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Poly(ADP-Ribose) Polymerase Inhibitor Hypersensitivity in Aggressive Myeloproliferative Neoplasms

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Abstract

Purpose—DNA repair defects have been previously reported in myeloproliferative neoplasms (MPNs). Inhibitors of poly(ADP-ribose) polymerase (PARP) have shown activity in solid tumors with defects in homologous recombination (HR). The present study was performed to assess MPN sensitivity to PARP inhibitors *ex vivo*.

Experimental Design—HR pathway integrity in circulating myeloid cells was evaluated by assessing formation of RAD51 foci after treatment with ionizing radiation or PARP inhibitors. Sensitivity of MPN erythroid and myeloid progenitors to PARP inhibitors was evaluated using colony formation assays.

Results—Six of 14 MPN primary samples had reduced formation of RAD51 foci after exposure to ionizing radiation, suggesting impaired HR. This phenotype was not associated with a specific MPN subtype, *JAK2* mutation status or karyotype. MPN samples showed increased sensitivity to the PARP inhibitors veliparib and olaparib compared to normal myeloid progenitors. This hypersensitivity, which was most pronounced in samples deficient in DNA damage-induced RAD51 foci, was observed predominantly in samples from patients with diagnoses of chronic

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myelogenous leukemia, chronic myelomonocytic leukemia or unspecified myelodysplastic/MPN overlap syndromes.

Conclusions—Like other neoplasms with HR defects, MPNs exhibit PARP inhibitor hypersensitivity compared to normal marrow. These results suggest that further preclinical and possibly clinical study of PARP inhibitors in MPNs is warranted.

Keywords

Myeloproliferative neoplasm; poly(ADP-ribose) polymerase; targeted therapy; DNA repair; chronic myelomonocytic leukemia

Introduction

Myeloproliferative neoplasms (MPNs) represent a heterogeneous group of clonal diseases with a common propensity to progress to acute leukemia (1–4). Essential Thrombocythemia (ET), Polycythemia Vera (PV), Primary Myelofibrosis (MF), and mixed myelodysplastic/myeloproliferative neoplasms such as Chronic Myelomonocytic Leukemia (CMML) are all clonal neoplasms derived from aberrant early hematopoietic precursors but have varied clinical manifestations. Upon progression, they are uniformly refractory to standard acute leukemia therapies, with median survival less than six months.

One common mutation in MPNs, an activating V617F point mutation in the tyrosine kinase JAK2, is found in greater than 80% of cases of PV (5), 40% of cases of ET, and 30% of cases of MF (6). *JAK2* mutation and overexpression have been associated with increased homologous recombination (HR) and genomic instability (7–9). Other alterations conferring an MPN-like phenotype, such as *BCR/ABL* translocations in CML, *FIP1L1-PDGFR* rearrangements in eosinophilic leukemias, and *FLT3* mutations in acute myeloid leukemia, have also been associated with changes in the DNA repair pathways leading to increased genomic instability and drug resistance (9, 10). For example, even though early studies indicated that RAD51, a critical component of the homologous recombination (HR) pathway, is upregulated in *BCR/ABL*-positive CML cells (11), subsequent studies demonstrated that repair in these cells is error-prone and leads to mutations and large deletions or insertions (12). Further analysis traced this genomic instability to several changes, including (i) enhanced tyrosine phosphorylation of RAD51, leading to its aberrant function (13); (ii) downregulation of BRCA1 (14); (iii) stimulation of single-strand annealing, an error-prone DNA repair pathway (15); and (iv) other changes in the Fanconi anemia/BRCA pathway that can be reverted by ectopic BRCA1 expression (16).

Repair defects in *BCR/ABL*-negative MPNs are not as well characterized. Gross chromosomal lesions are common in MF and accelerating MPN; and SNP array karyotyping identified additional subcytogenetic abnormalities (17, 18). These types of changes are reminiscent of chromosomal aberrations observed in HR-deficient solid tumors such as *BRCA1*- or *BRCA2*-mutant breast and ovarian cancer (19). Although the upstream source and pathological consequences of these extensive genomic rearrangements are not as well understood in MPN, previous studies have reported increased error prone double-strand break repair in MPNs as well as CML (15).

