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BRCA1 and TP53 Co-Deficiency Causes a PARP-inhibitor Sensitive Erythrophilertive Neoplasm

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ABSTRACT

Mutations in the BRCA1 tumor suppressor gene, such as 5382insC (BRCA1\textsuperscript{5382insC}), give carriers an increased risk for breast, ovarian, prostate and pancreatic cancers. We have previously reported that, in mice, Brca1 deficiency in the hematopoietic system leads to pancytopenia and, as a result, early lethality. Here we explore the cellular consequences of Brca1 null and BRCA1 5382insC alleles in combination with Trp53 deficiency in the murine hematopoietic system. We find that Brca1 and Trp53 co-deficiency leads to a highly penetrant erythroproliferative disorder that is characterized by hepatosplenomegaly and expanded megakaryocyte erythroid progenitor (MEP) and immature erythroid blast populations. The expanded erythroid progenitor populations in both bone marrow and spleen have the capacity to transmit the disease into secondary mouse recipients, suggesting Brca1 and Trp53 co-deficiency provides a new murine model of hematopoietic neoplasia. This Brca1/Trp53 model replicates Poly (ADP-ribose) polymerase (PARP) inhibitor olaparib sensitivity seen in existing Brca1/Trp53 breast cancer models and has the benefits of monitoring disease progression and drug responses via peripheral blood analyses without sacrificing experimental animals. In addition, this erythroid neoplasia develops much faster than murine breast cancer, allowing for increased efficiency of future preclinical studies.
INTRODUCTION

*BRCA1* is a tumor suppressor gene that encodes for a DNA repair protein involved in double strand break (DSB) repair. Inherited mutations in *BRCA1* predispose to an autosomal dominant predisposition to breast, ovarian, and other cancers. In the absence of BRCA1, cells are unable to accurately repair DNA DSBs by homologous recombination (HR) (1-3) leading to genomic instability and cancer predisposition. *BRCA1* mutant cancer cells rely on alternative DNA-repair pathways in the absence of HR. Poly(ADP-ribose) polymerase (PARP) is an enzyme involved the single-strand break (SSB) repair pathway (4) that *BRCA1* mutant cells may rely on. This dependency leads to a synthetic lethal interaction between BRCA1 deficiency and PAPR inhibition, where increased genomic instability leads to cell death (5-7). PARP inhibitors are used to treat *BRCA1*-mutated ovarian, prostate and pancreatic cancers (8).

Knowledge of the role of BRCA1 in the hematopoietic system is lacking despite human data associating BRCA1 to hematologic malignancies. For example, *BRCA1* mutations have been linked to Fanconi Anemia (FA), an inherited bone marrow failure syndrome with hematopoietic phenotypes such as myelodysplastic syndrome, (pan)cytopenia, and acute myeloid leukemia (AML), since BRCA1 interacts with several FA proteins (9-13). *BRCA1* mutations are detected in AML patients (although at a low frequency) (14) and BRCA1 expression is decreased in chronic myelogenous leukemia (CML) cells (15, 16). There are studies that suggest patients carrying *BRCA1* mutations have shown more adverse hematologic outcomes following chemotherapy compared to *BRCA2* mutation carriers or non-carriers (17, 18). Together, these data suggest that BRCA1 is an important regulator of hematopoiesis in humans.
Prior publications by us and others identified that the conditional deletion of \( Brca1 \) leads to hematopoietic defects in mice (17, 19). We established a requirement for \( Brca1 \) in the murine hematopoietic system where hematopoietic stem cell (HSC) and progenitor cell-specific deletion of \( Brca1 \) under the control of either \( Vav1-iCre \) or \( Mx1-Cre \) transgenes led to bone marrow failure and early lethality (17). This \( Brca1 \) null phenotype was worsened by compound heterozygosity of the null allele with the humanized \( BRCA1 \) 5382\( \text{insC} \) allele \( (BRCA1^{\text{insC}}) \). The \( BRCA1 \) 5382\( \text{insC} \) mutation is one of the relatively common Ashkenazi Jewish \( BRCA1 \) founder mutations that are known to increase the risk for cancer (20). This allele is a knockin of human \( BRCA1 \) cDNA carrying the 5382\( \text{insC} \) mutation into the mouse \( Brca1 \) locus. Thus the \( BRCA1^{\text{insC}} \) allele is constitutively expressed under the control of endogenous mouse regulatory elements. Our data demonstrated that the \( BRCA1^{\text{insC}} \) allele is more detrimental to hematopoietic tissue than a simple \( Brca1 \) null allele.

In further exploration to the roles of \( Brca1 \) in hematopoeisis, we have now made the discovery that hematopoietic \( Brca1 \) deficiency in combination with \( Trp53 \) deficiency leads to an erythroid neoplasia. In mice, \( Trp53 \) germline nullizygosity predominantly leads to T-cell lymphomas at 3-5 months of age and heterozygous mice develop a greater spectrum of cancers (including lymphomas and sarcomas), but at a much later age than the homozygotes (21-23). However, \( Trp53 \) mutations have been shown to induce non-lymphoid neoplasms in combination with a variety of genetic lesions (22, 24-27). None have directly evaluated the outcomes of combined \( Brca1 \) and \( Trp53 \) deficiency in the hematopoietic system.
In this report, we characterize a Brca1/Trp53 double deficiency-associated hematopoietic neoplasia, using Brca1 null and BRCA1 5382insC mutant alleles. As was observed with Brca1 single deficiency, Brca1/Trp53 double deficiency was more detrimental to mice carrying the 5382insC allele compared to mice with only the null allele. Brca1/Trp53 double deficiency-associated erythroid neoplasia is rapid onset, highly penetrant, and can be transplanted to multiple syngenic immune-replete mice. As expected from a Brca1 deficiency-associated neoplasia, PARP inhibitor olaparib treatment attenuated disease phenotypes. This novel mouse model could allow for rapid in vivo screening for compounds of therapeutic potential, and even for the assessment of pathogenicity of BRCA1 variants of uncertain significance (VUS).
RESULTS

