Annals of Hematology (2019) 98:2703–2709
https://doi.org/10.1007/s00277-019-03836-2

ORIGINAL ARTICLE

Transcriptional alteration of DNA repair genes in Philadelphia chromosome negative myeloproliferative neoplasms

Martin Kirschner[1] · Anne Bornemann[1] · Claudia Schubert[1] · Deniz Gezer[1] · Kim Kricheldorf[1] · Susanne Isfort[1] · Tim H. Brümmendorf[1] · Mirle Schemionek[1] · Nicolas Chatain[1] · Tomasz Skorski[2] · Steffen Koschmieder[1]

Received: 31 July 2019 / Accepted: 28 October 2019 / Published online: 20 November 2019

Abstract

Philadelphia negative (Ph-neg) myeloproliferative neoplasms (MPN) are a heterogenous group of clonal stem cell disorders. Approved treatment options include hydroxyurea, anagrelide, and ruxolitinib, which are not curative. The concept of synthetic lethality may become an additional therapeutic strategy in these diseases. In our study, we show that DNA repair is altered in classical Ph-neg MPN, as analyzed by gene expression analysis of 11 genes involved in the homologous recombination repair pathway (HRR), the non-homologous end-joining pathway (NHEJ), and the single-strand break repair pathway (SSB). Altogether, peripheral blood-derived cells from 57 patients with classical Ph-neg MPN and 13 healthy controls were analyzed. LIG3 as an essential part of the SSB was significantly lower expressed compared to controls in all three entities (essential thrombocythemia (ET), polycythemia vera (PV), and myelofibrosis (MF)). In addition, while genes of other DNA-repair pathways showed—possibly compensatory—increased expression in ET (HRR, NHEJ) and PV (NHEJ), MF samples displayed downregulation of all genes involved in NHEJ. With regard to the JAK2 mutational status (analyzed in ET and MF only), no upregulation of the HRR was detected. Though further studies are needed, based on these findings, we conclude that synthetic lethality may become a promising strategy in treating patients with Ph-neg MPN.

Keywords DNA repair · Gene expression · Myeloproliferative neoplasms

Introduction/Background

Myeloproliferative neoplasms (MPN) are a heterogeneous group of clonal stem cell disorders. In Philadelphia chromosome negative (Ph-neg) MPN, despite considerable advances in symptomatic therapy in the last decade, therapeutic options are still limited, especially for myelofibrosis, and survival is decreased in most patients [1–4]. The concept of synthetic lethality provides an alternative strategy to overcome inherent treatment resistance by exploiting tumor-specific vulnerabilities. It is based on the mechanism that neoplastic cells accumulate genetic aberrations and become susceptible to therapeutic approaches, whereas non-neoplastic cells do not acquire these alterations [5, 6], and, thus, such treatment will not or only minimally affect non-neoplastic cells. The concept of synthetic lethality has been applied to—among others—impairment of cell cycle as well as DNA repair mechanism pathways [7, 8].

As an example, cells harboring BRCA1 and BRCA2 mutations, both part of the homologous recombination repair pathway (HRR), are dependent on PARP as part of the single-strand break repair (SSB) pathway. Previous studies revealed that inhibiting PARP in BRCA mutated but not non-mutated cells induce apoptosis. As a consequence, PARP inhibitors were developed and approved for the treatment of patients with BRCA-mutated ovarian cancer [9].

Altered DNA repair pathways have also been implicated in the pathogenesis of myeloproliferative neoplasms (MPN). Recent works demonstrate a downregulation of BRCA1 by

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00277-019-03836-2) contains supplementary material, which is available to authorized users.

✉ Martin Kirschner
mkirschner@ukaachen.de

✉ Steffen Koschmieder
skoschmieder@ukaachen.de

[1] Department of Hematology, Oncology, Hemostaseology and Stem Cell Transplantation, Faculty of Medicine, RWTH Aachen University, Aachen, Germany

[2] Sol Sherry Thrombosis Research Center and Fels Institute for Cancer Research and Molecular Biology, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, USA

BCR-ABL in chronic myeloid leukemia (CML) [10]. Furthermore, impairing RAD52 (part of HRR) in BRCA1/2-deficient cells induced accumulation of double-strand breaks and synthetic lethality in these cells, without significantly impairing normal cells [11–13].

