene expression and clinical data for 356 patients with MM who had received ASCT and 319 patients with MM who had never received a transplant from the MMRF (*Online Supplementary Figure S3*). In patients with MM who had received ASCT, we used a median expression cut-off to categorize high- and low-expression samples. High expression of *PARP1* (HR=2.15; 95% CI: 1.34-3.45; P=0.002) and *POLD2* (HR=1.67; 95% CI: 1.04-2.68; P=0.03) was significantly associated with reduced OS (Figure 3A). PARP3 (HR=0.88; 95% CI: 0.55-1.41; P=0.58) and MPG (HR=0.79; 95% CI:0.49-1.27; P=0.34) were not associated with OS.

Given the availability of extensive clinical data in the MMRF, we conducted univariable and multivariable Cox proportional-hazards regression using both gene expression and clinical variables (*Online Supplementary Table S3*). In a multivariable clinical-genomic model including patient age, revised International Staging System (R-ISS), International Myeloma Working Group classification (IMWG), *TP53* status, Eastern Cooperative Oncology Group (ECOG) performance status, pre-ASCT induction therapy, and post-ASCT maintenance therapy, *PARP1* and *POLD2* were independently associated with OS (Figure 3B). It is notable that in this cohort R-ISS staging was not statistically associated with OS, driven by lack of full staging data and a low number of death events in the patients with R-ISS staging data available. Future analysis should make use of full up-to-date staging systems in their analysis.

We then tested the prognostic value of *PARP1, POLD2, PARP3*, and *MPG* in patients with MM who had never received a transplant to test whether the association between expression of genes in the BER pathway and OS in MM was inherent to the disease biology or related to receipt of ASCT. For patients with MM who had never received a transplant, expression of *PARP1, POLD2, PARP3*, and *MPG* did not correlate with OS (Table 1). The expression levels of these four genes were not significantly different between patients with MM receiving ASCT and patients who had not received ASCT (*Online Supplementary Figure S4A-D*). It is important to note that the patients who received ASCT and did not receive ASCT cannot be directly compared as they represent populations selected by clinical features, making them inherently different. The findings here only underscore that the relationship between *PARP1* and *POLD2* are validated only for patients receiving ASCT.

A potential co-variate of these findings is the known prognostic effect of chromosome 1q duplications in MM.[22] Specifically, amplification of the 1q21 locus has been associated with poor clinical outcomes.[23] In the MMRF dataset, *PARP1* (1q42.12) and *POLD2* (7p13) were overexpressed in samples with gain of 1q21 in at least 20% of cells identified by sequential fluorescence *in situ* hybridization (seqFISH) (*Online Supplementary Figure S4E*). *PARP1* and *POLD2* remained weakly correlated in samples with (Spearman's correlation coefficient: 0.04) or without (Spearman's correlation coefficient: 0.03) gain of 1q21. In order to test the role of 1q gain in MM, we correlated the expression of genes located on 1q21 hypothesized to play a role in the mechanism of gain of 1q21 related clinical outcomes[24] (*Online Supplementary Figure S4F*), including *CKS1B* (1q21.3), *IL6R* (1q21.3), *MCL1* (1q21.2), and *BCL9* (1q21.2). In patients receiving ASCT, all 1q21 genes queried were increased in MM with gain of 1q21, while in univariable analysis gain of 1q21, *CSK1B*, and *IL6R* were statistically associated with reduced OS (*Online Supplementary Figure S4G, H*). Increased PARP1 expression were associated with poor OS in both 1q21 amplified and non-amplified disease (*Online Supplementary Figure S4I*).