Poly(ADP-ribose) polymerase (PARP) inhibitors are a class of antineoplastic agents being widely tested in solid tumors (20–25). These agents target PARP1, PARP2, and PARP3, three enzymes that contribute to various aspects of DNA repair (21, 23, 24, 26–28). PARP inhibition not only diminishes base excision repair, but also impairs alternative end-joining (29) and accelerates nonhomologous end-joining (30). In cells with diminished HR, these changes lead to error-prone DNA repair and cell death (21, 23, 31). In addition, PARP inhibition leads to trapping of PARP1 on DNA, preventing access of downstream repair proteins to sites of DNA damage (32–34) and providing a potential mechanism for PARP inhibitor-induced killing in HR proficient cells that contain high levels of PARP1 protein. Importantly, chromosome 1q (including the *PARP1* locus at 1q42) is amplified in a subset of chronic phase MPNs and even more commonly in transformed MPNs (17, 35), providing a potential opportunity for PARP1 trapping even in MPNs without HR defects.

Consistent with the *BCR/ABL*-induced DNA repair abnormalities described above, PARP inhibitor hypersensitivity has been reported in CML (36). In view of the repair defects observed in other MPNs, as well as the copy number increases of the *PARP1* locus in these disorders (17, 35), we have performed a survey comparing PARP inhibitor sensitivity of various MPNs, including CML, with that of normal hematopoietic progenitors. For this study, we have examined two PARP inhibitors that are undergoing extensive clinical testing. Veliparib, which is in National Cancer Institute-sponsored trials in a variety of neoplasms, including hematological neoplasms (www.clinicaltrials.gov), has recently been shown to exhibit favorable properties for combining with DNA damaging agents (34). Olaparib, which is roughly 10-fold more potent *in vitro*, has attained regulatory approval for the treatment of ovarian cancer (24, 25).

MATERIALS AND METHODS

Inhibitors

Veliparib (ABT-888, Enzo Life Sciences, Farmingdale, NY) and olaparib (ChemieTek, Indianapolis, IN) were dissolved in dimethyl sulfoxide (DMSO) at stock concentrations of 10 mM. Stocks were aliquoted in 10 μ L volumes, stored at -80°C , and thawed once immediately before use. All samples in any given experiment contained identical concentrations of DMSO (0.1% vol/vol).

Clinical samples

After patients provided informed consent, specimens were obtained by purifying mononuclear cells from the peripheral blood of patients with MPN on Ficoll-Hypaque gradients. The MPN cohort included fresh samples from cases of *BCR/ABL*-positive CML; the classical *BCR/ABL*-negative MPNs ET, PV, primary and secondary MF; and mixed MDS/MPN diagnoses (CMML, aCML, MDS-MPN-U) (Table 1).

RAD51 focus formation assay

Ten million Ficoll/Hypaque-purified mononuclear cells (MNCs) from normal controls and MPN patients were exposed to 10 Gy ionizing radiation (IR) from a Rad Source RS200 X-ray irradiator (Brentwood, TN), then allowed to recover for 6 h in a humidified 37°C tissue