Based on these results in CML and Philadelphia-neg MPN, the aim of our study was to investigate the activity of DNA repair mechanism in Ph-neg MPN by gene expression analysis to detect whether synthetic lethality could be a possible therapeutic concept in these entities. In line with this, genes of the HRR (BRCA1, BRCA2, RAD52, RPA1, RPA2), NHEJ (LIG4, PRKDC, XRCC5, XRCC6), and SSB (PARP, LIG3) were analyzed in a respective cohort of Ph-neg MPN patients.

Materials and methods

Patients and controls

Fifty-seven patients from the Aachen MPN-registry of patients with classical, Ph-neg MPN (essential thrombocythemia (ET), polycythemia vera (PV), and myelofibrosis (MF)) and 13 controls were included in our study. All patients and controls provided written informed consent, as approved by the ethics committee (EK 127/12 and EK 099/14).

Blood preparation and gene expression analysis

Peripheral blood was used to isolate mononuclear cell (MNCs) for further workup. In the next step, mRNA was isolated and the concentration was measured using the NanoDrop® system. 1 μg of mRNA was further used for cDNA synthesis according to the in-house standard protocol.

For expression analysis, real-time PCR (RT-PCR) was applied using the SYBR® Green technology. 11 genes of DNA repair pathways (BRCA1, BRCA2, LIG3, LIG4, PARP1, PRKDC, RAD52, RPA1, RPA2, PALP2, XRCC5, XRCC6) were analyzed as well as GAPDH as housekeeping and reference gene (primer list: Supplement Table 1). RT-PCR run on a 7500 Fast real-time PCR-platform, for control and analyzing the 7500 Software v2.0.3 was used.

Statistics

Gene expression data (each gene in percentage of GAPDH as comparator) of each entity was compared to expression levels of normal controls. For statistical analysis, the non-parametric Mann-Whitney *U* test was used (IBM SPSS statistics 23; Prism 7 for Windows, Version 7.04, © GraphPad Software Inc.) to test for statistically significant differences of expression level of each gene and entity (ET, PV, MF) versus controls though normal distribution was not given in the different cohorts.

Table 1 JAK2 positivity

Entity	*n* (total)	JAK2 positive, *n* (%)	JAK2 negative, *n* (%)
PV	17	16 (94)	1 (6)
ET	15	10 (67)	5 (33)
MF	14	12 (86)	2 (14)

Results

After exclusion of 12 samples due to insufficient sample quality, the final analysis included 46 evaluable samples of MPN patients (17 PV, 15 ET, 14 MF (9 primary MF, 5 secondary MF)) and 12 healthy controls. The JAK2 mutational status was obtained from all samples and was detected in approximately 94% of PV, 67% of ET, and 86% of MF cases (Table 1, suppl. Table 4).

Gene expression data from the ET samples revealed statistically significant different expression compared to controls in the following genes (Table 2; Fig. 1; suppl. Table 2): In the genes affecting the base excision repair for LIG3 a lower expression could be detected ($p < 0.05$) compared to controls, but no difference was seen for PARP. In the genes which are involved in NHEJ-pathway, a statistically significant higher expression was seen for PRKDC ($p < 0.05$) and XRCC5 ($p < 0.01$), but no difference for LIG4 and XRCC6. For genes of the HRR, statistically significant upregulation was detected for BRCA1 ($p < 0.05$) and highly significant for BRCA2 ($p < 0.001$), whereas no differences were seen for RAD52, RPA1, and RPA2 (see Table 2).

In PV, a different expression pattern was revealed compared to ET (Table 2, Fig. 2; suppl. Table 2). While LIG3 expression was lower and PRKDC expression was higher compared to controls, which was comparable to ET, genes of the HRR pathway were differentially regulated in PV, with expression of RAD52 being decreased, whereas no differences were seen for BRCA1, BRCA2, RPA1, and RPA2, which was different from ET and MF.

