Simultaneous Targeting of PARP1 and RAD52 Triggers Dual Synthetic Lethality in BRCA-Deficient Tumor Cells

Graphical Abstract

Highlights

- RAD52 inhibitors attenuate SSA and residual HR in BRCA-deficient cells
- PARP + RAD52 inhibitors exert dual synthetic lethality in BRCA-deficient cells
- RAD52 inhibitor improves the effect of PARP inhibitor in BRCA-deficient tumors

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In Brief

Sullivan-Reed et al. show that simultaneous treatment with PARP and RAD52 inhibitors exerts dual synthetic lethality in BRCA-deficient tumors. Addition of RAD52 inhibitor should improve therapeutic outcome of BRCA-deficient malignancies treated with PARP inhibitor.
Simultaneous Targeting of PARP1 and RAD52 Triggers Dual Synthetic Lethality in BRCA-Deficient Tumor Cells

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SUMMARY

PARP inhibitors (PARPis) have been used to induce synthetic lethality in BRCA-deficient tumors in clinical trials with limited success. We hypothesized that RAD52-mediated DNA repair remains active in PARPi-treated BRCA-deficient tumor cells and that targeting RAD52 should enhance the synthetic lethal effect of PARPi. We show that RAD52 inhibitors (RAD52is) attenuated single-strand annealing (SSA) and residual homologous recombination (HR) in BRCA-deficient cells. Simultaneous targeting of PARP1 and RAD52 with inhibitors or dominant-negative mutants caused synergistic accumulation of DSBs and eradication of BRCA-deficient but not BRCA-proficient tumor cells. Remarkably, Parp1−/−; Rad52−/− mice are normal and display prolonged latency of BRCA1-deficient leukemia compared with Parp1−/− and Rad52−/− counterparts. Finally, PARPi+RAD52i exerted synergistic activity against BRCA1-deficient tumors in immunodeficient mice with minimal toxicity to normal cells and tissues. In conclusion, our data indicate that addition of RAD52i will improve therapeutic outcome of BRCA-deficient malignancies treated with PARPi.

INTRODUCTION

Numerous reports indicate that tumor cells accumulate high levels of spontaneous and drug-induced DNA damage, but they survive because of enhanced or altered DNA repair activities (Bartkova et al., 2005). PARP1 may prevent accumulation of potentially lethal DNA double-strand breaks (DSBs) by playing a key role in base excision repair (BER), single-strand break (SSB) repair, and alternative non-homologous end-joining (Alt-NHEJ) and/or by facilitating MRE11-mediated recruitment of RAD51 to promote stalled replication fork restart (Metzger et al., 2013; Ying et al., 2012). Homologous recombination (HR), which depends mostly on BRCA1-PALB2-BRCA2-RAD51 paralogs-RAD51-RAD54 (BRCA-HR), and RAD52-dependent single-strand annealing (RAD52-SSA) play an important role in DSB repair in proliferating cells (Kass and Jasinska, 2010).

The hypothesis that cancer cells are addicted to particular DNA repair pathways is supported by selective targeting of tumor cells by recently developed novel drugs and compounds against specific DNA repair mechanisms (Nickoloff et al., 2017). The success of the PARP inhibitor (PARPi) olaparib in BRCA1- and BRCA2-deficient breast tumors has established a proof of concept of personalized cancer therapy using synthetic lethality (Lord et al., 2015). Unfortunately, therapeutic effect is usually short-lived, and tumor cells become unresponsive to PARPi because of compensatory mechanisms such as restoration of HR via secondary mutations in BRCA2, PALB2, RAD51 paralogs (RAD51C, RAD51D), or loss of 53BP1, impaired drug uptake, and/or enhanced drug efflux (Lord and Ashworth, 2013). In concordance, we showed that BRCA-deficient breast carcinoma cells and leukemia cells could not be completely eradicated by PARPi (Nieborowska-Skorska et al., 2017). Therefore, more robust and rapid elimination of BRCA-deficient tumor cells is required to prevent time-dependent emergence of PARPi-resistant or refractory clones.
It has been suggested that RAD52-dependent HR pathways involving RAD51 (RAD52-HR) and/or RAD52-SSA can act as backups to the main BRCA-mediated HR pathway (BRCA-HR) (Stark et al., 2004; Wray et al., 2008). We hypothesized that RAD52-HR and/or RAD52-SSA represent potential escape route(s) from PARPi-mediated synthetic lethality in BRCA-deficient cells and that simultaneous inhibition of PARP and RAD52-dependent DNA repair pathways would trigger more effective "dual" synthetic lethality.

**RESULTS**

**Inhibition of RAD52 Attenuated Residual HR Activity in PARPi-Treated BRCA-Deficient Tumor Cell Lines**

BRCA1/2-deficient and BRCA1/2-proficient cells carrying DR-GFP recombination reporter cassette were co-transfected with pCBAScel (encoding I-Sce1 endonuclease generating a DSB in the reporter cassette) and pDsRed (transfection efficiency control) expression plasmids. As expected, BRCA1 and BRCA2 deficiencies were associated with reduced HR measured by the percentage of GFP+ cells in DsRed+ population ± SD from three independent experiments; *p < 0.05 in comparison with untreated control.

**Figure 1. RAD52 Inhibitor 6-OH-Dopa Attenuated HR and SSA in BRCA1/2-Deficient Cells Treated with PARP Inhibitor Olaparib**

(A and B) BRCA2-mutated VC8 cells (BRCA2−) and BRCA2 wild-type V79 cells (BRCA2+) (A) and BRCA1−/− murine ES clone 17 cells (BRCA1−) and BRCA1 wild-type clone 92B cells (BRCA1+) (B) carrying DR-GFP cassette were co-transfected with I-SceI and DsRed cDNAs, followed by treatment with 5 μM olaparib (Ola), 50 nM talazoparib (Tala), and/or 10 μM 6-OH-dopa (Dopa), or were left untreated (Control). Results represent mean percentage of GFP+DsRed+ cells in DsRed+ population ± SD from three independent experiments; *p < 0.05 in comparison with untreated control.

(C) BRCA1-mutated HCC1937 cells (BRCA1−) and HCC1937 expressing wild-type BRCA1 (BRCA1+) were treated with 3 μg/mL cisplatin (Control) and cisplatin combined with 5 μM olaparib (Ola) and/or 10 μM 6-OH-dopa (Dopa). Results represent percentage of cells with more than ten RAD51 foci from three independent experiments (100 cells/experiment were evaluated); *p < 0.05 in comparison with untreated control.