**Brca1** and **Trp53** double deficiency in the bone marrow leads to an erythrophilic disorder

In this study, we investigated the compound effects of **Brca1** and **Trp53** deficiencies in hematopoietic tissue. We used the Mx1-Cre system (28), where induction of interferon following injection with polynosinic:polycytidylic acid (pIpC) drives Cre-mediated recombination in hematopoietic stem and progenitor cells to generate null alleles of **Brca1** in hematopoietic tissues (**Figure 1A**). To confirm deletion of the **Brca1** floxed allele and the generation of the null allele following pIpC treatment, we tested for loss of the non-recombined, floxed allele of **Brca1** (**Supplemental Figure 1**). The amount of this allele in **Mx1-Cre;Brca1**\(^{F/F}\);**Trp53**\(^{+/−}\) spleens was significantly decreased compared to control **Brca1**\(^{F/F}\);**Trp53**\(^{+/−}\) spleens (p=0.003), confirming recombination of the conditional **Brca1** allele. As predicted, no recombination was seen in **Mx1-Cre;Brca1**\(^{F/F}\);**Trp53**\(^{+/−}\) and control **Brca1**\(^{F/F}\);**Trp53**\(^{+/−}\) brain tissue. These data confirm the hematopoietic tissue-specific deletion of **Brca1** following pIpC treatment and thus the generation of a hematopoietic tissue-specific **Brca1/Trp53** double deficiency model.

Using this model, we compared the hematopoietic phenotypes of double **Brca1/Trp53** deficiency to those of single **Brca1** deficiency. As observed in prior work (17), **Brca1** deficiency in a **Trp53** wildtype background led to early mortality and pancytopenia, including low white blood cell (WBC) and red blood cell (RBC) counts. No significant difference in survival was seen between **Mx1-Cre;Brca1**\(^{F/F}\) vs. **Mx1-Cre;Brca1**\(^{F/F}\);**Trp53**\(^{+/−}\) (median survival 11.3 vs. 10.6 weeks, respectively) (**data not shown**). Similarly, **Trp53** heterozygosity did not alter the anemia observed in **Brca1** null mice (**Figure 1C**). However, following an initial phase of low WBCs, a majority (77.8%)
of *Brca1/Trp53* double deficient mice developed elevated WBCs (at >8 weeks post initial plpC) ([Figure 1B, Supplemental Figure 2A](#)). Despite elevated “WBC” parameters via automated analysis, smears of peripheral blood from the same mice showed that the abundant nucleated cells were not leukocytes, but had, in fact, an erythroblast morphology (29) ([Figure 1D-E](#)). Indeed, abnormal RBCs and nucleated RBCs are noted to read as WBCs in automated analyses, according to the manufacture’s manual (30). All *Mx1-Cre;Brca1*F/F;*Trp53*+/− mice developed hepatosplenomegaly ([Figure 1F,G](#)). At terminal stage, the spleens of *Mx1-Cre;Brca1*F/F;*Trp53*+/− mice were ~22-fold larger than controls (7.7% vs. 0.35% spleen/body weight; 1.78 g vs. 0.09 g absolute weight) and the livers ~2-fold larger (10.35% vs. 5.0% liver/body weight; 2.4g vs. 1.1g absolute weight) than controls. Enlarged spleens were effaced without the normal red/white pulp structure ([Figure 1H-I](#)). Similar to spleens, livers of diseased mice showed mononuclear cell infiltration ([Figure 1J-K](#)).

To characterize the cell types contributing to the disease, we analyzed hematopoietic cell compartments by flow cytometry. As we had reported before (17), Brca1 deficiency alone (in *Mx1-Cre;Brca1*F/F mice) led to significant decreases in bone marrow HSC (LSK, CD150−CD48−), multipotent progenitor (MPP; CD150−CD48−LSK), common myeloid progenitor (CMP; ScaLK CD34+CD16/32low) and granulocyte/monocyte myeloid progenitor (GMP; ScaLK CD34+CD16/32high) populations ([Figure 2A](#)). *Trp53* deficiency contributed to modest increases in HSC and progenitor (MPP, CMP, and GMP) frequencies as would be expected from *Trp53* deficiency alone through its regulation of cell cycle and apoptosis (26, 31). However,
none of these frequencies increased above that of control. On the other hand, megakaryocyte/erythroid progenitor (MEP; Sca-LK+CD34−CD16/32low) compartment was massively expanded (~110-fold increase, p=0.004) in Mx1-Cre;Brca1F/F;Trp53+/− mice. Consistent with bone marrow cytometry, there was no elevation in B220+ B cells, CD3+ T cells or CD11b+ Gr1+ myeloid cells in peripheral blood (**Supplemental Figure 2B-D**). Further analysis of peripheral blood, spleen, and bone marrow from diseased Mx1-Cre;Brca1F/F;Trp53+/− mice showed increases in SSCloCD45− gated cells (**Figure 2B**) which previous studies have shown to correspond with a population that lacks myeloid- or lymphoid-associated surface antigens and represents nucleated erythroid cells based on high expression of surface transferrin receptor (CD71) and glycoprotein A (Ter119) (32). Indeed the CD71 positive erythroid cells were elevated in all hematopoietic compartments tested. We further analyzed this expanded erythroid cell population using Ter119 - a marker for late stages of erythropoiesis (33) and activated cellular stem cell factor receptor c-kit - a marker for early hematopoietic developmental stages (34). A majority of CD71 cells (~80% in bone marrow and spleen) also expressed the c-kit marker suggesting the immature nature of the erythroid cells (**Supplemental Figure 2E**). Consistently, early-(CD71+ Ter119+) and mid-(CD71+ Ter119+) erythroid progenitor cell frequencies in Mx1-Cre;Brca1F/F;Trp53+/− mice were elevated compared to control and Mx1-Cre;Brca1F/F mice (**Figure 2C-D**). Despite immature erythroid cell hyperplasia, no corresponding increases were seen in CD71+ Ter119+ late erythroid stage (**Figure 2E**). Due to the block in differentiation to mature RBCs, a positive feedback cycle of inefficient erythropoiesis and anemia persists. In contrast, Mx1-Cre;Brca1F/F mice showed no significant differences from control in
frequencies for any of the populations tested. Of the tissues tested, the spleen had the highest frequency of erythroid blast cells suggesting the spleen as the major site of housing for the hyperplasia. Together, these data suggest this disease is characterized by a dysregulated erythroid, rather than lymphoid hematopathology.