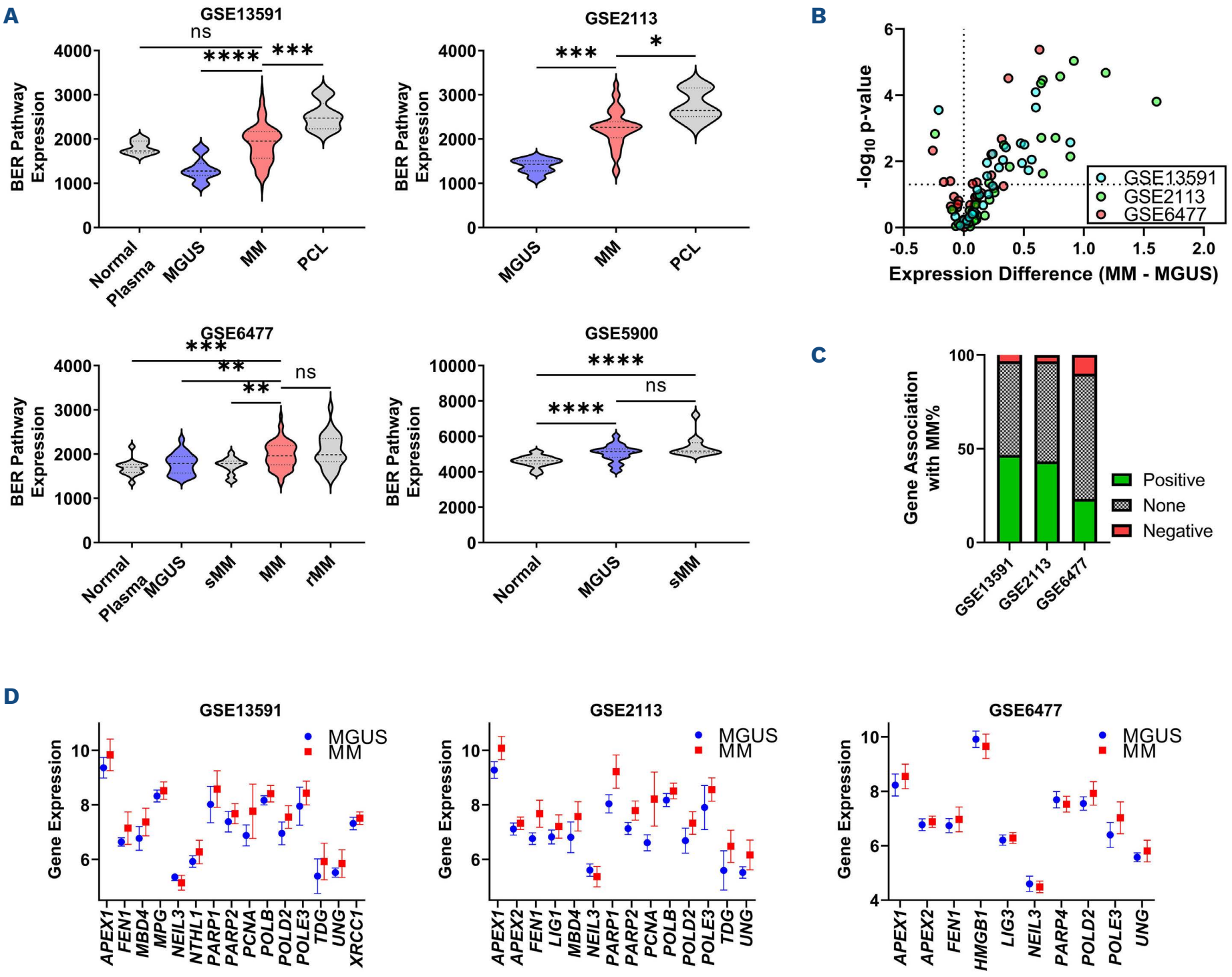

Figure 1. Base excision repair gene expression is increased across mupliple myeloma development. (A) Across datasets, the base excision repair (BER) pathway, as measured by single sample gene set enrichment analysis (ssGSEA), was consistently upregulated across the development of multiple myeloma (MM). *P* values were calculated using two-sided Mann Whitney Wilcoxon tests. ssGSEA is an extension of GSEA, which calculates gene set enrichment scores for each sample. Each ssGSEA enrichment score represents the degree of gene set up- or down-regulated within a sample. (B, C) Comparing monoclonal gammopathy of undetermined significance (MGUS) and MM in GSE13591, GSE2113, and GSE6477 demonstrated significant upregulation of genes in the BER pathway in MM. (D) Significant gene expression differences between MGUS and MM for genes in the BER pathway. All genes included are statistically significantly different as measures by Mann Whitney Wilcoxon test. Plot represents the center as the mean, error bars as ± standard deviation. ns: not significant; *$P \leq 0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.0001$.

For patients who had never received a transplant, all 1q21 genes queried remained increased in MM with gain of 1q21; however, no 1q21 gene or 1q21 status were associated with OS (*Online Supplementary Figure S4J, K*). *PARP1* was not associated with OS regardless of 1q21 status (*Online Supplementary Figure S4L*). While the role of *PARP1* expression cannot be dissected from 1q amplification in this dataset, this data suggests that 1q status and *PARP1* expression