(D) BRCA2−/−/− murine ES clone 42E cells (BRCA2−) and BRCA2 wild-type clone 40b cells (BRCA2+) carrying SA-GFP cassette were co-transfected with I-SceI and DsRed cDNAs, followed by treatment with 1.25 μM olaparib (Ola) and/or 20 μM 6-OH-dopa (Dopa), or were left untreated (Control). Results represent mean percentage of GFP+DsRed+ cells in DsRed+ population ± SD from three independent experiments; *p < 0.05 in comparison with untreated control. See also Figure S1.
Dopa, but not PARPi olaparib, inhibited cisplatin-induced RAD51 foci formation in BRCA1-deficient HCC1937 cells but not in BRCA1-proficient counterparts (Figure 1C). Moreover, Dopa reduced RAD51 foci formation in olaparib-treated BRCA1-deficient HCC1937 cells. As expected, RAD52i Dopa inhibited SSA activity in BRCA2-deficient and BRCA2-proficient cells and also in olaparib-treated cells (Figure 1D).

The importance of RAD52 was further supported by significantly higher relapse-free survival probability of the patients with leukemias displaying low expression levels of one of the BRCA-HR gene (BRCA1, BRCA2, PALB2, RAD51) and RAD52 gene compared with those with leukemias expressing high levels of these genes (Figure S1). In concordance, RAD52i reduced HR and SSA activities in BRCA-deficient tumor cells when applied alone or in combination with PARPi, suggesting that RAD52 promotes survival of PARPi-treated BRCA-deficient cells. This observation provided justification for the investigation of the potency of dual synthetic lethal effect exerted by simultaneous targeting of PARP and RAD52 in BRCA-deficient tumor cells.

**RAD52i Enhanced the Synthetic Lethal Effect of PARPi, Causing Complete Eradication of BRCA-Deficient Tumor Cells In Vitro**

Simultaneous treatment of BRCA1-deficient MDA-MB-436 tumor cells with PARPi olaparib and RAD52i Dopa (Chandramouly et al., 2015) resulted in enhanced accumulation of DSBs detected by neutral comet assay and γ-H2AX immunofluorescence and synergistic inhibition of cell growth in comparison with treatment with individual inhibitors, while BRCA-proficient counterparts were not affected by these inhibitors (Figure 2A). Similar growth inhibitory effect in BRCA1-deficient MDA-MB-436 cells was exerted by olaparib combined with RAD52i D-I03 (Huang et al., 2016) (Figure 2B). Moreover, combination of PARPi olaparib and RAD52i F79 aptamer (Cramer-Morales et al., 2013) synergistically inhibited the growth of BRCA1-deficient UWB1.289, MDA-MB-436, and HCC1937 tumor cell lines and BRCA2-deficient Capan-1 tumor cells compared with individual treatments (Figure 2C). BRCA-proficient counterparts and non-transformed NIH 3T3 cells were not affected by the inhibitors. PARPi and RAD52i did not cause downregulation of PARP1 and RAD52 proteins implicating functional inhibition of the targeted proteins (Figure S2A).

Hence, these data show consistent and selective killing of BRCA-deficient cells by dual treatment with PARPi (i.e., olaparib, talazoparib) and one of three different RAD52i (i.e., F79 aptamer, Dopa, D-I03), which validates RAD52 as an attractive target for inhibition along with PARPi.

The effect of dual synthetic lethality may be triggered selectively in BRCA-deficient cells. MLL-AF9-positive leukemia cells are BRCA proficient, but they are sensitive to PARPi, especially when combined with standard cytotoxic drug (Malfrede et al., 2017b). However, these cells did not respond favorably to the combination of PARPi + RAD52i (Figure S2B), most likely because PARP1-dependent but not RAD52-mediated mechanisms play a major role in their DNA repair.

Importantly, long-term continuous treatment (28 days) with PARPi (olaparib) + RAD52i (Dopa) led to eradication of BRCA1- and BRCA2-deficient cells, while individual agents generated partial inhibition of the growth rate (Figure 2D). At the end of 28 days of continuous treatment, double-treated cells were washed out of the drugs and incubated in drug-free medium. Remarkably, no living cells were retrieved even after 14 days of culture.

We also examined the effect of combination treatment on leukemia cells deficient in another BRCA-HR gene, RAD54 (Mazin et al., 2010). Simultaneous short-term treatment of RAD54−/− Nalm-6 human leukemia cell line but not RAD54+/+ parental cells with PARPi olaparib and RAD52i Dopa enhanced accumulation of DSBs assessed by neutral comet assay and γ-H2AX immunofluorescence and eliminated significantly more cells, as determined by trypan blue exclusion test, compared with individual drugs (Figures S2C–S2E). Moreover, long-term (35 days) combinatorial treatment resulted in complete elimination of RAD54−/− Nalm-6 cells, whereas individual inhibitors exerted only partial effect (Figure S2F). At the end of the experiment, living RAD54−/− Nalm-6 cells were not detected after a 14 day incubation of double-treated cells in drug-free medium.

In addition, a combination of olaparib and Dopa exerted a much stronger inhibitory effect against BRCA2-deficient Burkitt lymphoma (BL)-derived Epstein-Barr virus (EBV)-positive B cell lines Mutu and Raji (Malfrede et al., 2017a) compared with individual inhibitor treatment (Figure S2G). At the same time, BRCA2-proficient EBV-immortalized lymphoblastoid cell lines (LCL1 and LCL2) established from healthy donor cells were not sensitive to these inhibitors.

To rule out possible off-target effects of the small-molecule inhibitors, dominant-negative mutants of PARP1 and RAD52 were used. Ectopic expression of catalytically inactive PARP1(E988K) mutant downregulated protein PARylation and expression of DNA binding-defective RAD52i(F79A) mutant reduced RAD52 foci formation (Figure S3). PARP1(E988K) and RAD52i(F79A) when expressed individually were able to selectively inhibit the growth of BRCA1-deficient MDA-MB-436 cells and BRCA2-deficient EUFA423 cells (Figure 2E). Importantly, co-expression of PARP1(E988K) and RAD52i(F79A) mutants exerted synergistic growth inhibitory effect in MDA-MB-436 cells and EUFA423 cells, whereas BRCA1/2-reconstituted counterparts were not affected.

In conclusion, our data show that simultaneous targeting of PARP1 and RAD52 by small-molecule inhibitors or dominant-negative mutants facilitates accumulation of toxic DSBs, resulting in selective elimination of tumor cells displaying deficiencies in BRCA-mediated HR. Moreover, prolonged combinatorial treatment completely eradicated BRCA-deficient cells, demonstrating a highly robust method for selective killing of these cells.