Consistent with this pathology being an erythroid lineage proliferation, we noted a significant downregulation of CD45 on c-kit+, CD71+, and Ter119+ cells but not on B cells, T cells or Mac1+ myeloid cells in spleen and bone marrow of diseased Mx1-Cre;Brca1F/F;Trp53+/- mice (Figure 2F and data not shown). Under conditions of normal development, CD45 is expressed in all hematopoietic cells except terminally differentiated red blood cells. In addition, aberrant downregulation of CD45 has been reported in leukemia cells in mice (26). That we saw this aberrant loss of expression of CD45 selectively in the erythroid lineage further supports the erythroid origin of this hyperproliferation.

We confirmed that these phenotypes were not due to proliferative effects of plpC-induced interferon by using Vav1-iCre to drive recombination in embryonic and adult HSCs and progenitors independent of plpC (35). Vavi-Cre;Brca1F/F;Trp53+/- mice also developed enlarged spleens with abnormal histopathology (Supplemental Figure 3A-C), elevated WBC reads (Supplemental Figure 3D), and increased bone marrow MEP frequencies (Supplemental Figure 3E).

Erythroproliferative disease in Brca1/Trp53 deficient mice with the BRCA1 5382insC mutation
The oncogenic BRCA1 5382insC mutation was evaluated in Brca1/Trp53 deficiency to determine if it altered erythropoietic abnormalities as it had done with the pancytopenia in the setting of Brca1 deficiency. We compared Mx1-Cre;Brca1^{F/insC};Trp53^{-/} mice to Mx1-Cre;Brca1^{F/F};Trp53^{-/} mice and controls. These mice were in a 129S7/C57BL6 mixed background. The median survival of Mx1-Cre;Brca1^{F/F} and Mx1-Cre;Brca1^{F/F};Trp53^{-/} mice (12.3 and 12.0 weeks post initial pIpC induction respectively) (Figure 3A) was comparable to that of prior experiments using mice in a pure C57BL/6 background (11.3 and 10.6 weeks respectively). Mx1-Cre;Brca1^{F/insC} mice showed earlier lethality with a median survival of 3.3 weeks (Figure 3B). Although added Trp53 deficiency did not alter survival compared to simple Brca1 nullizygosity, it did extend the survival of mice carrying BRCA1^{insC} allele (median survival Mx1-Cre;Brca1^{F/insC};Trp53^{-/}, 6.7 weeks compared to Mx1-Cre;Brca1^{F/insC}, p=0.0392) (Figure 3B). Similarly to Mx1-Cre;Brca1^{F/F};Trp53^{-/} mice, Mx1-Cre;Brca1^{F/insC};Trp53^{-/} mice developed elevated WBC reads, indicative of the abberent presence of erythroid blasts, following a period of low WBC reads (Figure 3C). This peripheral blood erythroblast phenotype was slightly more penetrant in Mx1-Cre;Brca1^{F/insC};Trp53^{-/} mice (5/11, 45.45%) compared to Mx1-Cre;Brca1^{F/F};Trp53^{-/} mice (4/11, 36.4%) and occurred with significantly shorter latency (avg. time to elevated WBC reads, 8 vs. 10 weeks, respectively. p=0.0069) (Figure 3C, Supplemental Figure 4A-B). Regardless of Trp53 status, all Brca1 deficient mice displayed low red blood cell counts (Figure 3D). Mx1-Cre;Brca1^{F/insC};Trp53^{-/} mice also showed abnormal expansion of erythroid lineage cells in peripheral blood (Figure 3E), bone marrow (Figure 3F) and spleen (Figure 3G-H), as well as hepatosplenomegaly (Supplemental Figure 4C-D). The expansions in the
bone marrow MEP compartments (~32 fold increase in $Mx1$-$\text{Cre;Brca1}^{F/\text{insC};Trp53^{+/+}}$ and ~20 fold increase in $Mx1$-$\text{Cre;Brca1}^{F/F;Trp53^{+/+}}$) were not significant possibly due to small sample size. Enlarged spleens were effaced (Supplemental Figure 4E-G) and liver showed mononuclear cell infiltration (Supplemental Figure 4H-J). There were no elevations in B cell, T cell or myeloid cell frequencies in spleen (Supplemental Figure 4K). These phenotypes were not different from those of $Mx1$-$\text{Cre;Brca1}^{F/F;Trp53^{+/+}}$ mice. Therefore, although the disease developed significantly faster in the presence of the 5382insC allele compared to the null allele only, we conclude that both genotypes result in the same disease.