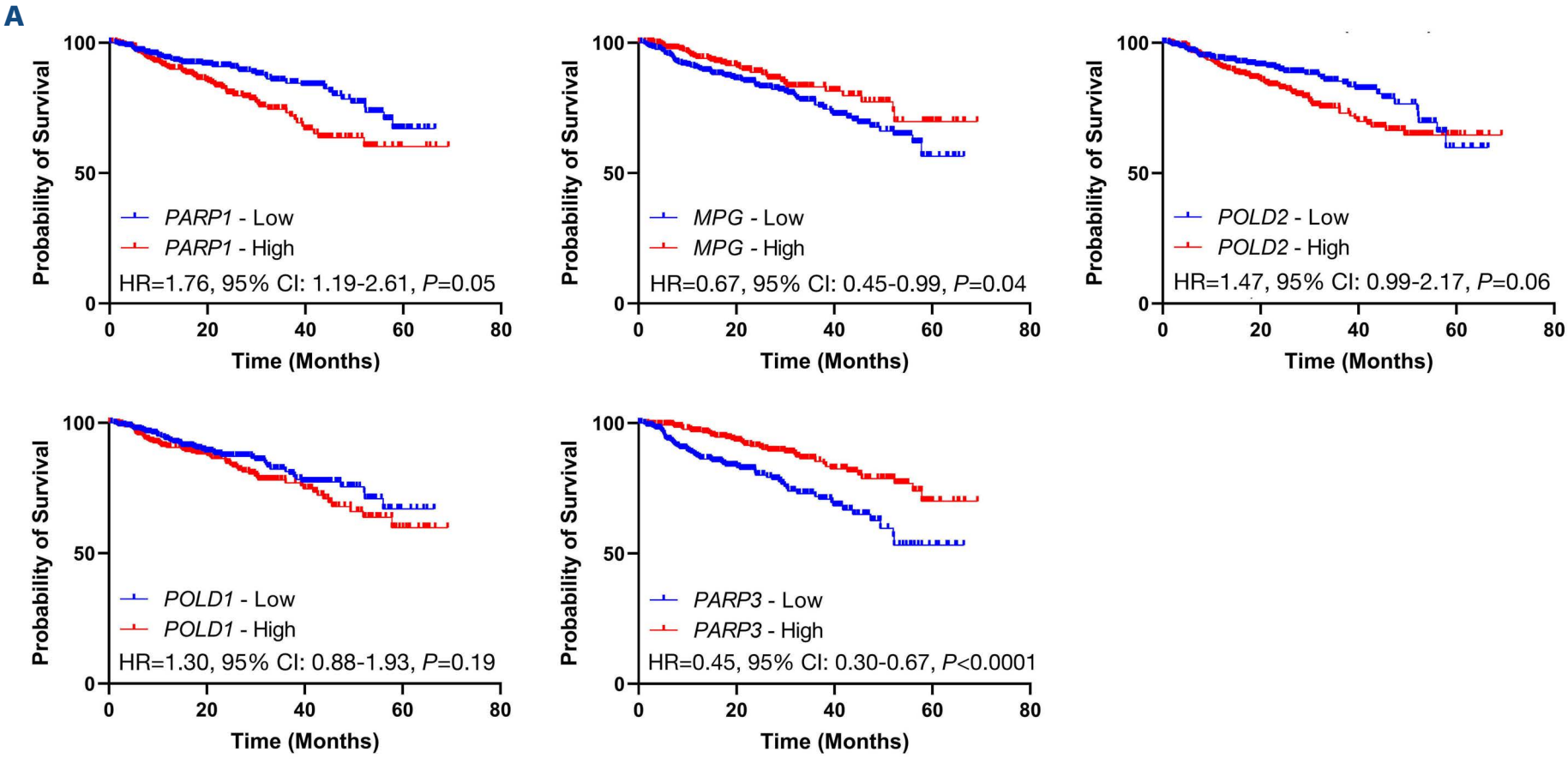

Figure 2. Base excision repair gene expression correlation with survival in training set. Gene expression and survival data for 559 pretreatment patients with multiple myeloma (MM) who received autologous stem cell transplant (ASCT) were collected from GSE2658. Stepwise Cox proportional-hazards regression on identified 5 genes correlated with overall survival (OS) as continuous variables. (A) Using a median expression to categorize high- and low-expression samples, high expression of *PARP1* and *POLD2* were associated with reduced OS. High expression of *PARP3* and *MPG* were associated with improved OS. Using median expression, *POLD1* was not associated with a change in OS. (B) In a multivariable Cox proportional-hazards regression model of PARP1, *POLD2*, *PARP3*, *MPG*, and *POLD1* as categorical variables using a median gene expression, *PARP1*, *POLD2*, *PARP3*, and MPG were significantly associated with OS. Points represent hazard ratios (HR), error bars represent 95% confidence interval (95% CI).

Table 1. Biomarkers of overall survival in patients with multiple myeloma.

Gene	Received ASCT (N=356)			Never received transplant (N=319)		
	HR	95% CI	*P*	HR	95% CI	*P*
PARP1 - High	2.15	1.31-3.55	0.003	1.03	0.77-1.45	0.85
PARP3 - High	0.88	0.55-1.41	0.58	0.83	0.59-1.17	0.28
POLD2 - High	1.67	1.03-2.71	0.04	1.06	0.75-1.48	0.75
MPG - High	0.79	0.49-1.27	0.34	0.88	0.63-1.24	0.47

ASCT: autologous stem cell transplant; HR: hazard ratio; CI: confidence interval.

may influence OS following ASCT and warrants further study.

PARP1 and *POLD2* co-expression define high-risk population specific to patients with multiple myeloma receiving autologous stem cell transplant

In order to determine the prognostic significance of *PARP1* and *POLD2* co-expression in patients with MM, we used a median gene expression cut-off to categorize high- and low-expression samples for each gene into four *PARP1/POLD2* categories: High/High, High/Low, Low/High, and Low/Low. High expression of both *PARP1* and *POLD2* was associated with poor OS in patients from GSE2658 (P=0.0001; Figure 4A) and MMRF (P=0.0006; Figure 4B) who had received ASCT. Multivariable analysis of *PARP1* and *POLD2* co-expression demonstrated significance for PARP1 in both sets while *POLD2* was only statistically significant in the MMRF dataset. For patients with MM in the MMRF dataset who never received transplant, *PARP1* and *POLD2* co-expression was not associated with OS (P=0.72;