MF samples showed a quite different expression pattern compared to the other two entities (Table 2, Fig. 3; suppl. Table 2). Nine of the analyzed 11 genes revealed a significant downregulation, only BRCA1 and BRCA2 were not significantly altered. None of the genes of the NHEJ (LIG4, PRKDC, XRCC5, XRCC6) and the SSB (LIG3, PARP1) showed expression levels comparable to healthy controls.

Furthermore, we analyzed differences in gene expression in our cohort according to JAK2 state (positive/negative). In ET, a significant downregulation of the HRR pathway was detected in JAK2 positive samples, whereas no difference was seen regarding the MF samples.

Table 2 Significance level of expression difference (MPN vs controls)

	BRCA1	**BRCA2**	**RAD52**	**RPA1**	**RPA2**	*LIG4*	*PRKDC*	*XRCC5*	*XRCC6*	***LIG3***	***PARP1***
ET	*↑	***↑	ns	ns	ns	ns	*↑	**↑	ns	*↓	ns
PV	ns	ns	*↓	ns	ns	ns	*↑	ns	ns	***↓	ns
MF	ns	ns	**↓	***↓	**↓	***↓	*↓	**↓	***↓	***↓	***↓

Statistically significant differences in gene expression in ET/PV/MF vs controls. Genes of the homologous recombination repair (HRR) are shown in bold, genes of the non-homologous end joining (NHEJ) are shown in italics, genes of the single strand break repair (SSB) are shown in bold italics

↑ = higher expression compared to control, ↓ = lower expression compared to control

*< 0.05

**< 0.01

***< 0.001

Discussion

In MPN, despite recent advances in symptomatic therapy, the options are still limited and further therapeutic approaches are desirable. Synthetic lethality as therapeutic concept shows promising preliminary data in the entity, so we analyzed a respective cohort of MPN samples with regard to DNA-repair mechanism.

Our study revealed a significant downregulation of LIG3 in all three analyzed MPN entities. LIG3 is an essential part of