**Combination of PARPi and RAD52i Eliminated BRCA-Deficient Primary Leukemia Cells More Efficiently Than Individual Inhibitors**

We and others reported previously that several oncopgenes, such as BCR-ABL1, AML1-ETO, and IGH-MYC, but not FLT3(TD) and MLL-AF9, inhibit BRCA1/2 protein expression, resulting in synthetic lethality triggered by PARP and/or RAD52 inhibitors used individually (Cramer-Morales et al., 2013; Fan et al., 2010; Malfrede et al., 2017a; Nieborowska-Skorska et al., 2017; Podszyalow-Bartnicka et al., 2014). Here we tested the effect of
Figure 2. Simultaneous Targeting RAD52 and PARP1 Enhanced Synthetic Lethality in BRCA-Deficient Carcinoma Cell Lines and Abrogated the Emergence of Resistant Clones

(A) BRCA1-deficient MDA-MB-436 cells and BRCA1-reconstituted counterparts were treated with 1 μM Ola and/or 5 μM Dopa. Results represent (left) mean percentage of comet tail DNA ± SD (100–150 cells) and (middle) mean percentage of γ-H2AX-positive cells ± SD (triplicate experiment) detected after 24 hr, and (right) mean percentage of viable cells ± SD relative to untreated counterparts (three experiments) detected at day 5. *p < 0.05 and **p = 0.06 in comparison with cells treated with individual drugs (olaparib, Dopa) using the response additivity approach.

(B) MDA-MB-436 BRCA1-deficient and BRCA1-proficient cells were treated on day 0 and day 2 with increasing concentrations of olaparib in absence or presence of D-O3 (1 μM). Results represent mean percentage of clonogenic cells ± SD relative to untreated counterparts (three experiments).

(C) Indicated BRCA1/2-deficient cells and BRCA1/2-reconstituted counterparts were treated with 1 μM olaparib (Ola) and/or 1 μM F79 aptamer (F79) added at 0 and 2 days, followed by trypan blue counting at day 5. Mean percentage of viable cells ± SD relative to untreated counterparts (three experiments). *p < 0.04 in comparison with cells treated with individual drugs using the response additivity approach.

(D) Indicated BRCA1/2-deficient cells were left untreated (black) or continuously treated with 2.5 μM olaparib (blue), 20 μM Dopa (green), and olaparib+Dopa (red) for 28 days. Results represent mean cumulative number of viable cells ± SD (triplicate experiments).

(E) BRCA1-deficient and BRCA1-reconstituted MBA-MB-436 cells and BRCA2-deficient and BRCA2-reconstituted EUFA423 cells were transfected with PARP1 and/or RAD52 wild-type or PARP1(E988K) and/or RAD52(F79A) dominant-negative mutants. Results represent relative growth of the cells expressing dominant-negative mutant(s) relative to these expressing wild-type proteins from at least three experiments. *p < 0.02 compared with BRCA1/2-proficient counterparts using Student’s t test; **p ≤ 0.01 compared with BRCA1/2-deficient cells transfected with individual mutants using the response additivity approach.

See also Figures S2 and S3.
combination PARPi+RAD52i treatment on BRCA1/2-deficient and BRCA1/2-proficient patient leukemia cells. Three different RAD52is and two PARPis were applied to exclude the possibility that the effect depends on non-specific properties of an individual inhibitor.

Combination of sub-optimal concentrations of olaparib and Dopa exerted a strong effect against primary BRCA1/2-deficient leukemia and lymphoma cells expressing BCR-ABL1, AML1-ETO, or IGH-MYC but not against BRCA1/2-proficient normal cells and leukemia cells expressing FLT3(ITD) (Figure 3A). Moreover, long-term continuous treatment (28 days) of the cells from very aggressive BCR-ABL1-positive CML-blast phase (CML-BP) with PARPi (olaparib) + RAD52i (Dopa) led to eradication of leukemia cells, while individual agents generated only partial inhibition (Figure 3B). At the end of 28 days of continuous treatment, double-treated cells were washed out of the drugs and incubated in drug-free medium. Remarkably, no living cells were retrieved even after 14 days of culture.

Using DR-GFP recombination reporter cassette and/or BRCA1/2 expression and foci formation, we were able to identify several BRCA-deficient and BRCA-proficient samples from individual acute myeloid leukemia (AML) and therapy-related myelodysplastic syndrome (t-MDS) patients (Cramer-Morales et al., 2013; Nieborowska-Skorska et al., 2017). These cells were treated with sub-optimal concentrations of the cytotoxic drug daunorubicin (DNR), PARPi olaparib, and/or RAD52i F79 aptamer. The combination of olaparib + F79 aptamer exerted much stronger inhibitory effect than individual compounds.
against AML cells from BRCA-deficient but not BRCA-proficient patients (Figures 3D and 3E). Triple combination of DNR + olaparib and F79 aptamer exerted synergistic effect compared with dual combinations of these compounds (Figure 3D).

In conclusion, combination of PARPi + RAD52i exerted stronger inhibitory effect than individual inhibitors specifically in BRCA-deficient leukemia and lymphoma cells. Addition of standard therapeutic drug (e.g., imatinib for CML-BP and DNR for AML) enhanced the effect of PARPi + RAD52i, suggesting a potentially beneficial therapeutic application of this approach.

**Simultaneous Targeting of PARP1 and RAD52 Exerted a Synergistic Effect against BRCA-Deficient Tumors in Mice**

BRCA-ABL1 oncogenic tyrosine kinase induces translational repression and degradation of BRCA1 protein, which makes leukemia cells sensitive to PARPi and RAD52i (Cramer-Morales et al., 2013; Dkhissi et al., 2015; Nieborowska-Skorska et al., 2017; Podszybalow-Bartnicka et al., 2014). To study the effect of PARPi + RAD52i against BRCA1-deficient BCR-ABL1-positive leukemias, we employed a tet-off SCLITAp210BCR-ABL1 transgenic mouse model, which upon withdrawal of tetracycline develops CML-CP-like disease (Koschmieder et al., 2005). Bone marrow cells from SCLITAp210BCR-ABL1 transgenic mice cultured in the absence of tetracycline were sensitive to PARPi olaparib and RAD52i Dopa, but the combination of these two inhibitors exerted a synergistic anti-leukemia effect (Figure 4A). As expected, BCR-ABL1 protein was downregulated in the absence of tetracycline (Figure 4B).

To test the effect of PARPi and RAD52i inhibition on BRCA1-deficient BCR-ABL1 leukemia in genetic settings, we generated mice (at least three mice per group) (results show mean ± SD number of BCR-ABL1-dependent colonies [tet− – tet+]; **p < 0.02 compared with BA and **p < 0.05 compared with BA;Parp1−− and BA;Rad52−−; and (D) Kaplan-Meier survival curves of BA;Parp1−−;Rad52−− (n = 19), BA;Parp1−−, BA;Rad52−−, and BA (n = 63) mice. See also Figure S4.)