culture incubator equilibrated at 5% (vol/vol) CO₂. Leukocytes were pelleted by centrifugation at 200×*g* for 10 min and fixed in 2% (wt/vol) paraformaldehyde in Dulbecco's calcium- and magnesium-free phosphate-buffered saline (PBS) for 10 min at 20–22 °C. Leukocytes were re-pelleted as above, washed with PBS, and stored in PBS at 4 °C. For analysis, 2.5 × 10⁴ leukocytes were deposited onto glass coverslips by cytocentrifugation and processed as previously described (37). Briefly, coverslips were washed 3 times with PBS, permeabilized in PBS + 0.25 % (vol/vol) Triton X-100 for 10 min, washed an additional 3 times with PBS, then incubated for 1 h in blocking buffer [PBS, 1 % (vol/vol) glycerol, 0.1% (wt/vol) gelatin from cold-water fish, 0.1% (wt/vol) bovine serum albumin, 5% (vol/vol) goat serum and 0.4 % (wt/vol) sodium azide] for 1 h at room temperature. Coverslips were incubated with RAD51 rabbit polyclonal (Active Motif; Carlsbad, CA) and phospho-Ser¹³⁹-H2AX mouse monoclonal (Millipore; Billerica, MA) antibodies diluted 1:500 in blocking buffer overnight at 4 °C. Coverslips were then washed 3 times with PBS, followed by incubation for 1 h in secondary Alexa Fluor 488-conjugated goat anti-mouse IgG and Alexa Fluor 568-tagged goat anti-rabbit IgG (Invitrogen; Carlsbad, CA) diluted 1:1000 in blocking buffer. Coverslips were further washed 3 times with PBS, counterstained with 1 µg/ml Hoechst 33258 in PBS, and mounted using UltraLong antifade reagent (Invitrogen, Carlsbad, CA). PEO1 and PEO4, cell lines with a truncating *BRCA2* mutation and a reversion mutation, respectively, were utilized as negative and positive controls for radiation-induced RAD51 foci. Confocal images were captured on a Zeiss LSM 710 scanning confocal microscope with a 100×/1.4 N.A. oil immersion objective. For quantitation, 100 cells per sample from greater than 5 fields were manually scored for RAD51 and phospho-H2AX foci by an investigator blinded to clonogenic assay results. Cells with >5 foci were graded as positive. Quantitation and image processing were performed with the Zeiss Zen software package and Adobe Photoshop CS3.

Colony forming assays

Colony forming assays were performed as previously described (38). Ficoll/Hypaque-purified MNCs were washed and resuspended in RPMI 1640 medium (Gibco). 0.5 ml aliquots were combined with 4.5 ml of MethoCult[®] medium (STEMCELL Technologies #H4435, Vancouver, BC) containing increasing concentrations of veliparib (0, 0.5, 1, 2.5, 5, 10, 20 µM) or olaparib (0, 0.25, 0.5, 1, 2 and 5 µM). Two or four 1 ml aliquots of each mixture were plated onto replicate 35 mm gridded culture plates to give final MNC concentrations of 1–5 × 10⁵ cells per plate. After incubation at 37 °C in 5% CO₂, most samples were scored for total colony number after 10–14 days; however, some CMMoL samples were scored earlier (5–7 days) due to hypersensitivity to growth factors. Colonies were defined as clusters of >40 cells. Drug concentrations at which total colony counts were inhibited to 50% of diluent treated controls (IC₅₀) were determined using linear regression analysis of the dose response curves after linear transformation using an exponential model (CalcuSyn software, Biosoft, Inc., Cambridge, UK).

Genomic analysis

DNA was extracted from Ficoll/Hypaque-purified MNCs using a DNA midi kit (Qiagen, Valencia, CA). To search for somatic mutations in HR pathway proteins, aliquots of DNA (1 µg) were subjected to targeted capture and massively parallel sequencing using BROCA as

previously described (39) for a panel of genes involved in HR (*ATM*, *ATR*, *BABAM1*, *BARD1*, *BAP1*, *BLM*, *BRCA1*, *BRCA2*, *BRE*, *BRIPI*, *BRCC3*, *CHEK1*, *CHEK2*, *FAM175A*, *FANCC*, *FANCP*, *MRE11A*, *NBN*, *PALB2*, *RAD50*, *RAD51*, *RAD51B*, *RAD51C*, *RAD51D*, *RBBP8*, *TOPBP1*, *UIMC1*, *XRCC2*, and *XRCC3*), regulation of HR (*CDK12*, *C11orf30*, *ID4*, *TP53BP1*, and *USP28*), or NHEJ (*LIG4*, *XRCC4*, *XRCC5*, *XRCC6*, *PRKDC*, *DCLRE1C*) and *TP53*.