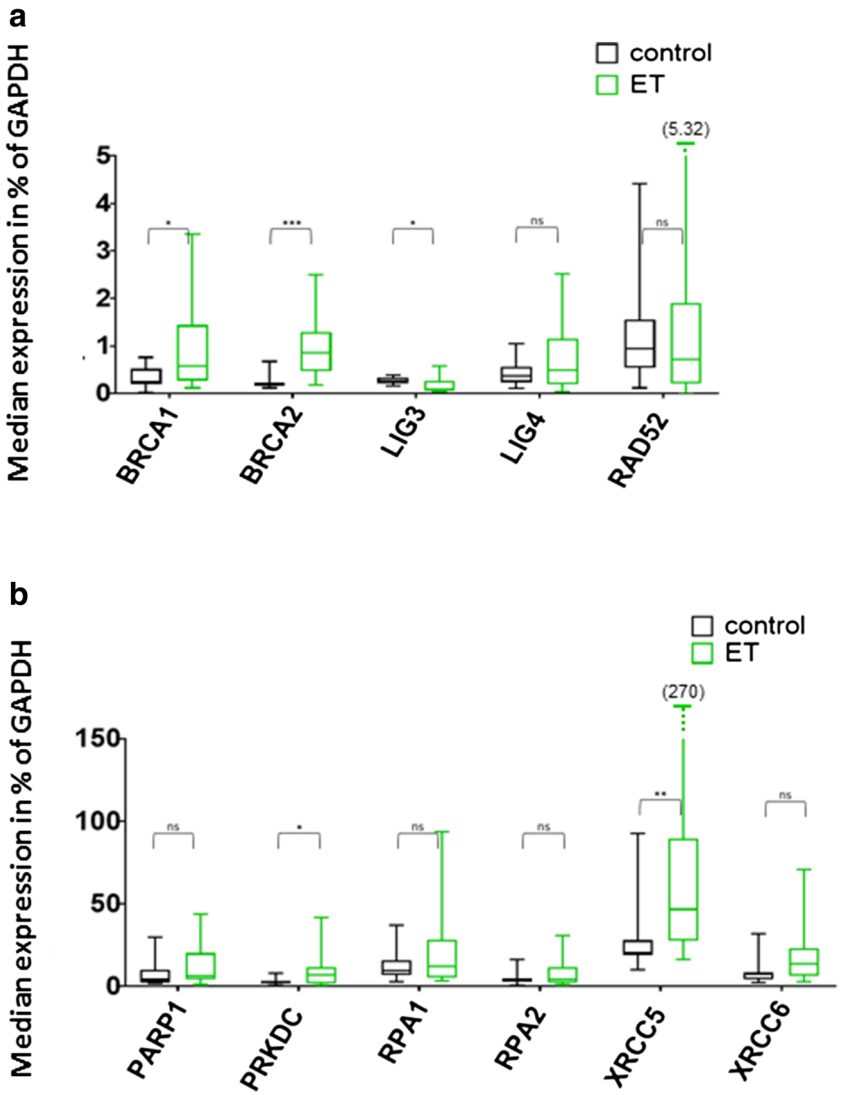

Fig. 1 a, b Expression of the analyzed gene set in ET. Expression in percentage of GAPDH as comparator is shown on the y-axis. For each gene, difference of expression compared to control is indicated with significance levels (ns = not significant; * = < 0.05; ** = < 0.01; *** = < 0.001. See also Table 2).

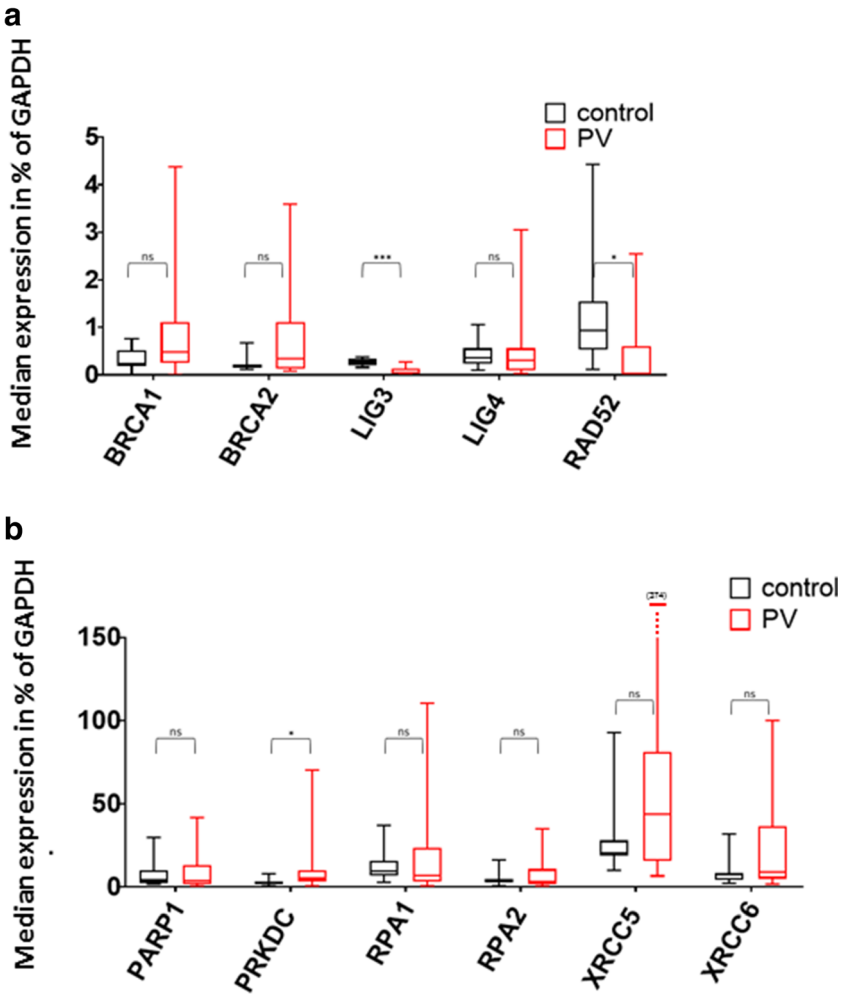

Fig. 2 a, b Expression of the analyzed gene set in PV. Expression in percentage of GAPDH as comparator is shown on the *y*-axis. For each gene, difference of expression compared to control is indicated with significance levels (ns = not significant; * = < 0.05; ** = < 0.01; *** = < 0.001. See also Table 2).