**Figure 4. The Effect of Simultaneous Inactivation of RAD52 and PARPi on Leukemogenesis in BRCA1-Deficient BCR-ABL1 Transgenic Mice**

(A) Sensitivity of clonogenic bone marrow cells from SCLITAp210BCR-ABL1 mice (n = 3) to 5 µM olaparib (Ola) and/or 20 µM 6-OH-dopa (Dopa) in the absence of tetracycline. Results represent mean percentage of clonogenic cells ± SD from three mice in triplicates; *p < 0.001 in comparison with untreated cells using Student’s t test; **p = 0.016 compared with individual drugs using the response additivity approach. (B–D) SCLITAp210BCR-ABL1;Parp1−−;Rad52−−; Rad52−−/C0, SCLITAp210BCR-ABL1;Parp1−−; Rad52−−/C0, SCLITAp210BCR-ABL1;Rad52−−/C0, and SCLITAp210BCR-ABL1 BA mice were assayed for (B) expression of BRCA1, BCR-ABL1, and actin proteins in the absence (−) or presence (+) of tetracycline; (C) clonogenic activity of bone marrow cells from BA;Parp1−−;Rad52−−, BA;Parp1−−, BA;Rad52−−, and BA mice (at least three mice per group) (results show mean ± SD number of BCR-ABL1-dependent colonies [tet− – tet+]; **p < 0.02 compared with BA and **p < 0.05 compared with BA;Parp1−− and BA;Rad52−−; and (D) Kaplan-Meier survival curves of BA;Parp1−−;Rad52−− (n = 19), BA;Parp1−−, BA;Rad52−−, and BA (n = 63) mice. See also Figure S4.
lethal disease in SCLtTA/p210BCR-ABL1/Parp1/C0 mice (120.0 ± 8.4 days) and SCLtTA/p210BCR-ABL1/Rad52/C0 mice (99.9 ± 17.6 days) was significantly prolonged in comparison with SCLtTA/p210BCR-ABL1 animals (p < 0.01 and p = 0.03, respectively). Importantly, mean survival time of SCLtTA/p210BCR-ABL1/Parp1−/−Rad52−/− mice (148.4 ± 11.7 days) was significantly prolonged in comparison with SCLtTA/p210BCR-ABL1/Parp1−/− (p < 0.04) and SCLtTA/p210BCR-ABL1/Rad52−/− (p < 0.05) animals. Remarkably, 33% of SCLtTA/p210BCR-ABL1/Parp1−/−Rad52−/− mice did not develop detectable leukemia during 200 days of observation, which demonstrates the ability of dual PARP1 and RAD52 inhibition to actually prevent onset of the disease.

To test the anti-tumor effect of PARPi+RAD52i against disseminated tumor, previously selected BRCA-deficient AML patient cells were injected into immunodeficient NGS mice (Cramer-Morales et al., 2013; Nieborowska-Skorska et al., 2017). Upon engraftment, the mice were treated with PARPi talazoparib, RAD52i D-I03, or talazoparib + D-I03 for 7 consecutive days. PARPi and RAD52i when used individually had diminished percentages of hCD45+ leukemia cells in peripheral blood leukocytes 3 weeks after leukemia injection; *p ≤ 0.005 and **p < 0.001 in comparison with Control and single-compound treatment, respectively, using Student’s t test. (A) Representative plots of peripheral blood leukocyte (PBL) from treated mice; mean percentage ± SD of human CD45+ AML cells in peripheral blood leukocytes 3 weeks after leukemia injection; *p < 0.05 compared with individual agents.

Although mice treated with talazoparib and F79 aptamer individually had diminished percentages of hCD45+ leukemia cells in peripheral blood, the combination of talazoparib and F79 aptamer caused an additional 2.6- to 3.8-fold reduction in the number of leukemia cells (Figure 5A). Untreated mice succumbed to leukemia after 37.3 ± 2.9 days, whereas those treated with F79 aptamer or talazoparib survived for 57.2 ± 3.5 days (p < 0.002) and 70.3 ± 2.5 days (p < 0.001), respectively (Figure 5B). Combinatorial treatment of talazoparib + F79 aptamer prolonged survival of leukemic mice to 116.7 ± 9.1 days (p < 0.001 compared with individual treatment).

In addition, we tested the effect of PARPi talazoparib combined with RAD52i D-I03 against BRCA1-deficient MDA-MB-436 and BRCA1-proficient MDA-MB-436 +BRCA1 carcinoma in nu/nu mice. Pharmacokinetic and toxicity studies indicated that maximal tolerated dose of D-I03 is ≥50 mg/kg, and t1/2 was 23.4 ± 17.4 hr, resulting in >1 μM maximal concentration in peripheral blood. Mice bearing subcutaneous tumors were treated with talazoparib, D-I03, or talazoparib + D-I03 for 7 consecutive days. PARPi and RAD52i when used individually
In BRCA-deficient cells, inhibition of PARP1 and RAD52 exerted stronger effect against BRCA-deficient tumors in vivo compared with individual inhibitors targeting PARP1 or RAD52. Single-cell RNA-sequencing (RNA-seq) analyses of untreated tumors revealed clonal heterogeneity in expression of DSB repair genes in tumor initiating cells and progenitor cell populations (Figure S6), suggesting that PARPi-resistant clones may be already present at diagnosis. However, at least some of these clones should be sensitive to the inhibitors such as RAD52i, which targets unrelated DSB repair pathway. In concordance, olaparib-resistant *BRCA1*-mutated/S3BP1-deficient SUM149PT breast carcinoma cells, olaparib-resistant *BRCA1*-mutated MDA-MB-436 breast carcinoma cells, and talazoparib-resistant RAD54–/– Nalm6 leukemia cells were sensitive to RAD52i Dopa (Figure S7).

**DISCUSSION**

Patients with BRCA-deficient tumors who initially respond to PARPi often develop resistance, leading to cancer relapse (Lord and Ashworth, 2013). In addition, PARPi increase the probability of accumulation of additional chromosomal translocations in BRCA-deficient cells (Bunting et al., 2010), which may facilitate disease progression. Altogether, because PARPi have become widely used to treat BRCA pathway-deficient tumors, there is an urgent need to develop novel strategies to prevent development of drug resistance, for example by killing tumor cells more rapidly and robustly before resistance mechanisms are selected for.

PARPi-mediated synthetic lethality in BRCA pathway-deficient cells is associated with increased number of lethal DSBs (Bryant et al., 2005; Farmer et al., 2005). In BRCA-deficient cells, some of these DSBs can still be repaired by the alternative mechanism of homologous recombination. In repression of PARPi-mediated recombination and repair and that RAD52 acetylation is required for sustained RAD51 colocalization at DSBs (Keskin et al., 2014; Sotiriou et al., 2016; Yasuda et al., 2018). We and others have shown that inhibition of RAD52 DNA binding activity exerted synthetic lethality in BRCA-deficient carcinomas and leukemia cells without affecting normal cells and tissues (Cramer-Morales et al., 2013; Feng et al., 2011). Therefore, RAD52 has proved to be an important target for therapeutic intervention in BRCA-deficient tumors.