**Loss of Trp53 heterozygosity (LOH) occurs in Brca1 deficient hematopoietic cells**

In patients with inherited TP53 mutations and mouse models of cancer with heterozygous germline Trp53 deficiency such as $\text{Brca1}$-deficient breast cancer models, the remaining wildtype allele is lost during tumorigenesis (23, 36-40). While interrogating the state of the genome in our $Mx1$-$\text{Cre;Brca1}^{F/F;Trp53^{+/+}}$ mice with whole exome sequencing (WES) of spleen and brain tissue we determined if the normal Trp53 allele in the Trp53 heterozygous knockout mice was intact. In enlarged $Mx1$-$\text{Cre;Brca1}^{F/F;Trp53^{+/+}}$ spleens, we found there was loss of heterozygosity in the region deleted in the Trp53 null allele as compared to spleens from Cre negative control $\text{Brca1}^{F/F;Trp53^{+/+}}$ mice (Supplemental Figure 5A). In contrast, brain tissue (where there was no expected Cre mediated deletion of $\text{Brca1}$) from the same mice did not show LOH. Thus the LOH of Trp53 was dependent on the conditional deletion of $\text{Brca1}$ in hematopoietic tissue. These data were confirmed with direct genotyping as well
(Supplemental Figure 5B). These data show that although there initially was a Trp53 heterozygous background, loss of the normal Trp53 allele occurred following Brca1 deletion and is part of the erythroproliferative process. Although we searched for other genetic alterations including single nucleotide polymorphisms (SNPs), insertions and deletions (InDels), structural variants (SVs), and copy number variants (CNVs), no other relevant alterations were found.

The erythroproliferative disease in Brca1 and Trp53 deficient mice is neoplastic

Expansion of erythroid progenitor cells and impaired erythroid terminal differentiation are considered hallmarks of human Acute Erythroid Leukemia (AEL) (41). Additionally, the erythroid phenotypes of our mice are reminiscent of those described in established murine models of acute erythroleukemia, including anemia, hepatosplenomegaly, infiltration of erythroblasts, and expansion of erythroid progenitors suggesting that this too is a murine erythroleukemia (41).

To characterize the leukemogenicity of the Brca1 and Trp53 deficiency-associated hematopoietic neoplasm and to identify leukemia-initiating cells (LICs) we carried out transplantation experiments. Two million unfractionated bone marrow cells from diseased Mx1-Cre;Brca1F/F;Trp53+/− mice together with 200,000 wildtype congenic CD45.2 support cells were transplanted into lethally irradiated wildtype CD45.1 recipient mice. There was a requirement for support bone marrow due to the necessity of Brca1 for the survival of HSCs (17). All recipients of unfractionated (UF) bone marrow developed the disease as indicated by splenomegaly, high WBC reads (indicative of erythroblasts in peripheral blood), and elevated erythroblast frequencies (CD71+c-kit+) in
bone marrow and spleen (Figure 4A, D; Supplemental Figure 6A, B). Elevated erythroblast frequencies in recipient bone marrow and spleen were comparable to those seen in the original \textit{Mx1-Cre;Brca1^{F/F};Trp53^{+/-}} mice, with the highest frequency seen in spleen (Supplemental Figure 6B, reference Figure 2C). The disease could be serially transplanted into secondary recipients (Figure 4A). As has been reported previously in murine erythroleukemia models (25), the disease developed significantly faster in secondary recipients compared to primary recipients (median survival, primary vs. secondary recipients, 5.7 vs. 3.0 weeks post-transplant. \textit{p}=0.0001). These transplant data demonstrate that the bone marrow cells of the diseased mice have transformed, and support the classification of this erythroblastic proliferation as a bona fide neoplastic disease.

To begin to identify the LICs, we sorted erythroblasts (CD71^+c-kit^+) from diseased \textit{Mx1-Cre;Brca1^{F/F};Trp53^{+/-}} mice and tested their ability to propagate disease. Twenty thousand CD71 and c-kit positive bone marrow cells from diseased \textit{Mx1-Cre;Brca1^{F/F};Trp53^{+/-}} mice initiated disease in all wildtype lethally irradiated recipients. Serial transplantation of disease with CD71^+ and c-kit^+ cells was also successful (Figure 4B, D; Supplemental Figure 6A, B). Thus, this CD71/c-kit positive population contains LICs. The capacity to propagate the disease through CD71^+ immature erythroblasts has been reported in another mouse model of erythroleukemia (25). Tsuruta-Kishino et al. showed that \textit{Jak2} mutant, \textit{Trp53} deficient model of erythroid leukemia was able to use 50,000 sorted immature erythroid cells (CD71^+Ter119^+) from bone marrow, along with 200,000 wildtype unfractionated support bone marrow, to consistently transmit the disease. Interestingly, in our model we found that 20,000 CD71 single positive cells
were less robust in transmitting disease compared to an equal number of CD71 and c-kit double positive cells as evidenced by a prolonged survival of 10.1 weeks (Figure 4B) and only 50% disease penetrance (Figure 4D, Supplemental Figure 6A, B). Due to the paucity of c-kit single positive cells, we were only able to successfully transplant one mouse with c-kit+ cells and this mouse did not display any disease phenotypes. In summary, we have found that the leukemia initiating capacity in *Mx1-Cre;Brca1<sup>F/F</sup>;Trp53<sup>+/−</sup>* mice is enriched in the CD71 and c-kit double positive cell population. The c-kit marker is expressed in leukemic blasts (26, 42), and here it helped select for a subpopulation of CD71+ cells with increased leukemia initiating capacity.