Methylation-specific PCR (MSP), quantitative MSP (qMSP)

Genomic DNA was bisulfite-treated with the EZ DNA Methylation Kit (Zymo Research, Irvine, CA). The initial MSP screen was performed as previously described (40). qMSP for *BRCA1* promoter methylation, normalized to β -actin levels, was carried out using the iTaq SYBR Green mix and 300 nM of each primer. qMSP, MSP primer sequences and annealing temperatures are listed in Supplemental Table 1. *BRCA1* qMSP primers were adapted from Estellar *et al.* and cover the region -175 to +9 relative to the TSS (41). Cycling conditions are 95 °C for 5 min, followed by 40 cycles of 95 °C for 5 sec and 64 °C for 60 sec. Melt curve readings were recorded at 0.5 °C increments from 65 °C to 95 °C. The controls for unmethylated and methylated templates were bisulfite-treated samples from normal lymphocyte DNA and CpG methylated Jurkat genomic DNA (New England Biolabs, Ipswich, MA), respectively. Methylated samples are defined as amplicons with melting temperatures matching that of the methylated control.

RESULTS

Some MPN primary samples have an abnormal DNA damage response

To assess whether PARP inhibitors might have any promise in non-CML MPNs, the present study examined primary samples from 41 patients with a variety of MPNs (Table 1). Following up on the observation that MPNs are associated with multiple genetic aberrations (17, 18), we examined a subset of 14 samples for integrity of the HR pathway. Upon exposure to ionizing radiation, cells with normal HR repair form foci of DNA repair complexes that can be detected by immunofluorescence after staining for RAD51, the recombinase responsible for pairing homologous sequences. Cells with impaired HR typically will not form RAD51 foci when exposed to ionizing radiation. Of 14 MPN samples examined using this assay, six (43%) displayed markedly diminished RAD51 foci formation (Figure 1 and Table 1). In contrast, 0 of 14 controls performed with these assays exhibited diminished RAD51 foci formation ($p = 0.016$, Fisher's exact test).

To search for potential explanations for this HR deficiency, we sequenced a panel of repair genes found to be mutated in a variety of malignancies, including *BRCA1*, *BRCA2*, *RAD51*, *RAD51* paralogs and several Fanconi anemia pathway genes, in MPN samples from 24 of these patients. Results of this analysis are summarized in Table 1. Mutations observed included a well-established *BRCA1* stop mutation (*BRCA1* c.5382insC) and *CHEK2* frameshift mutation (*CHEK2* c.1100delG) as well as a deleterious mutation in the *RAD50* gene (*RAD50* c.3476delA). While a variety of rare normal alleles and conservative single nucleotide variants were also detected in other genes (Table 1), no deleterious homozygous or hemizygous mutations were observed that could by themselves account for

the defect in RAD51 protein recruitment to sites of DNA damage. We also assessed *BRCA1*, *BRCA2*, *FANCC*, *FANCF*, and *FANCL* promoter CpG island methylation in these MPN samples. No hypermethylation was identified in the *BRCA2*, *FANCC*, *FANCF*, or *FANCL* promoters. However, six of 27 samples (22.2%) analyzed in the present study demonstrated hypermethylation of the *BRCA1* promoter (Table 1).

Inhibition of colony formation by the PARP inhibitor veliparib

With the observation that a substantial fraction of MPN samples have a deficient DNA repair response, as manifested by lack of formation of radiation-induced RAD51 foci, we performed colony forming assays with continuous exposure to the PARP inhibitor veliparib using MNCs from 41 MPN patients and 13 normal controls. As shown in Figure S1, the sensitivities of granulocyte and erythroid colonies generally paralleled each other in both normal and MPN samples. Accordingly, for the remainder of this work we tabulated various colony types with each assay but combined them at the time of graphing. Assay reproducibility was established by comparing results of assays performed on samples from several patients at two points in time in the absence of changes in treatment (Figure S2).