the SSB, since inhibiting LIG3 in a mouse model led to early death [14]. In addition, LIG3 may function as a backup for impaired function of the NHEJ pathway [15]. In line with this, downregulation of LIG3 may cause genetic aberrations in Ph-neg MPN especially in MF, in which also components of NHEJ show a lower expression. However, LIG3 is also part of the alternative non-homologous end-joining pathway (A-EJ). An upregulation of LIG3 was shown in BCR-ABL positive cell lines (CML in chronic phase), potentially replacing HRR by the more error-prone DNA-repair pathway (A-EJ) and subjecting these cells to genetic instability [16]. In contrast to that study, which analyzed cultured cell lines, we used "fresh" RNA from patients' blood samples which may explain these differences in results. In addition, A-EJ is not the only error-prone DNA repair mechanism. When both A-EJ and NHEJ are reduced, HRR may compensate for the efficient repair. However, excessive HRR, especially when combined with inefficient mismatch repair (MMR), can be responsible for genomic instability as described in CML [17–19]. This hypothesis is further supported by the report that the JAK2 kinase appears to regulate MMR [20]. Furthermore, downregulation of LIG3 may reduce base excision repair (BER) activity, resulting in genomic instability [21].

When studying the MPN subentities in detail, in ET samples, there was no downregulation of the analyzed genes detected except for LIG3. Compared to the control cohort, even an upregulation of the HRR pathway could be revealed in ET cohort. Our data show that DNA repair is not notably downregulated in ET. As cytogenetic aberrations may be caused by insufficient DNA-repair mechanisms [22], our finding is in accordance with the fact that additional mutations other than the three driver mutations (JAK2V617F, CALR, MPL) are infrequent in ET and cytogenetic aberrations are rare in ET (5–7%) [23]. In PV, cytogenetic abnormalities are detected in a slightly higher proportion (~ 20%) and the frequency can increase during clinical course [24]. We detected a significant downregulation of RAD52 as part of the HRR pathway abnormalities in PV.

In the MF samples, our analysis revealed a statistically significant downregulation of most of the analyzed genes, including all genes of the SSB and NHEJ pathways and three genes (RAD52, RPA1, RPA2) which are relevant in HRR