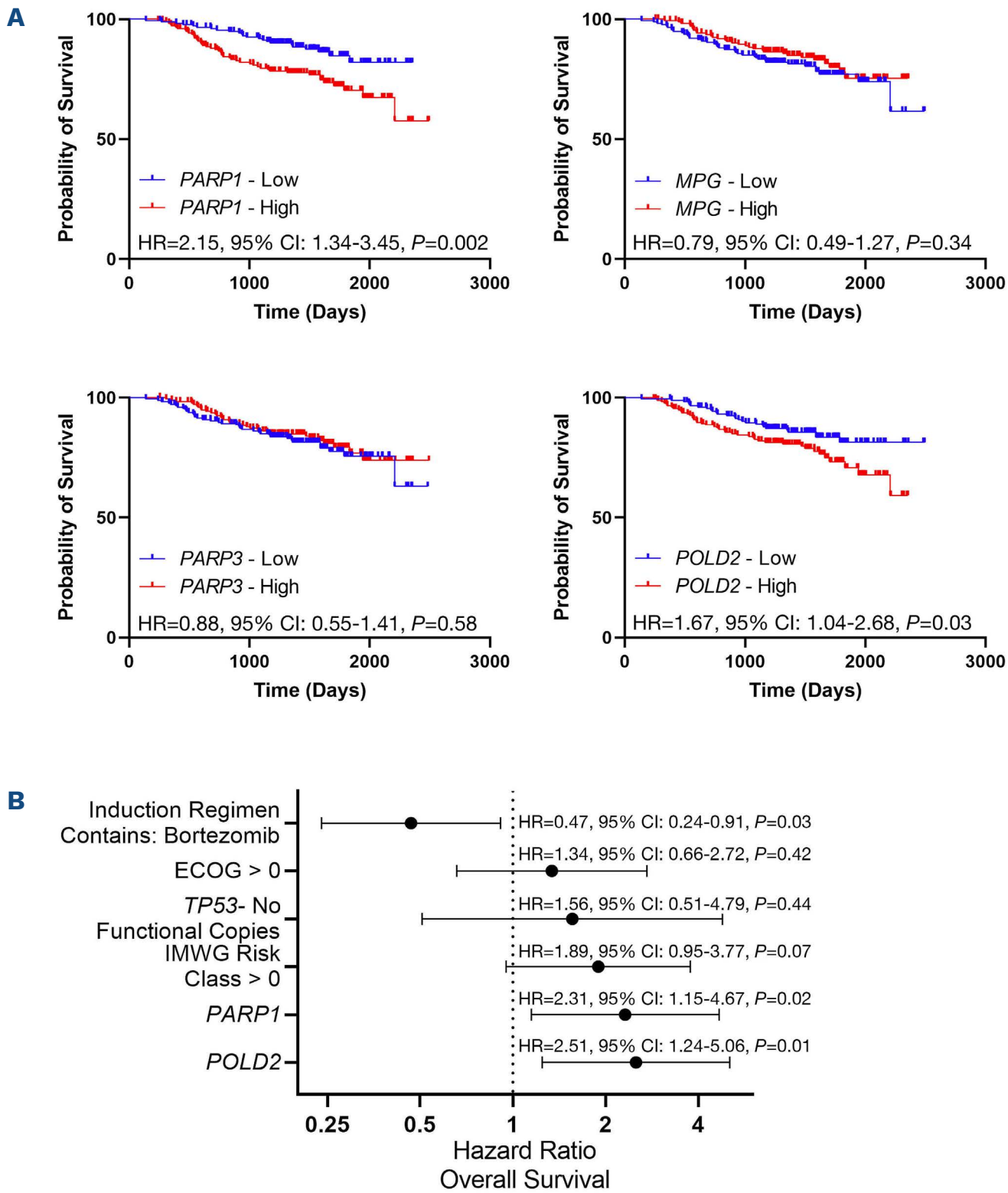

Figure 3. *PARP1* and *POLD2* expression correlate with survival in validation set. Gene expression, clinical, and survival data for 356 patients with multiple myeloma (MM) who received autologous stem cell transplant (ASCT) was collected from the Multiple Myeloma Research Foundation (MMRF). (A) Using a median expression to categorize high- and low-expression samples, high expression of *PARP1* and *POLD2* were associated with reduced overall survival (OS). High expression of *PARP3* and MPG were associated with improved OS. (B) In a multivariable Cox proportional-hazards regression model of *PARP1*, *POLD2*, and significant clinical variables, *PARP1* and *POLD2* maintained statistical significant independent of clinical factors. Points represent hazard ratios (HR), error bars represent 95% confidence interval (95% CI).

Figure 4C). Across the three cohorts, *PARP1* and *POLD2* were similarly, but weakly co-expressed (Spearman's correlation coefficients 0.19, 0.14, and 0.16).

PARP inhibition potentiates melphalan cytotoxicity in preclinical multiple myeloma models

Given the link between upregulation of *PARP1* and *POLD2* and poor OS in patients with MM receiving ASCT, we hypothesized that attenuation of the BER pathway using PARP Food and Drug Administration-approved inhibitors (olaparib, talazoparib) increase melphalan sensitivity in preclinical MM models (*Online Supplementary Figure S5A*). In order to focus on the effect of BER pathway attenuation in MM, we selected two MM cell lines with the wild-type *TP53* gene, MM1S and NCI-H929, to evaluate the effect of BER pathway attenuation.[25,26] mRNA expression of *PARP1* and *POLD2* in these cell lines is representative of low-expressing (NCI-H929) and high-expressing (MM1S) MM cell lines (*Online Supplementary Figure S5B*). Despite the difference in *PARP1* and *POLD2* expression between MM1S and NCI-H929, we identified no significant difference in melphalan half maximal inhibitory concentration (IC_{50}s) (*Online Supplementary Figure S5C*). As expected, PARP inhibition had no intrinsic anti-MM effect and did not exhibit cytotoxicity in either cell line (*Online Supplementary Figure S5D*). The combination of PARP inhibitors with melphalan was highly synergistic as measured by cell viability (Figure 5A, B) and apoptotic assays (Figure 5C).

We then tested the drug combination in a subcutaneous xenograft model using the MM1S cell line (Figure 6A). Mirroring melphalan administration in ASCT, mice assigned to receive melphalan received a single intravenous (IV) dose on day 0. Mice assigned to receive the PARP inhibitor talazoparib received two doses daily from day -3 to day +3. There was no difference in tumor size or mouse weight between treatment groups (vehicle control, talazoparib, melphalan, talazoparib + melphalan) from day -3 to day 0 (Figure 6B). Mice treated with melphalan + talazoparib demonstrated smaller tumor volume from day +3 until the end of the study (mean