In conclusion, simultaneous genetic and pharmacological inhibition of PARP1 and RAD52 exerted stronger effect against normal tissues and organs (Figure S5; Table S1). In BRCA-deficient solid tumor cells and leukemia cells. At the same time, BRCA-proficient tumor cells and normal cells and tissues were not significantly affected by the combinatorial treatment.

PARPi resistance is a common phenomenon (Sonnenblick et al., 2015), and PARPi+RAD52i-mediated dual synthetic lethality is an aggressive approach, which may lead to more effective elimination of BRCA-deficient malignant cells, thus limiting or preventing time-dependent emergence of preexisting and/or drug-induced resistant clones. In concordance, our data indicate that inhibition of RAD52 will improve therapeutic outcome of BRCA-deficient malignancies treated with PARPi while causing minimal toxicity to normal cells and tissues.

**EXPERIMENTAL PROCEDURES**

Further details about experimental procedures used in this work can be found in Supporting Information.

**Primary Cells**

FLT3(TI)-positive AML samples (bone marrow or blood) were obtained during routine investigation at the Department of Internal Medicine I, Division of Hematology and Hemostaseology, Medical University of Vienna. Isolated mononuclear cells were stored as intact cells in a biobank in liquid nitrogen until used for in vitro experiments and/or in xenotransplantation experiments. BCR-ABL1-positive CML, AML1-ETO-positive AML, IGH/MYC-positive BL primary samples, and BRCA1/2-deficient and BRCA1/2-proficient AML and t-MDS samples have been previously characterized (Maifreda et al., 2017a; Nieborska-Skorska et al., 2017). Samples of normal hematopoietic cells were purchased from Cambrex Bio Science. Lin–/CD34+ cells were obtained from mononuclear fractions by magnetic sorting using the EasySep negative selection human progenitor cell enrichment cocktail followed by human CD34+ positive selection cocktail (StemCell Technologies) as described previously (Nieborska-Skorska et al., 2017).

**Transgenic and Knockout Mice**

RAD52–/– mice were obtained from Dr. M. Jasin (Memorial Sloan Kettering Cancer Center), and Parp1–/– mice and SCLITAP210BCR-ABL1 mice were used previously (Boiton-Gillespie et al., 2013; Nieborska-Skorska et al., 2017). RAD52–/– were cross-bred with Parp1–/– mice to generate Parp1–/–; Rad52–/– mice. These animals were cross-bred with SCLITAP210BCR-ABL1 mice to generate SCLITAP210BCR-ABL1;Parp1–/–; Rad52–/– mice. Transgenic and knockout mice were identified by PCR of tail snap DNA. Mice were provided with drinking water supplemented with 0.5 g/L 6-OH-dopa (Chandramouly et al., 2015), and D-I03 (Huang et al., 2016), DNR (Selleckchem), and BCR-ABL1 tyrosine kinase.
inhibitor imatinib (Selleckchem) were added to indicated cells for 3–5 days. Cell count and viability were determined by trypan blue exclusion. Clonogenic activity was assessed 7 days after plating of treated cells. Cell death and γ-H2AX staining were examined by flow cytometry after staining with Fixable Viability Dye eFluor 780 (eBioscience) and Alexa Fluor 647 anti-γ-H2AX (BD Biosciences) as described previously (Maifrede et al., 2017b). For long-term experiments, fresh inhibitors were added every 3–4 days, and cells were expanded in fresh medium every 7 days.

**Clonogenic Assay**

Freshly harvested Lin− murine bone marrow cells were plated in serum-free MethoCult-SF HA236 (StemCell Technologies) supplemented with TET System Approved Fetal Bovine Serum (Takara Bio USA) with and without 10 μg/mL tetracycline hydrochloride in the presence of a threshold concentrations (0.1 U/mL) of recombinant murine IL-3, IL-6, and SCF. Colonies were scored after 5–7 days. BRCA1-deficient and BRCA1-proficient counterpart MDA-MB-436 cells, cultured in RPMI + 10% fetal bovine serum (FBS), were seeded on day 0 in triplicate at 5,000 cells/well. On day 1, the cells were treated with 0, 10, 20, and 50 nM olaparib in the absence or presence of 1 μM D-I03. On day 3, the treatment was repeated with media refreshment. Cells were counted on day 4 using trypan blue exclusion and were immediately plated at a density of 500 cells/well in a six-well plate, in RPMI + 10% FBS. After 2 weeks, cell survival was determined using the clonogenic assay. For this purpose, the colonies were fixed and stained with 0.05% of 10 mg/mL ethidium bromide in 50% ethanol and visualized using an Alphaimager 3400 gel documentation system.

**In Vivo Treatment**

NSG mice (10- to 12-week-old males and females) were total-body irradiated (250 cGy) and inoculated intravenously (i.v.) with 1 × 10^6 BRCA-deficient AML primary leukemia xenograft cells. Two weeks later, mice were treated with vehicle (control), talazoparib (0.33 mg/kg/day) by oral gavage for 7 days; Nieborowska-Skorska et al., 2017), F79 aptamer (2.5 mg/kg i.v.; Cramer-Morales et al., 2013), and a combination of talazoparib + F79 aptamer. Leukemia burden was analyzed by flow cytometry 7 days after the end of treatment. Human leukemia cells were detected by anti-human CD45 antibody as described previously (Nieborowska-Skorska et al., 2017). Median survival time was determined. Nude mice (10- to 12-week-old females) were injected subcutaneously (s.c.) with 1 × 10^6 BRCA1-deficient MDA-MB-436 cells or BRCA1-reconstituted counterparts. Once tumors reached a volume of 100 mm^3, mice were treated with vehicle (control), talazoparib (0.33 mg/kg/day by oral gavage; Nieborowska-Skorska et al., 2017), D-I03 (50 mg/kg/day intraperitoneally [i.p.],) and a combination of talazoparib and D-I03 for 7 days. Tumors were measured weekly, and tumor volumes were calculated using the ellipsoid volume formula (V = length × width × height).

**Statistical Analysis**

Data are expressed as mean ± SD and were compared using the unpaired Student’s t test; p values less than 0.05 were considered to indicate statistical significance. Mean survival time of the mice ± SE was calculated using Kaplan-Meier log-rank survival analysis. The response additivity approach was used to study the synergistic effects (Slinker, 1996). This approach shows a positive drug combination effect when the observed combination effect (CI) is greater than the expected additive effect by the sum of the individual effects (E_a + E_b). The combination index (CI) was calculated as CI = (E_a + E_b)/E_ab. The p value for the possible synergistic effect is given by the significance of the interaction effect in a factorial ANOVA of the individual and combination effects.

**Study Approval**

Studies involving primary leukemias were approved by the ethics committee of the Medical University of Vienna and Temple University Lewis Katz School of Medicine and met all requirements of the Declaration of Helsinki. Animal studies were approved by the Temple University Institutional Animal Care and Use Committee.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures, seven figures, and one table and can be found with this article online at https://doi.org/10.1016/j.celrep.2018.05.034.