Similar to bone marrow cells, spleen cells from diseased *Mx1-Cre;Brca1<sup>F/F</sup>;Trp53<sup>+/−</sup>* mice transplanted the disease. Five million unsorted splenocytes with 200,000 wildtype support bone marrow cells was readily transplanted into lethally irradiated wildtype recipients. As in bone marrow transplantation, there was a necessity for support bone marrow due to the HSC requirement for *Brca1*. All recipients died with splenomegaly, elevated WBC reads, anemia, and elevated erythroblasts by four weeks of age (Figure 4C, D; Supplemental Figure 6C-E). As seen with bone marrow, 20,000 CD71 and c-kit double positive cells from diseased *Mx1-Cre;Brca1<sup>F/F</sup>;Trp53<sup>+/−</sup>* spleens were capable of transmitting the disease to lethally irradiated wildtype recipients with full penetrance. In contrast, none of the mice that received CD71 single positive cells or c-kit single positive cells developed disease phenotypes at the completion of the experiment at 10 weeks post-transplant (Figure 4C, D; Supplemental Figure 6C-E). These transplant data show that the *Brca1/Trp53* deficiency-associated erythroid neoplasia is transplantable via CD71 and c-kit double positive cell populations indicating
that this population in the spleen contains LICs similar to those found in the bone marrow.

The time it took for the disease to develop in recipients correlated with the number of cells used to transplant the disease. Average times to disease onset when 2.0x10^6, 0.2x10^6 or 0.02x10^6 unfractionated (UF) Mx1-Cre;Brcar1F/F;Trp53+/− spleen cells were used were 3.14, 4.07, and 5.57 weeks respectively (Supplemental Figure 6F). Similarly, average time to disease was significantly prolonged from 4.7 weeks with 20,000 cells to 9.14 weeks with 1,000 cells when CD71+/c-kit+ cells were used (Supplemental Figure 6G). Despite this difference, there were no differences in terminal disease state as indicated by terminal WBC reads or degree of splenomegaly (Supplemental Figure 6H, I). These transplant data show that hundreds of recipients with disease can be readily generated by transplanting spleen cells, thus eliminating the requirement to maintain large cohorts of syngeneic mice.

The transplantation experiments described to this point were done with cells isolated from Mx1-Cre;Brcar1F/F;Trp53+/− mice at an advanced state of disease when they have developed hepatosplenomegaly plus high levels of erythroblasts in peripheral blood (indicated by high WBC reads). However, we have found that the first indicator of disease is palpable splenomegaly, which occurs approximately 6 weeks post initial plpC treatment. To investigate transplantability prior to full blown disease, we transplanted lethally irradiated wildtype mice with 2 million unfractionated bone marrow or spleen cells from Mx1-Cre;Brcar1F/F;Trp53+/− mice 3.5 weeks or 6.5 weeks post initial plpC treatment. Neither donor groups showed abberant presence of erythroblasts in peripheral blood (no high WBC reads) but did have anemia and modest splenomegaly
Neither bone marrow nor spleen cells from the 3.5 week group transferred the disease into recipients (Supplemental Figure 7B, D). In contrast, both bone marrow and spleen from the 6.5 week donor group transferred the disease with full penetrance (Supplemental Figure 7C, E). Spleen transplant recipients developed the disease significantly faster than bone marrow transplant recipients (spleen vs. bone marrow average time to disease 6.3 vs. 8.7 weeks post transplant, p<0.05) (Supplemental Figure 7C), and therefore spleen is more suitable for rapid disease propagation in recipient cohorts.

Olaparib attenuates the erythroproliferative phenotypes in Brca1/Trp53 deficient mice

Olaparib is a PARP inhibitor approved for use in BRCA-mutated ovarian, prostate and pancreatic cancers (8). Olaparib treatment delays mammary tumor development in Brca1/Trp53 deficient mice (43). We hypothesized that olaparib may similarly inhibit the Brca1 and Trp53 deficiency-associated erythroid neoplasia. After the final plpC mediated induction of Cre, we initiated olaparib treatment (Figure 5A). Mice received IP injections of either olaparib or vehicle every other day and were evaluated when moribund. A majority (90.9%) of vehicle-treated Mx1-Cre;Brca1F/F;Trp53+/- mice developed the expected elevated white blood cell counts (avg. 68.4K/ul) indicative of disease (Figure 5B, D). In contrast, of the olaparib-treated Mx1-Cre;Brca1F/F;Trp53+/- mice, only two (13.3%) developed elevated white blood cell counts (Figure 5C, D), and these elevations were modest (16.6 K/ul and 28.8 K/ul). Olaparib was also able to abrogate the hepatosplenomegaly associated with Brca1/Trp53 deficiency (Figure 5E).
Olaparib was non-toxic as control mice showed no changes in WBC reads, spleen and liver weights, or viability. These data show that treatment with olaparib prior to disease development significantly prevented the highly penetrant neoplastic phenotypes observed in untreated $Mx1$-$Cre;Brca1^{F/F};Trp53^{-/-}$ mice.