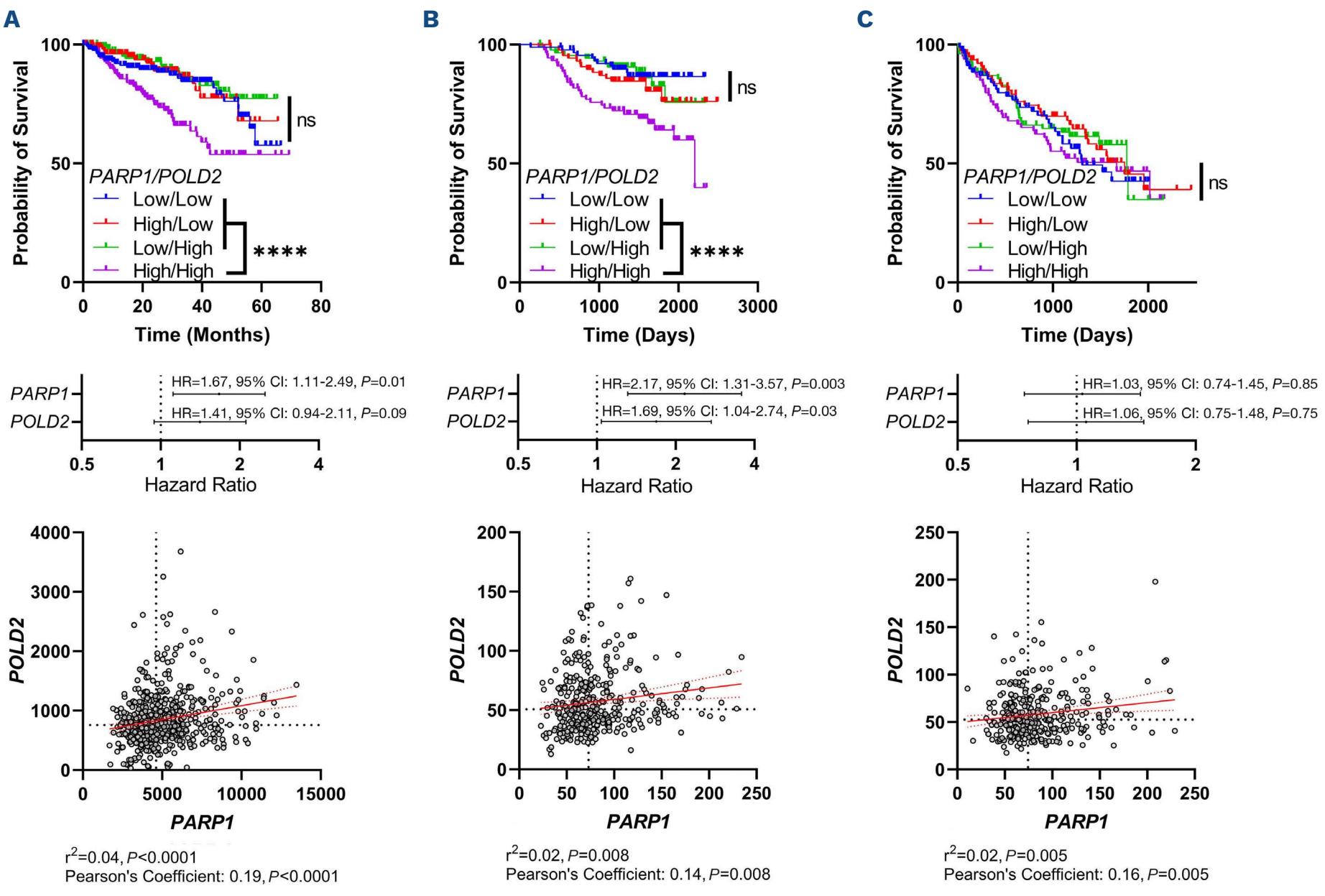

Figure 4. *PARP1* and *POLD2* co-expression highly prognostic in the setting of autologous stem cell transplant. For patients with multiple myeloma (MM) who received autologous stem cell transplant (ASCT) (GSE2658 (A), Multiple Myeloma Research Foundation (MMRF) (B)) high expression of both *PARP1* and *POLD1* was associated with poor overall survival (OS). Multivariable analysis demonstrated significance for *PARP1* in both sets while *POLD1* was only trended towards significance in GSE2658. For patients with MM who never received transplant (C), *PARP1* and *POLD1* were not associated with OS. Points represent hazard ratios (HR), error bars represent 95% confidence interval (95% CI). ns: not significant; ****P<0.0001.

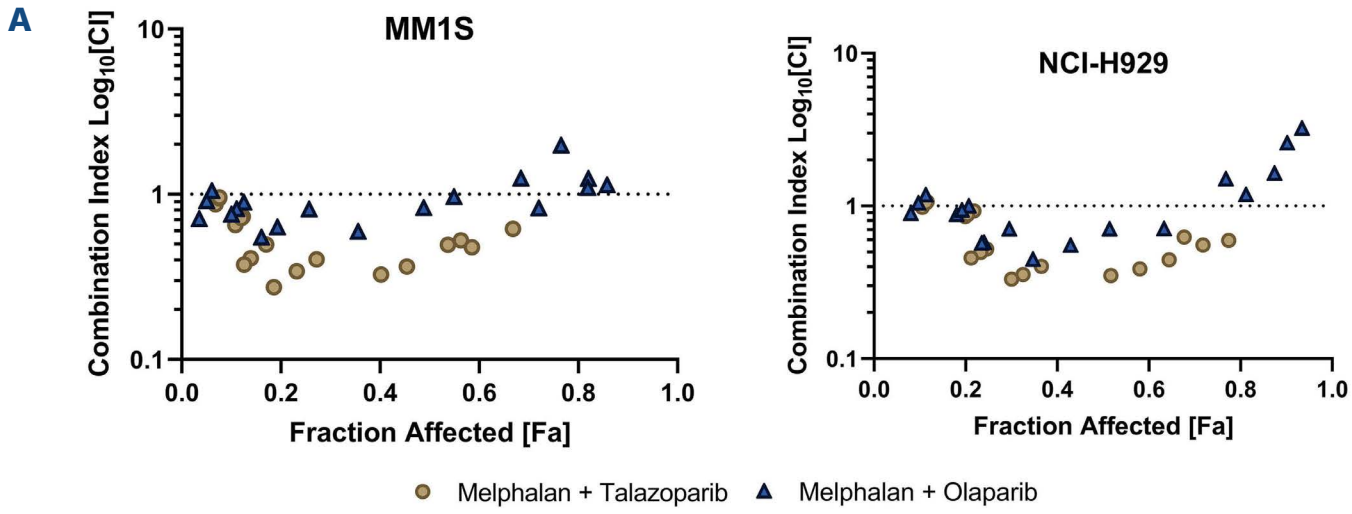

Figure 5. PARP inhibition potentiates melphalan-mediated cytotoxicity for *in vitro* multiple myeloma models. (A) Combination index for olaparib or talazoparib and melphalan for multiple myeloma (MM) cell lines calculated using CompuSyn. (B) Combination index for olaparib or talazoparib and melphalan for MM cell lines calculated using Combenifit. (C) Apoptosis, measured by flow cytometry, was increased with combination of talazoparib and melphalan for 2 of 4 cell lines. Plots represent the center as the mean, error bars as ± standard error of the mean. ns: not significant; *P≤0.05; to.

± standard error of the mean [SEM]; melphalan 313.6±25.1 mm^2 *vs.* melphalan + talazoparib 185.4±16.8 mm^2; *P*=0.0001; Figure 6C; *Online Supplementary Figure S6A*). The combination arm did experience severe adverse effects from their treatment with 40% of mice in this arm being removed from the study due to extreme weight loss (Figure 6D; *Online Supplementary Figure S6B*). It is notable that no other treatment arm experienced this effect and that the mice receiving melphalan + talazoparib with severe weight loss had no measurable disease at the time of their removal from the study.

Discussion

Despite significant therapeutic advances for MM, ASCT following high-dose, single-agent melphalan conditioning remains a cornerstone of therapy.[27] Understanding ASCT and melphalan resistance is therefore vitally important. DNA repair capability, particularly the BER pathway, has unsurprisingly been associated with response to melphalan and thus ASCT.[28] Using a big-data, clinical-genomic approach, we investigated the role of the BER pathway in MM and its prognostic relevance. Similar to previous findings, we identified an increase in the expression of genes in the BER pathway across the development of MM.[29–32] Prior studies demonstrated upregulation of specific members of the BER pathway such as *APEX1*, *APEX2*, and *PARP1*, across the development of MM.[33–36] Our findings suggest that these results are not limited to single genes, but are demonstrable across a majority of genes in the BER pathway.