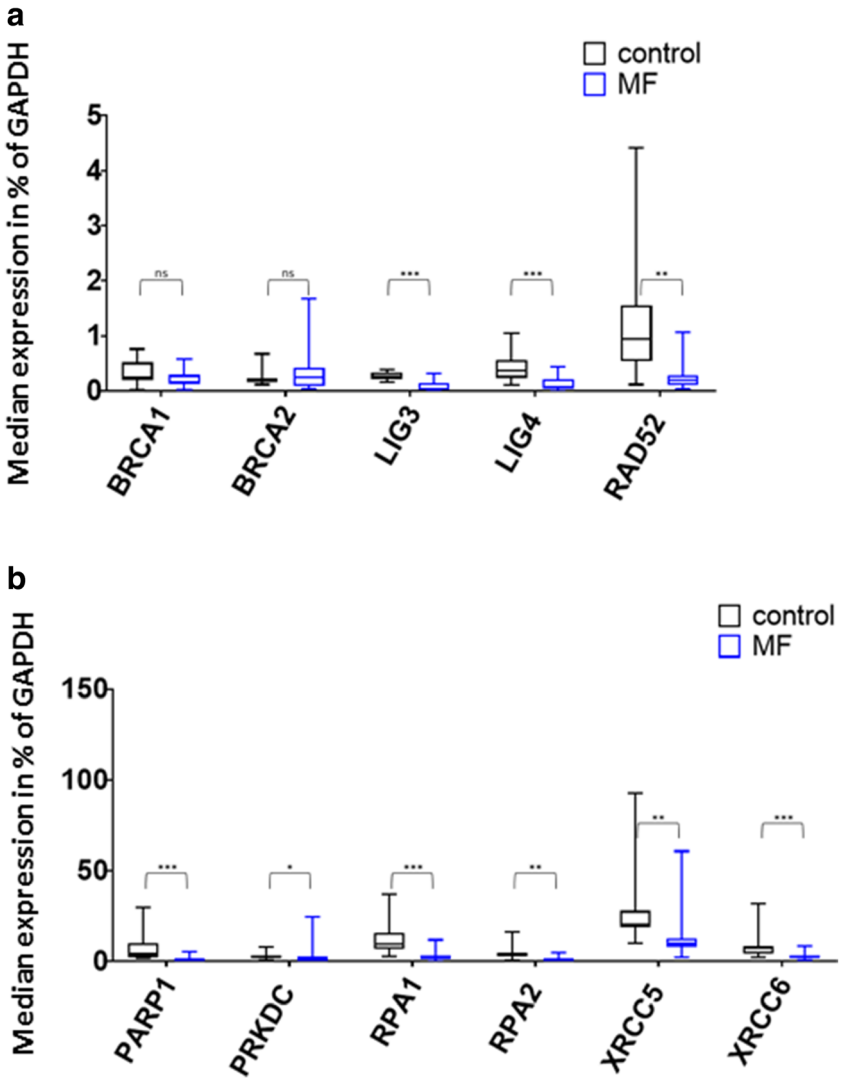

Fig. 3 a, b Expression of the analyzed gene set in MF. Expression in percentage of GAPDH as comparator is shown on the *y*-axis. For each gene, difference of expression compared to control is indicated with significance levels (ns = not significant; * = < 0.05; ** = < 0.01; *** = < 0.001. See also Table 2).

pathway. Comparing MF to ET/PV, cytogenetic aberrations are more frequent and complex in patients with MF [25, 26]. In addition, further somatic mutations besides the classical MPN defining mutations (JAK/CALR/MPL) are detected more frequently in MF compared to ET/PV [27]. The distinct downregulation of DNA repair mechanisms in our cohort may be an explanation for these findings.

Previous studies could reveal synthetic lethality as possible therapeutic approach in CML [10]. Even in Ph-neg MPN, recent works showed sensitivity to PARP inhibitors in ruxolitinib-treated patients, as ruxolitinib causes HRR and NHEJ pathway impairment [12]. Since patients' samples in our study were obtained in the pre-ruxolitinib era, ruxolinitib was excluded as confounding factor in our analysis.

As data exist that JAK2 V617F activates the HRR pathway [28], we analyzed expression differences among JAK2 positive and negative samples in ET and MF (PV was excluded due to small number of JAK2 negative samples). To conclude our analysis regarding this point, JAK2 positive and negative samples in the complete cohort showed no statistically significant differences in gene expression levels (Suppl. Table 3). With regard to the already mentioned previous data [28], this discrepancy may be caused by different analyzed material as well as different methodic approaches.

Finally, we have to address some limitations and advantages of our study. Although we analyzed a respective cohort of MPN samples, the number of samples is still small, and so far only mRNA expression data was analyzed, but for better understanding of DNA regulation in Ph-neg MPN and proving our hypotheses, further experimental evaluation on protein level is essential. As JAK2 can influence DNA repair, it would have been interesting to investigate the correlation of our DNA repair gene expression data with JAK2 allele burden. However, since allele burden measurements were not yet routinely assessed, these data are not available. While this may be a limitation of our cohort, its advantage is that it reflects a real-world setting without pre-selection.

Conclusion

In conclusion, our study demonstrated significantly different gene expression in DNA-repair pathways in Ph-neg MPN compared to controls as well as marked differences among the three entities. Influence of JAK2 V617F mutational state on gene expression could not be shown in our cohort, but especially in MF samples all analyzed DNA repair mechanisms are distinctly downregulated. Even though further studies are needed, data of this study suggests synthetic lethality as potential new approach in the therapy of Ph-neg MPN.

Acknowledgments We thank Kristina Feldberg for the excellent technical assistance.

The data presented in this publication will be part of the doctoral thesis of Anne Bornemann (co-author) which will be published in future.

Authorship MK: performed parts of the experiments, analyzed and interpreted the data, and wrote the manuscript. AB: performed parts of the experiments, analyzed the data, and revised the manuscript. CS: performed parts of the experiments, analyzed the data, and revised the manuscript. DG: Collected the clinical data and revised the manuscript. KK: Collected the clinical data and revised the manuscript. SI: interpreted the data and revised the manuscript. THB: analyzed and interpreted the data and revised the manuscript. MS: analyzed and interpreted the data and revised the manuscript. NC: analyzed and interpreted the data and revised the manuscript. TS: analyzed and interpreted the data and revised the manuscript. SK: conceived and planned the study design, interpreted the data, and revised the manuscript.