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**AUTHORS CONTRIBUTIONS**

Conceptualization, T.S.; Methodology, T.S., E.B.-G., and A.J.B.; Software, J.L. and K.M.-W.; Validation, E.B.-G. and K.S.-R., Formal Analysis, H.Z.; Investigation, K.S.-R., E.B.-G., Y.D., S.L., M.S., M.N.-S., K.H., E.A.B., and M. Moore; Resources, A.J.B., P.V., S.B., and R.B.; Writing – Original Draft, T.S.; Writing – Review & Editing, T.S., A.V.M., and N.J.; Visualization, K.S.-R., E.B.-G., Y.D., K.H., E.A.B., J.L., and K.M.-W.; Supervision, M. Muschen, N.J., M.A.W., A.V.M., and T.S.; Project Administration, T.S.; Funding Acquisition, K.S., N.J., A.V.M., and T.S.

**DECLARATION OF INTERESTS**

The authors declare no competing interests.

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Supplemental Information

Simultaneous Targeting of PARP1 and RAD52 Triggers Dual Synthetic Lethality in BRCA-Deficient Tumor Cells

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Figure S1 related to Figure 1. Prognostic value of the expression levels of RAD52 and/or BRCA1, BRCA2, PALB2.

(A) Kaplan-Meier estimates of relapse-free survival (RFS) for patients with pediatric B-precursor ALL based on higher or lower than the median gene expression values for RAD52 mRNA expression level; p values were calculated from the logrank test. (B) Survival of MDS patients displaying downregulation of BRCA2 mRNA (25 percentile) combined with downregulation (25 percentile) or upregulation (75 percentile) of RAD52 mRNA. (C) Kaplan-Meier estimates of RFS for patients with pediatric B-precursor ALL based on simultaneous higher or lower than the median mRNA expression values of the indicated pairs of genes. p values were calculated from the logrank test. Analyses were performed using previously published arrays: (A, C) (Kang et al., 2010), and (B) PROGene V2 – Pan Cancer Prognostic Database (http://watson.compbio.iupui.edu/chirayu/proggene/database/?url=proggene).
Figure S2 related to Figure 2. Additional biological effects of PARP1i and RAD52i. (A) Indicated cells were left untreated (-) or treated with 5μM olaparib (O), 10μM Dopa (D), or olaparib + Dopa (O+D). After 24 hours whole cell lysates were analyzed by Western blotting to detect RAD52, PARP1, and actin (loading control). Results are representative of 2-3 independent assays. (B) Murine bone marrow cells expressing MLL-AF9 were treated with the indicated concentrations of olaparib in the absence (vehicle) or presence of 2.5 μM Dopa for 72 hrs followed by Trypan blue exclusion test. Results represent mean ± SD of living cells from 2 independent experiments. (C-E) Nalm-6 parental (grey bars) and Nalm-6 RAD54-/- (black bars) cells were left untreated (Control) and treated with 0.3 μM olaparib, 5 μM Dopa, and olaparib + Dopa for 24 h (C, D) and 48 h (E). DSBs were detected by (C) neutral comet assay and (D) γ-H2AX immunofluorescence. Results represent mean % of tail DNA ± SD from 100-150 cells and mean % increase of γ-H2AX in cells ± SD from triplicate experiment. (E) Mean % of trypan blue-negative living cells ± SD relative to untreated counterparts from triplicate experiment. (F) Nalm-6 RAD54-/- cells were left untreated (black) and treated with 5 nM talazoparib (blue), 5 μM Dopa (green), and talazoparib + Dopa (red) for 35 days. Mean cumulative number ± SD of trypan blue-negative living cells. (G) BRCA2-deficient BL-derived B-cell lines Mutu and Raji, and control LCL1 and LCL2 cells were treated with 2.5 μM olaparib and/or 5 μM Dopa for 72 h. Results represent mean % of trypan blue-negative living cells ± SD relative to untreated counterparts from triplicate experiments. Results are from 3 independent experiments. * p<0.05 in comparison to the cells treated with individual drugs (Olaparib, Dopa) using Student t test.
Figure S3 related to Figure 2. Ectopic expression of DNA binding-defective RAD52(F79A) mutant (Kurumizaka et al., 1999) or catalytically inactive PARP1(E988K) mutant (Rolli et al., 1997) inhibited RAD52 foci formation and downregulated protein PARylation, respectively. (A) BRCA1-deficient leukemia cells expressing GFP-RAD52 fusion protein and RAD52(F79A) or RAD52(wt) were treated with 3μg/ml cisplatin for 16 hours. Results represent mean number ± SD of GFP-RAD52 foci/nucleus in 25 cells/group containing >5 foci; *p<0.05 using Student t test. Representative nuclei with foci are shown. (B) BRCA1-deficient leukemia cells expressing PARP1(E988K) or PARP1(wt) were left untreated (black line) or treated with 10Gy (red line). Protein PARylation was determined by flow cytometry. Representative PARylation plots are shown. Results represent mean +/- SD increase of protein PARylation after irradiation (triplicate data); *p<0.05 when compared to PARP1(E988K) using Student t test.
Figure S4 related to Figure 4. Characterization of Parp1-/-;Rad52-/- double-knockout mice and BCR-ABL1-induced leukemias. (A) Peripheral blood parameters: number of white blood cells (WBC)/μL, and % of differentiated myeloid cells and B cells. (B) Spleen parameters: spleen weight, and % of differentiated myeloid cells and B cells. (C) Bone marrow parameters: % of myeloid cells and B cells, % of Lin-c-Kit+ stem/progenitor cells, number of colonies per 5,000 BMCs formed by untreated cells and these treated with 1 Gy X-ray, 0.01 μg/ml daunorubicin or 0.4 μg/ml cisplatin, and % of LSK cells with DSBs detected by γ-H2AX immunofluorescence as described before (Bolton-Gillespie et al., 2013) 24 hrs after LSK cells were treated with 1 Gy, 0.01 μg/ml daunorubicin, or were left untreated. Results in A-C represent mean ± SD from 3-5 mice/group. (D) Representative hematoxylin-eosin stained tissue sections magnified 100x (bone marrow and spleen) and 40x (liver, kidney, brain, lung, heart, and intestine). (E-I) Hematological parameters in mice succumbed to the disease: (E) spleen weight, (F) number of peripheral blood mononuclear cells (PBMC)/μL, and (G-I) percent of Gr1+/Mac1+ cells in bone marrow (BM), spleen (SP) and peripheral blood (PB).
Figure S5 related to Figure 5. Combination of talazoparib + D-I03 was not toxic to major internal organs. C57Bl/6 mice were treated with vehicle (Control) and talazoparib + D-I03 as described in Figure 5C, D. Tissue samples were harvested one day after the end of treatment, fixed in formalin, and embedded in paraffin for microscopic evaluation. Control: H&E sections of: A) kidney (20x), B) liver (20x), C) tibia bone marrow (20x), D) heart (20x), E) intestine (20x), F) spleen (20x), and G) lung (20x). Talazoparib + D-I03: H&E sections of: A) kidney (20x), B) liver (20x), C) tibia bone marrow (20x), D) heart (10x), E) intestine (20x), F) spleen (20x), and G) lung (20x). Results are representative of 5 mice/group.
Figure S6 related to Figure 5. Heterogeneous gene expression patterns of DSB repair genes in single tumor cells identified by single-cell RNA sequencing (scRNAseq). Heatmaps show gene expression values as Z-scores calculated on log2-transformed (RPKM+1) values. (A, B) Expression heatmap for DNA repair genes in 34 cells (A), and in the replicate batch of 43 cells (B) from patient-derived xenograft (PDX) of a lung adenocarcinoma patient (GSE69405) (Kim et al., 2015). (C) Gene expression profiles in BCR-ABL1-positive CML stem cells (n = 477) obtained from 18 patients with chronic-phase CML at diagnosis (GSE76312) (Giustacchini et al., 2017). (D) Unsupervised hierarchical clustering of selected DNA repair genes for 96 cells from the c-Kit+ leukemic splenocytes of two independent Flt3ITD/ITD;Dnmt3afl/flMxCre AML mice (GSE77847) (Meyer et al., 2016); yellow, macrophage/dendritic/neutrophil precursors; blue, hematopoietic stem/progenitor-like cells.
Figure S7 related to Figure 5. PARP1i-resistant tumor cells are sensitive to RAD52i Dopa. (A) Nalm6 RAD54−/− leukemia cells and BRCA1-mutated MDA-MB-436 breast carcinoma cells were continuously cultured in the growing concentrations of talazoparib (Tala) and olaparib (Ola) until they were resistant to 10nM and 10 μM of the inhibitors, respectively. PARP1i resistant (R) and sensitive (S) cells were left untreated or treated with the indicated concentrations of Tala or Ola and/or Dopa on day 0 and +2. (B) BRCA1-mutated SUM149PT breast carcinoma cells expressing endogenous 53BP1 (53BP1+) and these with downregulated 53BP1 (53BP1-) (Western blots, left panel) were left untreated or treated with the indicated concentrations of Ola and/or Dopa on day 0 and +2. Living cells were counted in trypan blue on day +5. Results represent mean ± SD number of living cells from triplicate experiments; *p<0.05 when compared to corresponding untreated cells, **p<0.05 when compared to the cells treated with Ola/Tala or Dopa.
Table S1 related to Figure 5. Talazoparib + D-I03 were not toxic for hematopoietic organs in C57Bl/6 mice.