In order to test the prognostic value of genes in the BER

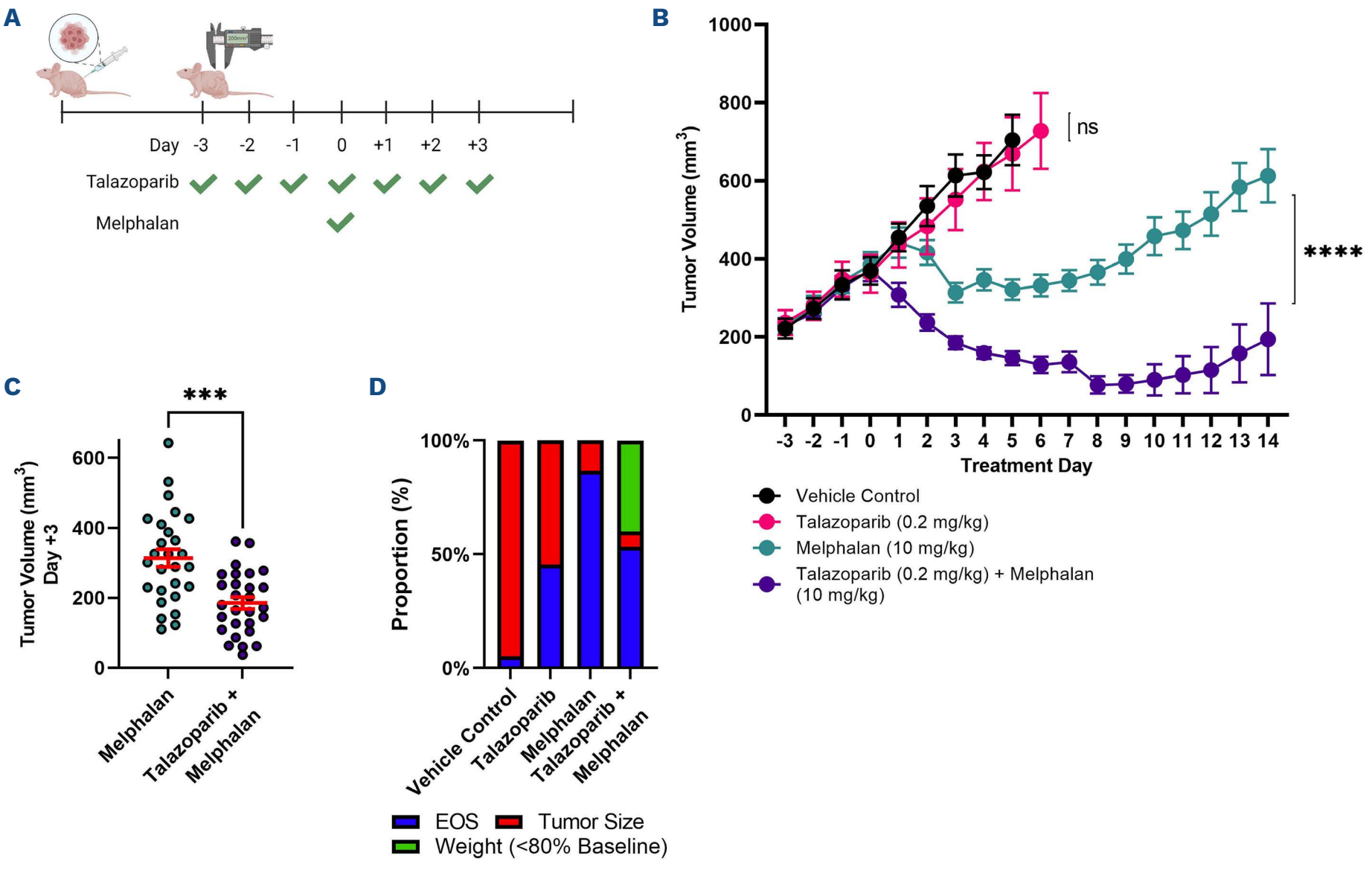

Figure 6. PARP inhibition potentiates melphalan-mediated cytotoxicity for *in vivo* multiple myeloma models. An *in vivo* subcutaneous xenograft model of using the MM1S cell line. (A) Mirroring melphalan administration in autologous stem cell transplant (ASCT), mice assigned to receive melphalan received a single intravenous dose on day 0. Mice assigned to receive the PARP inhibitor talazoparib received 2 doses daily from day -3 to day +3. Graphic created with BioRender.com. (B) Tumors in mice treated with vehicle control or talazoparib were not significantly different over the course of treatment (q=0.82, ANOVA with repeated measures). The combination of talazoparib with melphalan reduced tumor burden to a greater extent than melphalan alone (q<0.0001). (C) By day +3, melphalan + talazoparib had significantly reduced tumor size compared to melphalan alone (*P*=0.0001; Mann Whitney Wilcoxon test). (D) Mice were removed from the study due to excessive tumor growth, excessive weight loss or at the end of the study period (EOS). Graph represent the proportion of mice in each treatment arm and the reason they were removed from the study. The melphalan + talazoparib arm experienced severe adverse effects from treatment with 40% mice in this arm removed due to extreme weight loss. Plot represent the center as the mean, error bars as ± standard error of the mean. ns: not significant; ***P<0.001; ****P<0.0001.

pathway in MM, we collected data for patients with MM who had received ASCT from two large datasets. These datasets represent clinically homogenous (GSE2658) and heterogeneous (MMRF) treatment courses from patients in controlled clinical trials as well as in real-world data. We found that elevated expression of both *PARP1* and *POLD2* were consistently associated with poor survival outcomes, independent of other clinical factors.[35,37,38] This confirms prior association of *PARP1* expression as a negative prognostic biomarker in MM and extends the current knowledge of this biomarker as well as *POLD2*.[28] While the correlation between *PARP1/POLD2* expression with ASCT-related OS is purely prognostic, it is notable that these findings do not extend to patients with MM who did not receive ASCT. This may suggest that our findings may be due to the relationship between P*ARP1/POLD2* expression and resistance to melphalan-based ASCT. The direct mechanism relating *PARP1/POLD2* expression with clinical outcomes for patients receiving ASCT requires further study.