Funding information Steffen Koschmieder was supported by a grant of the German research Foundation (DFG KO 2155/6-1).

Compliance with ethical standards

Conflict of interest THB: Consultancy: Novartis, Pfizer, Janssen, Merck; research funding: Novartis, Pfizer. SK: reports funding from Novartis, Bristol-Myers Squibb and Janssen, as well as consultancy, honoraria, and travel support from Novartis, Incyte, Ariad, Bristol-Myers Squibb, AOP, CTI, Pfizer, Celgene, and Shire. The other authors declare that they have no conflict of interest.

References

1. Barbui T, Barosi G, Birgegard G, Cervantes F, Finazzi G, Griesshammer M, Harrison C, Hasselbalch HC, Hehlmann R, Hoffman R, Kiladjian JJ, Kröger N, Mesa R, McMullin M, Pardanani A, Passamonti F, Vannucchi AM, Reiter A, Silver RT, Verstovsek S, Tefferi A, European LeukemiaNet (2011) Philadelphia-negative classical myeloproliferative neoplasms: critical concepts and management recommendations from European LeukemiaNet. *J Clin Oncol* 29(6):761–770
2. Tefferi A, Cortes J, Verstovsek S, Mesa RA, Thomas D, Lasho TL, Hogan WJ, Litzow MR, Allred JB, Jones D, Byrne C, Zeldis JB, Ketterling RP, McClure R, Giles F, Kantarjian HM (2006) Lenalidomide therapy in myelofibrosis with myeloid metaplasia. *Blood* 108(4):1158–1164
3. Tefferi A (2012) JAK inhibitors for myeloproliferative neoplasms: clarifying facts from myths. *Blood* 119(12):2721–2730
4. Tefferi A, Lasho TL, Finke CM, Knudson RA, Ketterling R, Hanson CH, Maffioli M, Caramazza D, Passamonti F, Pardanani A (2014) CALR vs JAK2 vs MPL-mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparisons. *Leukemia* 28(7):1472–1477
5. Iglehart JD, Silver DP (2009) Synthetic lethality–a new direction in cancer-drug development. *N Engl J Med* 361(2):189–191
6. Friend SH, Oliff A (1998) Emerging uses for genomic information in drug discovery. *N Engl J Med* 338(2):125–126
7. Kamb A (2003) Mutation load, functional overlap, and synthetic lethality in the evolution and treatment of cancer. *J Theor Biol* 223(2):205–213
8. Kaelin WG Jr (2005) The concept of synthetic lethality in the context of anticancer therapy. *Nat Rev Cancer* 5(9):689–698
9. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, Scott CL, Meier W, Shapira-Frommer R, Safra T et al (2014) Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. *Lancet Oncol* 15(8):852–861
10. Deutsch E, Jarrousse S, Buet D, Dugray A, Bonnet ML, Vozenin-Brotons MC, Guilhot F, Turhan AG, Feunteun J, Bourhis J (2003) Down-regulation of BRCA1 in BCR-ABL-expressing hematopoietic cells. *Blood* 101(11):4583–4588
11. Cramer-Morales K, Nieborowska-Skorska M, Scheibner K, Padget M, Irvine DA, Sliwinski T, Haas K, Lee J, Geng H, Roy D et al (2013) Personalized synthetic lethality induced by targeting RAD52 in leukemias identified by gene mutation and expression profile. *Blood* 122(7):1293–1304
12. Nieborowska-Skorska M, Maifrede S, Dasgupta Y, Sullivan K, Flis S, Le BV, Solecka M, Belyaeva EA, Kubovcakova L, Nawrocki M et al (2017) Ruxolitinib-induced defects in DNA repair cause sensitivity to PARP inhibitors in myeloproliferative neoplasms. *Blood* 130(26):2848–2859
13. Bolton-Gillespie E, Schemionek M, Klein HU, Flis S, Hoser G, Lange T, Nieborowska-Skorska M, Maier J, Kerstiens L, Koptyra M et al (2013) Genomic instability may originate from imatinib-refractory chronic myeloid leukemia stem cells. *Blood* 121(20):4175–4183
14. Puebla-Osorio N, Lacey DB, Alt FW, Zhu C (2006) Early embryonic lethality due to targeted inactivation of DNA ligase III. *Mol Cell Biol* 26(10):3935–3941
15. Wang H, Rosidi B, Perrault R, Wang M, Zhang L, Windhofer F, Iliakis G (2005) DNA ligase III as a candidate component of backup pathways of nonhomologous end joining. *Cancer Res* 65(10):4020–4030
16. Tobin LA, Robert C, Rapoport AP, Gojo I, Baer MR, Tomkinson AE, Rassool FV (2013) Targeting abnormal DNA double-strand break repair in tyrosine kinase inhibitor-resistant chronic myeloid leukemias. *Oncogene* 32(14):1784–1793
17. Nowicki MO, Falinski R, Koptyra M, Slupianek A, Stoklosa T, Gloc E, Nieborowska-Skorska M, Blasiak J, Skorski T (2004) BCR/ABL oncogenic kinase promotes unfaithful repair of the reactive oxygen species-dependent DNA double-strand breaks. *Blood* 104(12):3746–3753
18. Stoklosa T, Poplawski T, Koptyra M, Nieborowska-Skorska M, Basak G, Slupianek A, Rayevskaya M, Seferynska I, Herrera L, Blasiak J et al (2008) BCR/ABL inhibits mismatch repair to protect from apoptosis and induce point mutations. *Cancer Res* 68(8):2576–2580
19. Slupianek A, Dasgupta Y, Ren SY, Gurdek E, Donlin M, Nieborowska-Skorska M, Fleury F, Skorski T (2011) Targeting RAD51 phosphotyrosine-315 to prevent unfaithful recombination repair in BCR-ABL1 leukemia. *Blood* 118(4):1062–1068