| Parameter               | Control               | talazoparib+D-I03   |
|-------------------------|-----------------------|---------------------|
| **Peripheral blood**    |                       |                     |
| WBC 10⁹/μL              | 6.644±1.877           | 7.020±2.684         |
| NE 10⁹/μL               | 1.366±0.699           | 1.402±0.545         |
| LY 10⁹/μL               | 4.952±1.051           | 5.236±1.999         |
| MO 10⁹/μL               | 0.198±0.086           | 0.220±0.129         |
| EO 10⁹/μL               | 0.094±0.104           | 0.116±0.127         |
| BA 10⁹/μL               | 0.032±0.036           | 0.050±0.065         |
| RBC 10⁹/μL              | 9.114±0.646           | 8.640±0.630         |
| HB g/dL                 | 13.240±0.942          | 12.660±1.555        |
| HCT %                   | 44.540±3.370          | 43.400±3.279        |
| MCV fl                  | 48.860±0.607          | 50.140±0.598        |
| MCH pg                  | 14.500±0.339          | 14.620±0.841        |
| PLT 10⁹/μL              | 641.000±204.808       | 825.600±413.964     |
| **Bone marrow**         |                       |                     |
| White cells/femur (x10⁶) | 11.030±4.260         | 13.000±1.823        |
| Lin cKit⁻ (x10⁶)        | 0.203±0.082           | 0.541±0.181 *       |
| Lin Sca1⁻ (x10⁶)        | 0.076±0.035           | 0.152±0.063         |
| Lin cKit⁻ Sca1⁻ (x10⁶)  | 0.015±0.006           | 0.065±0.030 *       |
| **Spleen weight (g)**   | 0.077±0.008           | 0.072±0.006         |
| **Body weight (g)**     | 19.5±1.400           | 20.74±0.422         |

C57Bl/6 mice (5 mice/group) were treated with vehicle (Control) and talazoparib [0.33mg/kg/day by oral gavage, (Nieborowska-Skorska et al., 2017b)] + D-I03 (50 mg/kg i.p. for 7 days, see Figure 5C, D). Hematological parameters were examined one day after the end of treatment. Blood was collected into heparinized syringes by cardiac puncture. Peripheral blood parameters (WBC = white blood cells, NE = neutrophils, LY = lymphocytes, MO = monocytes, EO = eosinophils, BA = basophils, RBC = red blood cells, HB = hemoglobin, HCT = hematocrit, MCV = mean red blood cell volume, MCH = mean corpuscular hemoglobin, PLT = platelets) were tested using Hemavet 950FS (Drew) as described before (Nieborowska-Skorska et al., 2017a). Lin cKit⁺, Lin Sca1⁺, and Lin cKit⁺ Sca1⁻ cells in bone marrow were counted by flow cytometry as described in Materials and Methods. *p<0.05 in comparison to Control.
**Supplemental Experimental Procedures**