In addition to successful suppression of gross disease in hematopoietic tissues by olaparib treatment, flow cytometric analysis of hematopoietic tissues showed absence of the disease-associated expansion of erythroid populations in spleen (Figure 5F), bone marrow (Figure 5G), and peripheral blood (data not shown). The elevated frequencies of c-kit$^+$ cells and CD71$^+$ early/mid erythroid progenitors in the spleen were reduced to control levels. In contrast, the frequencies of CD71$^+$Ter119$^+$ late erythroid progenitors (that were not elevated in diseased tissue) were not changed by olaparib treatment (Figure 5F). Similar to immature erythroblasts, the increased frequencies of MEPs in bone marrow were significantly reduced by olaparib treatment (2.9-fold, $p=0.0146$) (Figure 5G). Genetic analysis of spleens from olaparib- or vehicle-treated animals showed that the cells diminished by olaparib treatment were, as expected, the $Brca1$-and $Trp53$-deficient cells. The unrecombined floxed $Brca1$ allele and the wild-type $Trp53$ allele were predominant in olaparib-treated spleens compared to vehicle-treated spleens (Supplemental Figure 8A-C). In contrast, no differences were found in our genetic analysis of brain tissue when comparing olaparib vs. vehicle (Supplemental Figure 8D-F).

$Vav1$-$Cre Brca1^{F/F}$ mice were also sensitive to olaparib (Supplemental Figure 8). In this case, olaparib treatment further reduced the already low peripheral blood counts of $Vav1$-$Cre Brca1^{F/F}$ mice (white blood cells (WBCs), 2.55-fold, $p=0.0015$ and red blood
cells (RBCs), 2.64-fold, p=0.0001) compared to no effect on WBCs and a mild 1.16x effect on RBCs in controls (Supplemental Figure 8G-H). Spleen weights were also significantly decreased (2.24-fold, p=0.0055) by olaparib treatment in Vav-Cre Brca1i
mice but not in controls (Supplemental Figure 8I). In sum, these data demonstrate that pre-treatment with olaparib can prevent the development of Brca1/Trp53 double deficiency-associated erythroproliferative neoplasm through selective elimination of Brca1 deficient cells.

These data together indicate that the hematologic disease in double Brca1 and Trp53 deficient mice is a rapidly fatal, olaparib sensitive erythroid neoplasia. The olaparib sensitivity, the ability to transplant the disease to multiple recipients, and the ability to monitor disease through peripheral blood counts all suggest that this Brca1/Trp53 deficiency syngenic immune-replete mouse model will be useful for achieving rapid in vivo screens for new anti-cancer agents against BRCA1- or TP53-deficient tumors.
DISCUSSION

In this report and in our prior publication (17), we show that hematopoietic Brca1 deficiency in mice leads to bone marrow failure. Expanding on these discoveries, we now report that Brca1 deficiency when combined with Trp53 deficiency, leads to the development of a murine erythroid leukemia that is rapid onset, aggressive, and ultimately, fatal. It is established that BRCA1’s role in HR-mediated DNA DSB repair is important in the maintenance of genomic stability and tumor suppression (10, 44). We and others have previously shown that, as would be expected, deletion of Brca1 in the hematopoietic cells leads to increased DNA damage and genomic instability (17, 19). Brca1 deficiency and associated defects in DNA repair are not tolerated in the hematopoietic system and leads to bone marrow failure and lethality. These data are consistent with the “transform or die” hypothesis (17), where Brca1 loss is lethal to hematopoietic cells unless there are secondary genetic changes, such as Trp53 loss of function, which can drive neoplastic transformation. Although, in our model, we started with a Trp53 heterozygous background, wildtype Trp53 allele was lost in enlarged Mx1-Cre;Brca1^{F/F};Trp53^{+/−} spleens of leukemic mice (Supplemental Figure 5). As seen with other models, loss of heterozygosity of Trp53 could be a necessary early step in the development of leukemia. In a JAK2^{V617F} mouse model of erythroleukemia, Trp53 nullizygosity was essential for disease development; in a Trp53 heterozygous background JAK2^{V617F} mice did not develop the erythroleukemia of JAK2^{V617F};Trp53^{-/-} mice, but were similar to JAK2^{V617F} mice (25). Unlike with the JAK2^{V617F} mutation, Brca1 deficiency created a background permissive for the loss of the normal Trp53 allele to initiate tumorigenesis, consistent with BRCA1 functions in
maintaining genome integrity (44). We originally thought that Brca1 and Trp53 deficient cells would accumulate additional mutations that support proliferation and self-renewal but didn’t find additional meaningful changes. It is possible that alterations that effect these processes are present in the non-coding regions not covered by WES. Additionally, WES has low sensitivity for certain structural variants such as rearrangements. Therefore, our analysis may have missed relevant alterations.

A single spontaneous case of erythroleukemia has been reported in a Mx1-cre driven Brca1 deficient mouse model but (19). However, this erythroleukemia developed at a low frequency (1/13 mice) and without mutations in Trp53 suggesting that this erythroleukemia is probably of different etiology to ours, and that Brca1 deficiency-associated erythroleukemia can develop via other mechanisms independent of Trp53 deficiency.

In this study, we show that the Brca1/Trp53 double deficiency-associated hematopoietic neoplasia can be prevented with the PARP inhibitor Olaparib. Prior in vivo mouse studies have shown that Brca1/Trp53 deficiency-driven mammary tumors are sensitive to PARP inhibition (7, 43). PARP inhibitor-sensitivity suggests that leukemia cells share the synthetic lethality of combined Brca1 deficiency and PARP inhibition demonstrated in mice with Brca1/Trp53 deficient mammary tumors. Although the Brca1/Trp53 deficient breast tumor models are important in that they recapitulate features of human breast cancer (39, 40, 45-52), they are inefficient for testing drug responses due to long tumor latency (>6-12 months). In addition, the BLG-Cre system requires repeated pregnancies to induce recombination of the Brca1 allele which makes
the system cumbersome. Our Brca1/Trp53 deficient hematopoietic model has advantages over existing Brca1 models, and could provide a novel preclinical platform to test putative drugs targeting Brca1- (or Trp53-) deficient cancers in vivo. The ability to transplant the disease to multiple recipients and generate large cohorts of syngenic mice, the ability to monitor disease through peripheral blood counts, the rapid disease onset and the high penetrance all suggest that this Brca1/Trp53 deficiency mouse model will be useful for achieving rapid in vivo drug screening.