As shown in Figure 2A and B, the drug concentrations that inhibited colony formation by 50% (IC₅₀) were an average of 4-fold lower in the MPN samples than in normal controls (2.5 vs 9.6 μ M, $P < 0.0001$). Focusing on the subset of samples that were subjected to assays for both RAD51 foci and colony formation (Figure 2C), PARP inhibitor sensitivity was greater (IC₅₀ on average 3-fold lower) in MPN samples with impaired RAD51 foci formation compared to those without (mean 9.3 vs. 2.8 μ M, $p = 0.028$). In contrast, *BRCA1* promoter methylation status did not track with veliparib sensitivity (Figure S3).

MPN primary samples demonstrate veliparib sensitivity across clinical subtypes

To determine whether PARP inhibitor sensitivity was linked to clinical characteristics, we analyzed colony-forming assay results based on clinical descriptions of disease at the time of sample acquisition. This analysis suggested broad sensitivity across clinical subtypes, with samples from MF patients demonstrating the greatest heterogeneity (Figure 3A). Although the number of samples in any MPN category was somewhat limited, CML, CMMoL and MDS/MPN-U samples were most sensitive, with IC₅₀ values averaging 3, 3 and 1.5 μ M, respectively.

Activating *JAK2* mutations correlate with decreased veliparib sensitivity

We also examined whether *JAK2* mutation status, which was suggested as a surrogate marker for impaired DNA repair (15), correlated with PARP inhibitor sensitivity. In our sample set (Figure 3B), *JAK2* non-mutated samples were significantly more sensitive to PARP inhibition than *JAK2*V617F mutated samples (mean IC₅₀ 2.8 vs. 5.7 μ M, $P = 0.04$). Notably, however, 5 of 16 individual *JAK2* mutated samples demonstrated veliparib IC₅₀s below 2 μ M despite the relative insensitivity of this subset as a whole.

MPN hypersensitivity to the PARP inhibitor olaparib

While this work was in progress, it was reported that the PARP inhibitors PJ-34 (a preclinical tool compound) and veliparib might also inhibit certain kinases when assayed at

high concentrations under cell-free conditions (42, 43). Importantly, the structurally dissimilar PARP inhibitor olaparib lacks this off-target kinase inhibitory activity (43). To provide assurance that the veliparib hypersensitivity observed in MPN samples was due to PARP inhibition, olaparib sensitivity was also examined using colony-forming assays (e.g., Figure 4A) in a subset of samples. For samples assayed using both veliparib and olaparib, there was a strong correlation ($R^2 = 0.61$, $p = 0.003$) between sensitivity to the two agents (Figure 4B). Accordingly, as was the case with veliparib, a substantial fraction of these MPN samples were also hypersensitive to olaparib when compared to normal controls (Figure 4C).

DISCUSSION

Because genomic instability is a hallmark of advanced MPNs, we examined whether clinical MPN specimens exhibit HR defects and whether they are hypersensitive to PARP inhibitors, which are known to be particularly effective in neoplasms with HR deficiency (20–24). Here we demonstrate PARP inhibitor hypersensitivity for the first time in a large subset of MPN samples.

An impaired DNA damage response, as evidenced by lack of development of RAD51 foci in response to ionizing radiation, was observed in ~40% of MPN samples assayed (Figure 1 and Table 1). This observation suggests that the HR pathway is impaired in those samples. These results are consistent with prior observations of extensive chromosomal and subchromosomal copy number changes in MPN (17, 18), which are another hallmark of HR deficiency (19). Because formation of RAD51 foci only provides an assessment of HR pathway integrity upstream of RAD51 and is unaffected by changes downstream, e.g., loss of RAD51C (44), it is important to emphasize that the present observations might underestimate the true frequency of HR repair defects in MPNs.