In order to test the potential for targeting the BER pathway during melphalan administration, we attenuated PARP1 pharmacologically using FDA-approved PARP inhibitors in combination with melphalan in preclinical models of MM. Prior studies by Patel *et al*. and Xiong *et a*l. found that PARP1 inhibitors are highly synergistic with melphalan in some, but not all, cell line models of MM.[28,39] Our data here validates these findings and extends them to additional cell lines and treatment strategies. The clinical applicability of these findings, however, are unclear.[28,39] Combination of PARP inhibition with traditional cytotoxic chemotherapy has been tested in a large number of clinical trials; however, few have been determined to be successful due to high rates of dose-limiting myotoxicity. This effect can be seen in our *in vivo* studies with 40% of mice receiving both melphalan and talazoparib experiencing severe weight loss and failure to thrive. It is unclear if this adverse event is a complication of the intended myeloablation, or an unintended site of action such as gut toxicity. In the setting of ASCT however, myotoxicity is a primary goal of conditioning therapy with transplant rescue vital to patient survival. Mice in our study did not receive stem cell transplants, which would better mirror the clinical setting of ASCT. Further study is necessary to determine the effect on minimal residual disease and adverse event rates if combining melphalan with a PARP inhibitor.

Our data suggests that *PARP1* is vital to the efficacy of this standard treatment regimen. However, there are key limitations to the broader application of our findings. This study is limited by the retrospective nature of the computational analysis conducted. Because our study did not include other relevant disease endpoints such as rate of PFS, disease-free survival, or minimal residual disease status, it is not possible to determine the predictive effect of *PARP1* and *POLD2* in patients with MM receiving ASCT. Given the results of the DETERMINATION trial, PFS will be a vital endpoint for future study. Further research is necessary to fully elucidate the mechanism of the BER pathway in MM. Despite the limitations of our analysis, the stability of the *PARP1* and *POLD2* signal identified over multiple large datasets and both microarray and RNA-sequencing methods suggests that the external validity of our findings may still be strong. Finally, it is clear that *PARP1* and *POLD2* alone do not explain the total biological or clinical heterogeneity present in MM. Further research is necessary to understand the role of the BER pathway in the greater molecular context of MM.[40,41]

There remains a significant need to develop predictive and prognostic biomarkers in MM. In this study, we found that elevated expression of genes in the BER pathway, specifically the *PARP1* and *POLD2* genes, correlated with poor survival in patients with MM who received ASCT. Furthermore, targeting of the BER pathway during melphalan therapy may be a potential clinical strategy to address melphalan-resistance mediated via *PARP1* and *POLD2*. Prospective clinical evaluation will be required to validate these findings.

Disclosures

No conflicts of interest to disclose.

Contributions

JL, MJP and NDS designed the research. MT, JL, KK, AKP, ED, ZV and NDS performed the research. MT, JL, ACT, BLF, MJP and NDS analyzed data. MT, JL and NDS wrote the manuscript. MT, JL, KK, AKP, ED, ZV, CCH, JKL, ACT, BLF, MJP and NDS critically reviewed the manuscript.

Acknowledgments

Parts of the data presented in this report were generated as part of the Multiple Myeloma Research Foundation Personalized Medicine Initiatives (https://research.themmrf.org and www.themmrf.org).

Funding

This study was supported by a Pelotonia IDEA award (46050-502048) (to CCH and MJP) and startup research grants from the College of Pharmacy, The Ohio State University (to MJP) and the College of Pharmacy, The University of Florida (to NDS).

Data-sharing statement

All publicly available data is noted within the manuscript with directions to obtain raw and processed data as appropriate. For all original data generated in this manuscript, please contact the corresponding author.