Whether mouse models can be used to understand the pathogenicity of human BRCA1 mutations has not been extensively explored. Using our humanized BRCA1 5382insC allele, we have established the pathogenicity of this mutation in hematopoietic tissue. We have preliminary data suggesting that this mutation is pathogenic in mammary tissue as well. Similarly to BLG-cre;Brca1F/F;Trp53+/− mice, BLG-cre;Brca1F/insC;Trp53+/− mice developed mammary tumors with characteristics of basal-like breast adenocarcinoma (49, 51) with near-absent mRNA expression of estrogen and progesterone receptors, as has been reported before for Brca1 null, Trp53 deficient mammary tumors (Supplemental Figure 9 and data not shown, respectively). This data show for the first time that the human BRCA1 5382insC mutation causes breast cancer when in combination with Trp53 deficiency.

A previous study had shown that a mouse Brca1 allele with a premature stop codon at an analogous human 5382 position predisposes mice to breast cancer (53). However, in humans, the 5382insC mutation does not lead to a simple truncation, but instead it is a frameshift that replaces the last 69 amino acids with a novel peptide sequence at the C-terminus. Whereas this mouse Brca1 5382stop truncation resulted in
loss of expression of the BRCA1 protein, the human 5382insC mutant protein is not lost (54, 55). Therefore, this mouse Brca1 mutant model may not reflect the tumorigenic behavior of an expressed human BRCA1 5382insC protein product. In contrast to this allele, our BRCA1 5382insC allele leads to the expression of a detectable, stable protein in mouse cells (17).

It is interesting that the Brca1 null phenotype is exacerbated by the presence of the BRCA15382insC alleles, although it doesn’t show dominant negative effects in a Brca1 wildtype background (17). This suggests that the mutant protein is detrimental only in the absence of wildtype Brca1. Multiple activities of BRCA1, including those of DNA repair, involve its interaction with other proteins. The BRCA1 5382insC mutation results in the expression of a truncated BRCA1 missing the C-terminal BRCT repeat that interacts with phosphopeptides and promotes HR, which is necessary for tumor suppression (56). The greater pathogenicity of the BRCA1 5382insC allele suggests that the mutant protein effects cellular functions in other ways as well. The BRCA1 5382insC mutant protein retains a large part of the wildtype sequence and may, therefore, retain wildtype properties. It may bind and sequester key proteins and DNA sequences, disrupting their proper function. Alternatively or additionally, the mutation may lead to changes in its structure that interfere with its normal function. For example, studies in the breast cancer cell line HCC1937 that carries the BRCA1 5382insC mutation have shown that structural changes near the N-terminal RING domain leads to changes in post-translational modifications under conditions of cell stress (57). The distinct effects of the BRCA1 5382insC mutation in hematopoietic cells, in comparison to the Brca1 null mutation, will only be understood with more research.
Our analysis of the \textit{BRCA1} 5382insC mutation shows that our hematopoietic model could serve as a genetic model to assess the pathogenicity of \textit{BRCA1} variants. In humans, \textit{BRCA1} 5382insC mutation is one of the relatively common inherited mutations that predispose to hereditary cancer syndromes and therefore, it is well established as a pathogenic mutation. With the increase in sequencing, many other less common variants/mutations of \textit{BRCA1} have been identified (58). A significant number of these variants are classified as variants of unknown significance (VUS) as their clinical significance, whether pathogenic or benign, cannot be determined due to the lack of sufficient evidence. Many attempts have been made to develop \textit{in vitro} functional assays to determine the pathogenicity of the VUSs (59), but they don't address the complexity of \textit{in vivo} biology. Similar to improved \textit{in vivo} drug screening, assessing \textit{BRCA1} variants in the murine mammary tumor system is a laborious task and our hematopoietic \textit{Brca1/Trp53} leukemia model could provide an alternative model.

In future studies, it will be of interest to carry-out molecular and genetic experiments to understand the mechanisms of how \textit{Brca1} deficiency-associated genomic instability and other defects lead to the hematopoietic phenotypes. For example, TP53BP1 is a DNA damage response protein that can reverse phenotypes of \textit{Brca1} mutant cells (59). TP53BP1 promotes NHEJ repair and suppresses HR (60). TP53BP1 loss has been shown to partially restore HR defects and reduce genomic instability seen in \textit{Brca1} deficient cells (61). We would expect that the co-deletion of \textit{Tp53bp1} would at least partially rescue the bone marrow failure associated with \textit{Brca1} deletion. Such a rescue will confirm that it is the \textit{Brca1}-deficiency associated HR defects that lead to bone marrow failure. Restoration of HR and alleviation of genomic
instability would create a milieu less permissible to tumorigenesis, and reduce the likelihood that LOH of *Trp53*, which we assume is an early driver of leukemic transformation, would occur.