20. Ding N, Miller SA, Savant SS, O'Hagan HM (2019) JAK2 regulates mismatch repair protein-mediated epigenetic alterations in response to oxidative damage. *Environ Mol Mutagen* 60(4):308–319
21. Dianov GL, Hubscher U (2013) Mammalian base excision repair: the forgotten archangel. *Nucleic Acids Res* 41(6):3483–3490
22. Khanna KK, Jackson SP (2001) DNA double-strand breaks: signaling, repair and the cancer connection. *Nat Genet* 27(3):247–254
23. Gangat N, Tefferi A, Thanarajasingam G, Patnaik M, Schwager S, Ketterling R, Wolanskyj AP (2009) Cytogenetic abnormalities in essential thrombocythemia: prevalence and prognostic significance. *Eur J Haematol* 83(1):17–21
24. Tang G, Hidalgo Lopez JE, Wang SA, Hu S, Ma J, Pierce S, Zuo W, Carballo-Zarate AA, Yin CC, Tang Z, Li S, Medeiros LJ, Verstovsek S, Bueso-Ramos CE (2017) Characteristics and clinical significance of cytogenetic abnormalities in polycythemia vera. *Haematologica* 102(9):1511–1518
25. Okamura T, Kinukawa N, Niho Y, Mizoguchi H (2001) Primary chronic myelofibrosis: clinical and prognostic evaluation in 336 Japanese patients. *Int J Hematol* 73(2):194–198
26. Bacher U, Haferlach T, Kern W, Hiddemann W, Schnittger S, Schoch C (2005) Conventional cytogenetics of myeloproliferative diseases other than CML contribute valid information. *Ann Hematol* 84(4):250–257
27. Singh NR (2015) Genomic diversity in myeloproliferative neoplasms: focus on myelofibrosis. Transl Pediatr 4(2):107–115
28. Plo I, Nakatake M, Malivert L, de Villartay JP, Giraudier S, Villeval JL, Wiesmuller L, Vainchenker W (2008) JAK2 stimulates homologous recombination and genetic instability: potential implication in the heterogeneity of myeloproliferative disorders. *Blood* 112(4):1402–1412