References

1. Kumar SK, Callander NS, Adekola K, et al. Multiple myeloma, version 3.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2020;18(12):1685-1717.
2. Hamed RA, Bazarbachi AH, Malard F, Harousseau JL, Mohty M. Current status of autologous stem cell transplantation for multiple myeloma. Blood Cancer J. 2019;9(4):44.
3. Kumar S, Paiva B, Anderson KC, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lancet Oncol. 2016;17(8):e328-e346.
4. Gu J, Liu J, Chen M, Huang B, Li J. Longitudinal flow cytometry identified "minimal residual disease" (MRD) evolution patterns for predicting the prognosis of patients with transplant-eligible multiple myeloma. Biol Blood Marrow Transplant. 2018;24(12):2568-2574.
5. Richardson PG, Jacobus SJ, Weller EA, et al. Triplet therapy, transplantation, and maintenance until progression in myeloma. N Engl J Med. 2022;387(2):132-147.
6. van Kan M, Burns KE, Helsby NA. A systematic review of inter-individual differences in the DNA repair processes involved in melphalan monoadduct repair in relation to treatment outcomes. Cancer Chemother Pharmacol. 2021;88(5):755-769.
7. Pascal JM. The comings and goings of PARP-1 in response to DNA damage. DNA Repair (Amst). 2018;71:177-182.
8. Yang J, Yang D, Cogdell D, et al. APEX1 gene amplification and its protein overexpression in osteosarcoma: correlation with recurrence, metastasis, and survival. Technol Cancer Res Treat. 2010;9(2):161-169.
9. Weaver DA, Crawford EL, Warner KA, Elkhairi F, Khuder SA, Willey JC. ABCC5, ERCC2, XPA and XRCC1 transcript abundance levels correlate with cisplatin chemoresistance in non-small cell lung cancer cell lines. Mol Cancer. 2005;4(1):18.
10. Higuchi F, Nagashima H, Ning J, Koerner MVA, Wakimoto H, Cahill DP. Restoration of temozolomide sensitivity by PARP inhibitors in mismatch repair deficient glioblastoma is independent of base excision repair. Clin Cancer Res. 2020;26(7):1690-1699.
11. Givechian KB, Garner C, Garban H, Rabizadeh S, Soon-Shiong P. CAD/POLD2 gene expression is associated with poor overall survival and chemoresistance in bladder urothelial carcinoma. Oncotarget. 2018;9(51):29743-29752.
12. Rahmanian S, Taleei R, Nikjoo H. Radiation induced base excision repair (BER): a mechanistic mathematical approach. DNA Repair (Amst). 2014;22:89-103.
13. Lee KJ, Piett CG, Andrews JF, Mann E, Nagel ZD, Gassman NR. Defective base excision repair in the response to DNA damaging agents in triple negative breast cancer. PLoS One. 2019;14(10):e0223725.
14. Persaud AK, Li J, Johnson JA, et al. XRCC1-mediated DNA repair is associated with progression-free survival of multiple myeloma patients after autologous stem cell transplant. Mol Carcinog. 2019;58(12):2327-2339.
15. Chen Q, Van der Sluis PC, Boulware D, Hazlehurst LA, Dalton WS. The FA/BRCA pathway is involved in melphalan-induced DNA interstrand cross-link repair and accounts for melphalan resistance in multiple myeloma cells. Blood. 2005;106(2):698-705.
16. Saitoh T, Oda T. DNA damage response in multiple myeloma: the role of the tumor microenvironment. Cancers (Basel). 2021;13(3):504.
17. Nath CE, Trotman J, Tiley C, et al. High melphalan exposure is associated with improved overall survival in myeloma patients receiving high dose melphalan and autologous transplantation. Br J Clin Pharmacol. 2016;82(1):149-159.
18. Seligson ND, Warner JL, Dalton WS, et al. Recommendations for patient similarity classes: results of the AMIA 2019 workshop on defining patient similarity. Am Med Inform Assoc. 2020;27(11):1808-1812.
19. Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M. KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res. 2016;44(D1):D457-D462.
20. Zhang N, Fu JN, Chou TC. Synergistic combination of microtubule targeting anticancer fludelone with cytoprotective panaxytriol derived from panax ginseng against MX-1 cells in vitro: experimental design and data analysis using the combination index method. Am J Cancer Res. 2016; 6(1):97-104.
21. Di Veroli GY, Fornari C, Wang D, et al. Combenefit: an interactive platform for the analysis and visualization of drug combinations. Bioinformatics. 2016;32(18):2866-2668.
22. Schmidt TM, Barwick BG, Joseph N, et al. Gain of chromosome 1q is associated with early progression in multiple myeloma patients treated with lenalidomide, bortezomib, and dexamethasone. Blood Cancer J. 2019;9(12):94.
23. Weinhold N, Salwender HJ, Cairns DA, et al. Chromosome 1q21 abnormalities refine outcome prediction in patients with multiple myeloma - a meta-analysis of 2,596 trial patients. Haematologica. 2021;106(10):2754-2758.
24. Burroughs Garcìa J, Eufemiese RA, Storti P, et al. Role of 1q21 in multiple myeloma: from pathogenesis to possible therapeutic targets. Cells. 2021;10(6):1360.
25. Iorio F, Knijnenburg TA, Vis DJ, et al. A landscape of pharmacogenomic interactions in cancer. Cell. 2016;166(3):740-754.
26. Gaballa S, Saliba RM, Srour S, et al. Outcomes in patients with multiple myeloma with TP53 deletion after autologous hematopoietic stem cell transplant. Am J Hematol. 2016;91(10):E442-E447.
27. Koomen DC, Meads MB, Magaletti DM, et al. Metabolic changes are associated with melphalan resistance in multiple myeloma. J Proteome Res. 2021;20(6):3134-3149.
28. Patel PR, Senyuk V, Sweiss K, et al. PARP inhibition synergizes with melphalan but does not reverse resistance completely. Biol Blood Marrow Transplant. 2020;26(7):1273-1279.
29. Beksac M, Balli S, Akcora Yildiz D. Drug targeting of genomic instability in multiple myeloma. Front Genet. 2020;11:228.
30. Teoh PJ, An O, Chung TH, et al. Aberrant hyperediting of the myeloma transcriptome by ADAR1 confers oncogenicity and is a marker of poor prognosis. Blood. 2018;132(12):1304-1317.
31. Taiana E, Cantafio MEG, Favasuli VK, et al. Genomic instability in multiple myeloma: a "non‐coding RNA" perspective. Cancers (Basel). 2021;13(9):2127.
32. Gourzones-Dmitriev C, Kassambara A, Sahota S, et al. DNA repair pathways in human multiple myeloma: role in oncogenesis and potential targets for treatment. Cell Cycle. 2013;12(17):2760-2773.
33. Kumar S, Talluri S, Pal J, et al. Role of apurinic/apyrimidinic nucleases in the regulation of homologous recombination in myeloma: mechanisms and translational significance. Blood Cancer J. 2018;8(10):92.
34. Liao C, Talluri S, Kumar S, et al. Base excision repair and homologous recombination pathway intermediates drive genomic instability and evolution in Myeloma. Blood. 2020;136(Suppl 1):S27-28.

35. Neri P, Ren L, Gratton K, et al. Bortezomib-induced "BRCAness" sensitizes multiple myeloma cells to PARP inhibitors. Blood. 2011;118(24):6368-6379.
36. Shen HY, Tang HL, Zheng YH, Feng J, Dong BX, Chen XQ. The PARP1 inhibitor niraparib represses DNA Damage repair and synergizes with temozolomide for antimyeloma effects. J Oncol. 2022;2022:2800488.
37. Krokan HE, Bjoras M. Base excision repair. Cold Spring Harb Perspect Biol. 2013;5(4):a012583.
38. Alagpulinsa DA, Ayyadevara S, Yaccoby S, Shmookler Reis RJ. A cyclin-dependent kinase inhibitor, dinaciclib, impairs homologous recombination and sensitizes multiple myeloma cells to PARP inhibition. Mol Cancer Ther. 2016;15(2):241-250.
39. Xiong T, Wei H, Chen X, Xiao H. PJ34, a poly(ADP-ribose) polymerase (PARP) inhibitor, reverses melphalan-resistance and inhibits repair of DNA double-strand breaks by targeting the FA/BRCA pathway in multidrug resistant multiple myeloma cell line RPMI8226/R. Int J Oncol. 2015;46(1):223-232.
40. Schjesvold FH, Dimopoulos MA, Delimpasi S, et al. Melflufen or pomalidomide plus dexamethasone for patients with multiple myeloma refractory to lenalidomide (OCEAN): a randomised, head-to-head, open-label, phase 3 study. Lancet Haematol. 2022;9(2):e98-e110.
41. Bashir Q, Thall PF, Milton DR, et al. Conditioning with busulfan plus melphalan versus melphalan alone before autologous haemopoietic cell transplantation for multiple myeloma: an open-label, randomised, phase 3 trial. Lancet Haematol. 2019;6(5):e266-e275.