**Cell lines**

The triple-negative breast cancer cell line MDA-MB-436 contains *Brca1* 5396 + 1G>A mutation in the splice donor site of exon 20 that results in a BRCT domain-truncated protein, and MDA-MB-436 cells ectopically express wild-type BRCA1 (MDA-MB-436 BRCA1+). SUM149PT breast cancer cells carrying *Brca1* 2288delT mutation and the loss of other allele were transfected with pLKO.1 lentiviral vector (Dharmacon) carrying 53BP1 shRNA (AAACGATGAAGCCAAATATCC) or scrambled. Clones with knocked down 53BP1 (shRNA) or expressing endogenous 53BP1 protein (scrambled) were selected in puromycin followed by Western blot analysis with anti-53BP1 antibody (Abcam, ab21083) and anti-lamin B (Santa Cruz) as a loading control. Capan1, a pancreatic carcinoma cell line with truncated *Breca2* (Breca2Δ51) as well as Capan1 cells in which BRCA2 expression has been restored (Capan1 BRCA2+) were obtained from Dr. Simon Powell (Feng et al., 2011). Human ovarian carcinoma cell line UWB1.289 carrying a germ-line *BRCA1* mutation within exon 11 (2594delC) and a deletion of the wild-type allele (*Breca1-null*), and UWB1.289 cells with restored BRCA1 expression (UWB1.289 BRCA1+) were described before and purchased from ATCC (DelloRusso et al., 2007). Breast cancer cell line, HCC1937, (5382insC germ-line mutation generating the truncated protein and no wild-type allele, *Breca1-null*) and cells with restored BRCA1 expression (BRCA1+) were obtained from Dr Ralph Scully (Scully et al., 1999). EUFA423 cells are derived from a Fanconi anemia patient with biallelic mutations (7691 insAT and 9901 insA) in *Breca2* that result in two different truncated forms of *Breca2*, and EUFA423 cells with restored BRCA2 expression were obtained from Dr Simon Powell (Feng et al., 2011). *Breca1*-/- (clone 17) and *Breca1*+/+ (clone 92B) murine embryonic (ES) cells and *Breca2*-/-VC8 and *Breca2*+/+ V79 hamster cell lines carrying DR-GFP reporter cassette, and *Breca2*+/+ (clone 40b) and *Breca2*-/- (clone 42E) mouse ES cell lines carrying SA-GFP reporter cassette were obtained from Drs. Maria Jasim and Jeremy Stark (Moynahan et al., 2001; Saeki et al., 2006; Stark et al., 2004). Nalm-6 parental cells and RAD54/-/- isogenic cells were purchased from Horizon (Cambridge, UK). Burkitt lymphoma-derived Epstein-Barr virus (EBV)-positive B-cell lines Mutu and Raji and EBV-immortalized lymphoblastoid cell lines (LCLs) from healthy donors were described before (Maifrede et al., 2017).

**Mice genotyping**

DNA isolation and purification from mice tails were performed using the REDExtract-N-Amp Tissue PCR Kit (Sigma-Aldrich). Genotyping for the SCLtTA and p210BCR-ABL1 transgenes and *Parp1* was performed using transgene/knockout-specific primers (Operon) and 2X GoTaq polymerase Master Mix (Promega). *BCR-ABL1*-specific primers (forward: 5'-GAGCGGTAGAGTGAGGGAAGGAA-3'; reverse: 5'-GGTACCAGAGGTGGTTTTTCAGACTG-3') amplified a 500 bp fragment using amplification conditions of 50 cycles at 94°C for 45 seconds, 55°C for 1 min, and 72°C for 1 min. SCLtTA-specific primers (*ta*: 5'-TTTCGATCTGGACATGTTGG-3'; *sl*: 5'-AGAACAGAATTCAGGGTCTTC-3') yielded a 750 bp product using amplification conditions consisting of 40 cycles at 94°C for 40 s, 60.5°C for 1 min, and 72°C for 1 min. *Parp1* specific primers: forward: 5'-CATGTTGATGGAAAGTCCC-3'; wild type reverse: 5'-CGCCAGCGTCGAGAGAAGCCA-3'; mutant reverse: 5'-CATGTTGATGGGAAAGTCCC-3'. The primers amplified a 112 bp fragment if wild type, a 350 bp fragment if *Parp1* null, and both 112 and 350 bp fragments if heterozygous using amplification conditions consisting of 35 cycles at 94°C for 1 min, 60°C for 1 min, and 72°C for 3 min. The *Rad52*-specific primers were: forward: 5'-AGCAGATACAGCGGATG-3'; wild type reverse: 5'-CACTGATACATGCCCAGC-3'; mutant reverse: 5'-CGCATGCTTCTCTACTGCT-3'. The amplification conditions consisted of 35 cycles at 93°C for 1 min, 55°C for 1 min, and 72°C for 3 min. PCR products (120 bp fragment if wild type, a 320 bp product if *Rad52* null, and both 120 and 320 bp fragments if heterozygous for *Rad52*) were run in a 1.5% agarose gel containing ethidium bromide, and visualized using the Gel Doc™ XR+ Molecular Imager™ System (Bio-Rad).

**Transfections**

All transfections were performed using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer’s instructions. MDA-MB-436 and EUFA423 cells and their BRCA1 and BRCA2 reconstituted counterparts were transfected with pLSXP-GFP-RAD52wt, pLSXP-YFP-RAD52(F79A), pMIG-mCherry-Parp1wt and pMIG-mCherry-Parp1(E988K). Double-positive cells were sorted 72 h after transfection with BD Biosciences Influx™ Sorter, cultured in MDA-MB-436 BRCA1+). SUM149PT breast cancer cells carrying DR-GFP reporter cassette, and *Breca2*+/+ (clone 40b) and *Breca2*-/- (clone 42E) mouse ES cell lines carrying SA-GFP reporter cassette were co-transfected with pCBAsec1 (encoding I-Sce1) and pDsRed (transfection efficiency control) plasmids using Lipofectamine 2000 (Invitrogen) for 12 hours as described before (Nieborowska-Skorska et al., 2017b).
Olaparib (AZD2281, Selleckchem), 6-OH-dopa, (Sigma) or vehicle (DMSO) were added immediately after removing the transfection complexes and the percentage of GFP+DsRed+ cells in DsRed+ population was detected after 60 hours by flow cytometry to assess HR and SSA repair activity.

**RAD51 foci**
Cells were treated for 24 h with 3 µg/ml cisplatin combined with 5µM olaparib (Ola) and/or 10µM 6-OH-dopa (Dopa). RAD51 nuclear foci were detected after 12 hrs as described before (Slupianek et al., 2006).

**GST-RAD52 foci**
BRCA1-deficient leukemia cells stably expressing GFP-RAD52 fusion protein (Chandramouly et al., 2015) were co-transfected with retroviral constructs carrying mCherry and RAD52(F79A) or RAD52(wt). GFP-RAD52 foci were counted in mCherry+ cells treated with 3µg/ml cisplatin for 16 hours as described before (Chandramouly et al., 2015).

**Protein PARylation**
Protein PARylation was examined as described before with modifications (Kunzmann et al., 2006; Sun et al., 2016). Briefly, BRCA1-deficient BCR-ABL1-32DcI3 murine hematopoietic cells were nucleofected with pMIG-mCherry-PARP1wild-type or pMIG-mCherry-PARP1(E988K) mutant expression plasmids. Seventy two hours after transfection the cells were exposed to 10 Gy irradiation followed by fixation in 2% formaldehyde and subsequent permeabilization in ice-cold 100% MeOH. After blocking with 2% BSA in PBS + 0.02% Triton-X, the cells were stained with anti-PAR antibody (10H, Enzo Life Sciences, Inc.) followed by a secondary anti-mouse antibody conjugated with AlexaFluor 647. AlexaFluor 647+/mCherry+ double-positive cells were immediately analyzed with BD Biosciences Influx™.
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