The causes of this HR pathway dysfunction in MPNs are incompletely understood at present. Some of the samples with impaired RAD51 foci formation also exhibited diminished formation of H2AX foci (Fig. 1B and Table 1). Because H2AX phosphorylation after double strand breaks typically reflects activation of ATM and phosphorylation of its substrate MDC1 (45), diminished formation of both phospho-H2AX and RAD51 foci might reflect a defect in ATM or its activation as described in other neoplasms. Other samples formed phospho-H2AX foci but nonetheless failed to form RAD51 foci, suggesting one or more defects between these two events in the DNA damage response. Consistent with this heterogeneity, we have previously examined 144 cases of MPN via SNP arrays and found that 26% have heterozygous deletions in genes encoding one or more DNA repair pathway proteins such as *BRCA2*, *ATM*, *FANCC* or *FANCL* (46). Because the other copy remains intact, however, it is unclear whether these heterozygous deletions cause sufficient changes in protein expression to impact the HR pathway. Accordingly, we have examined a subset of MPN samples for methylation changes in FANC proteins, *ATM*, and *BRCA2* but did not observe frequent changes in methylation that would confer increased genomic instability. *BRCA1* promoter methylation was found in 6 of 27 samples analyzed (Table 1) but was not associated with increased sensitivity to PARP inhibition *ex vivo* (Figure S3). Given the results of drug sensitivity testing in the present study, further investigation of repair defects in MPNs appears warranted.

PARP inhibitors are currently undergoing extensive testing in other neoplasms with HR deficiencies (20–25). Here we found that MPN samples with impaired RAD51 foci formation exhibited increased sensitivity to the PARP inhibitor veliparib, suggesting that these cells depend on alternative repair pathways containing one or more veliparib-sensitive PARPs for their survival (Figure 2C). Further examination of MPN samples as a group revealed broad sensitivity to PARP inhibition across many subtypes of MPN (Figure 3A). This hypersensitivity relative to normal myeloid progenitors might reflect multiple alterations in MPNs. In addition to the sensitizing effects of HR deficiency, the presence of high concentrations of PARP1, e.g., as a consequence of chromosome 1q gains in copy number seen in a subset of MPNs (see Introduction), might also sensitize cells to the PARP trapping that accompanies treatment with PARP inhibitors (32, 37). Accordingly, there are multiple potential explanations for the observed hypersensitivity of MPNs relative to normal myeloid progenitors, just as there are in solid tumors (20–25); and further investigation is required to understand this hypersensitivity on a case by case basis in both instances.

While this work was in progress, it was reported that veliparib can inhibit purified kinases when applied under cell-free conditions at concentrations 100- to 1000-fold higher than those required to inhibit purified PARP1 and PARP2 (43). Importantly, the PARP inhibitor olaparib was shown to lack these off-target kinase inhibitor effects (43). In our study, there was a strong correlation between olaparib and veliparib sensitivity (Figure 4B), suggesting that the PARP inhibitor hypersensitivity of MPNs (Figures 2B and 4C) reflects PARP inhibition rather than an off-target effect.

To our knowledge the present study provides the first examination of PARP inhibitors in non-CML MPN. Because hypersensitivity of some MPN samples was apparent within the first few samples, we surveyed a broad range of MPN samples in an attempt to determine the types of diseases lumped under the categories of MPN and mixed MDS/MPN that might display this hypersensitivity. Further studies examining additional samples with each type of MPN or MDS/MPN syndrome at various stages of disease (e.g., initial diagnosis versus advanced disease requiring treatment versus recurrence after therapy or stem cell transplant) are required to assess whether PARP inhibitor hypersensitivity persists after current treatments, as appears to be the case in a subset of ovarian cancers, for example (23).

Our results indicate that MPN samples with the activating *JAK2* V617F mutation are, on average, less sensitive than MPN samples without *JAK2* mutations. Because the assays measured the sensitivity of proliferating cells, this difference is unlikely to reflect any difference in the rate of proliferation in *JAK2* wildtype vs. *JAK2* mutant samples. Instead, it is possible that the difference reflects the ability of *JAK2* V617F to activate STAT-mediated transcription and inhibit apoptosis (47).

On the other hand, the therapeutic window for PARP inhibition in MPN, e.g., as measured by differences in mean IC₅₀ of RAD51 foci-deficient MPNs and normal progenitors (Figure 2C), is somewhat narrower than the hundreds-fold difference in IC₅₀ observed when comparing *BRCA1*- or *BRCA2*-deficient murine embryonic stem cells and their HR proficient counterparts (48). However, it is important to point out that the difference in sensitivity between *BRCA2*-deficient human ovarian cancer cells and their isogenic

BRCA2-restored counterparts is also only 5- to 10-fold (30), yet clinical activity of PARP inhibitors is observed in ovarian cancer (20–25). The limited, albeit easily observed, sensitivity difference between normal and MPN progenitors suggests that activity of PARP inhibitors as single agents in MPN might merit further investigation, particularly in *JAK2* wildtype MPNs, which sometimes lack therapeutic options (1–4). On the other hand, we have previously examined the impact of adding veliparib to topotecan and carboplatin (37), two agents that have exhibited some activity when combined to treat relapsed AML (49), and have observed synergistic cytotoxic effects with the topotecan/veliparib combination in AML lines *in vitro* (37). Moreover, because *JAK2* mutations were associated with diminished PARP inhibitor sensitivity (Figure 3B), we have begun examining the effect of combining PARP inhibitors with other agents used in the treatment of MPNs, looking for synergistic cytotoxic effects with PARP inhibitor/hydroxyurea and PARP inhibitor/ruxolitinib combinations. These observations will provide the impetus for further study of PARP inhibitors, alone and in combination, in MPNs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Translational Relevance

Myeloproliferative neoplasms (MPNs) are a heterogeneous group of clonal hematological disorders with limited current therapeutic options. Previous studies have shown that MPNs often have impaired DNA repair pathways. Poly(ADP-ribose) polymerase (PARP) inhibitors have shown promising activity in solid tumors with defects in homologous recombination repair. Here we compare the sensitivity of clinical isolates from several BCR/ABL-negative chronic myeloid neoplasms, including chronic myelomonocytic leukemia, essential thrombocythemia, and primary myelofibrosis, to normal controls using two different PARP inhibitors in colony forming assays *ex vivo*. Results of this analysis demonstrate that myeloid progenitors from many patients with *JAK2* wildtype MPNs exhibit enhanced PARP inhibitor sensitivity, which is greatest in those with defective formation of RAD51 foci after DNA damage. These observations support the further study of PARP inhibitors, alone or in combination with other therapies, in certain MPNs.

Figure 1. Lack of radiation-induced RAD51 foci in a subset of MPNs
A, RAD51 foci formation in circulating myeloid cells from the indicated samples before and after ionizing radiation. **B**, summary results from 14 MPN cases, 3 normal controls and 2 de novo acute myelogenous leukemia cases.

Figure 2. Veliparib sensitivity in MPN samples and normal controls

A, results of colony forming assays in three MPN samples and four normal controls. Each line and corresponding symbols represent the mean results of replicate plates from one assay performed as described in the Methods. * indicates sample with no colony growth at the next higher veliparib concentration. **B**, summary of IC_{50} values in MPN samples and normal controls from results in panel A and additional samples run at veliparib concentrations up to 20 μM . **C**, relationship between IC_{50} values and formation of RAD51 foci in MPN samples. Open triangles represent samples with impaired formation of both phospho-H2AX and RAD51 foci (Table 1).

Figure 3. Relationship between veliparib sensitivity and MPN biology

A, IC₅₀ values for veliparib in various MPN subsets. **B**, relationship between IC₅₀ values for veliparib and *JAK2* mutation status.

Figure 4. Olaparib sensitivity in MPN samples and normal controls

A, results of colony forming assays for 7 MPN samples and 4 normal controls. Each line and corresponding symbols represent the mean results of replicate plates from one assay performed as described in the Methods. * indicates sample with no colony growth at the next higher olaparib concentration. All samples assayed for olaparib sensitivity were also assayed using veliparib. **B**, correlation between IC_{50} for veliparib and olaparib in the 12 samples that overlapped. **C**, comparison of olaparib IC_{50} values in normal and MPN samples.

Table 1

Cancer type	BRCA1 methylation ^c	JAK2 V617F Mut ^{b,c}	RAD51 foci Formation ^{b,d}	H2Ax foci formation ^b	Veliparib IC ₅₀ (μM)	Olaparib IC ₅₀ (μM)	BROCA analysis ^{b,e,f}	
							Deleterious alterations	Variants of unknown significance
11q-	No	-	Normal	Normal	6.9	<i>b</i>	CHEK2 c.1100delG	None
13q+ +14	No	+			15			
5q-	No	-	Impaired	Normal	1.3	0.35	None	LIG4 p.A857T PALB2 p.R414Q
inv9	Yes	48%	Normal	Normal	8.6	0.83	None	PRKDC p.M333I RAD51D p.R232Q
		+			1.0	0.56	None	CDK12 p.P1275L, MSH2 p.R55G
		-	Impaired	Normal	4.8	0.4		
+1p	Yes	-	Normal	Normal	2.4	0.64	None	PRKDC p.P695S TP53BP1 p.V1031A
+8	No	45%	Normal	Normal	9.3	1.8		
		-	Impaired	Impaired	0.8	0.14	None	No alterations
		-	impaired	Normal	0.7	1.0		
t(11;14)		+	Normal	Normal	>20	5.2		
		-	Normal	Normal	2.6	0.30		
		58%	Normal	Normal	22	1.8		
+9 t(12;13)		+	Impaired	Normal	7.0	1.1		
Y t(9;22)			Impaired	Impaired	2.2			
20q-		+			2.7		None	PRKDC p.A3904V
		-	Normal	Normal	2.9			
		+			0.3			
		+			4.6			
	No	-			ovgrth			
	No				4.1		None	No alterations
del(2)(q)	No	-			1.3		XRCC4 c.24 delC DCLRE1C p.G38R	

Cytotype	BRCA1 methylation ^c	JAK2 V617F Mut ^{b,c}	RAD51 foci Formation ^{b,d}	H2Ax foci formation ^b	Veliparib IC ₅₀ (μM)	Olaparib IC ₅₀ (μM)	BROCA analysis ^{b,e,f}	
							Deleterious alterations	Variants of unknown significance
XX	Yes				2.5		None	PIK3CA p.Y644H
XXY der(1;2)		+			4.1			
XX	No	-			3.2			
XX					5.5			
XY	Yes	+			5.4		None	SLX4 p.P385T, p.P957L, p.E942Q
XX	No	-			1.9			
t(9;22)	No				4.0		None	MSH6p. I1054F PTEN p.H397R
t(4;7)	No				4.4		None	CDK12 p.L988S, NBN p.I439M SLX4 p.R1372Q and p.A916S
XX	No				2.2		BRCA1 5382insC (c.5263_5264insC)	RAD51B p.K243R TOPBP1 p.N1042S,
t(1;7) +9 1q-	Yes	+			4.2		None	TOPBP1 p.R309C XRCC5 p.A550S
XX	No	+			1.7		None	LIG4 p.T9I
XX +8 +14	No				0.8		None	CDK12 p.L1189Q MLH1 p.H718Y, PALB2 p.D134N, PRKDC p.R1253H, SLX4 p.E701D, TOPBP1 p.M293V
XX	No	34%			1.5		None	XRCC5 p.R184H,
XX	Yes	-			4.3		None	ATR L274F, RBBP8 C485
XX 20q-	No	+			1.9		None	BARD1 p.Q11H, BLM p.I366T, PIK3CA p.R524K
XX	No				4.3		RAD50 c.3476delA	ATR p.H117R, CDK12 p.P645S, NBN p.N142S, SLX4 p.S1271F, UIMC1 p.Y564H
t(9;22)		-			4.8		None	ATR p.197F, MRE11 p.S334R,

Cell type	BRCA1 methylation ^c	JAK2 V617F Mut ^{b,c}	RAD51 foci Formation ^{b,d}	H2Ax foci formation ^b	Veliparib IC ₅₀ (μM)	Olaparib IC ₅₀ (μM)	BROCA analysis ^{b,e,f}	
							Deleterious alterations	Variants of unknown significance
CLL	No	—			3.2		None	RBBP8 p.K357N, SLX4 p.K1635E, TP53BP1 p.H58R
								ATM p.S1691R, FAM175A p.T141I, TOPBP1 p.S817L, TP53BP1 p.E1019G
CLL		43%			3.8	.78		