In this report and in our prior publication (17), we show that hematopoietic *Brca1* deficiency in mice leads to pancytopenia. Expanding on these discoveries, we now report that *Brca1* deficiency when combined with *Trp53* deficiency, leads to the development of a murine erythroid leukemia that has a rapid onset, aggressive, and ultimately, fatal.
METHODS

Mice

The Brca1\textsuperscript{F} (Brca1\textsuperscript{F22:24}) (49), Brca1\textsuperscript{insC} (17), Mx1-Cre (28), Vav1-iCre (35) and BLG-cre (49) alleles have been described previously. The generation and characterization of the BRCA1\textsuperscript{insC} allele was described in detail in our prior publication (17) that reported this allele for the first time. Briefly, we first generated mice that had wildtype human BRCA1 cDNA knocked in to the mouse Brca1 locus (BRCA1\textsuperscript{WT}). These mice expressed wildtype human BRCA1 instead of mouse Brca1. This wildtype human BRCA1 allele was designed in such a way, upon Cre-mediated recombination it expressed the 5382insC mutant version (BRCA1\textsuperscript{insC}) instead of the wildtype BRCA1. Mice with BRCA1\textsuperscript{insC} allele in the germline was generated by crossing mice carrying BRCA1\textsuperscript{WT} allele to CMV-Cre deleter mice (62). The BRCA1\textsuperscript{insC} allele has since been maintained through breeding in our colony. All animals were generated on a C57BL/6 pure background except for the mice in Figure 3 and Supplemental Figures 2, 3, 4, 8G-I, and 9 which are in a BL6/129S mixed background.

Mx1-cre recombination was induced by administering eight intraperitoneal polyinosinic:polycytidylic acid (pIpC) injections (10mg/kg), every other day. Following pIpC injections, mice were monitored regularly for evidence of disease and sacrificed when moribund. Expression of BLG-cre recombinase was induced in female mice undergoing, on average, four pregnancies. Animals were monitored for tumor development and sacrificed for analysis when maximum tumor size allowable was
reached (~1/10 body weight) or when the health of the mouse was otherwise compromised.

Mice were weaned around four weeks of age and genotyped from tail snips using real-time PCR assays designed by Transnetyx. All experimental animals were housed in the Unit for Laboratory Animal Medicine at the University of Texas Southwestern Medical Center under specific pathogen-free conditions. Experimental procedures were conducted after approval by the University of Texas Southwestern Institutional Animal Care and Use Committee (APN 2017-102119).

**Histology**

Tissue samples were fixed in 4% paraformaldehyde/PBS; bones (femur and tibia) were fixed in Cal-Rite decalcifying solution. The HistoPathology Core at UT Southwestern Medical Center then performed paraffin processing and embedding, mounted 5 µm thick sections on slides, and stained the slides with haematoxylin and eosin for morphological analysis. Blood smears were prepared for haematoxylin and eosin staining following manufacturer’s directions.

**Hematopoietic Analysis**

For flow experiments, bone marrow and spleen single cells suspensions were created as described (63). For HSC and progenitor analysis cells were incubated with biotinylated lineage specific antibodies (CD3ε, CD4, CD5, CD8a, CD45R, CD11b, Gr-1, Ter119) and fluorophore-conjugated antibodies against progenitor surface markers (Sca1, c-kit, CD16/32, CD34, CD48, CD150). PE-CF594 streptavidin was used to
identify lineage positive cells and 4’, 6-diamidino-2-phenylindole (DAPI, Sigma) to exclude dead cells. For lineage analysis samples were incubated with combinations of fluorophore-conjugated antibodies to the following cell surface markers: CD117, CD3ε, CD4, CD8a, CD45, CD45R, CD11b, Gr-1, Ter119, and CD71. Antibodies used in flow cytometric analysis are included in the Supplement (Supplemental Table 1).

Flow samples were analyzed using the FACS Canto RUO analyzer (BD). Gating schemes for hematopoietic progenitors were performed as previously described (17, 63, 64) using fluorescence minus one control. Complete peripheral blood counts were assessed using the HemaVet HV950 with MULTI-TROL Mouse as an equilibration control (Drew Scientific). Blood smear slides were stained using Hemacolor Stain Set (Harleco) according to manufacturer’s instructions.

**Whole Exome Sequencing (WES)**

Sample preparation, sequencing, and bioinformatics analysis was performed by CD Genomics, NY. WES data from each spleen was compared to respective paired brain tissue. DNA was extracted using the Qiagen DNeasy kit. Quantity of DNA was measured by picogreen method using Victor X2 fluorometry. Integrity of DNA were checked by Agilent genomic DNA screentape, offering a numeric measurement DNA Integrity Number (DIN). Sequencing libraries were generated using Agilent SureSelectXT Kit (Agilent Technologies, CA, USA) following manufacturer’s recommendations and index codes were added to attribute sequences to each sample. Read length for paired-end reads was 151bp. Barcoded WES libraries were sequenced on Illumina-based platform. Average depth was 136x. Paired-end clean reads were
aligned to mouse reference genome GRCm38.p6 using Burrows-Wheeler Aligner (BWA) software (65). Picard was used to mark duplicates. GATK Best Practices were followed for preprocessing BAM files (66). GATK and SAMtools were used for variant calling. SnpEff program was used to examine structural changes. Sequences were aligned and graphically visualized using the Integrative Genomic Viewer (IGV) (http://software.broadinstitute.org/software/igv/). WES data were deposited in the Sequence Read Archive (SRA) database (accession number PRJNA887455).

Drug treatment

Olaparib (AZD2281, LC Laboratories) was dissolved in DMSO (Fisher Scientific) and stored as a 50 mg/mL stock. Prior to use, the olaparib stock was diluted with 10% 2-hydroxyl-propyl-β-cyclodextrine/PBS (Fisher Scientific) in a 1:10 ratio (v/v). Vehicle preparation was the same minus olaparib. All mice began treatment 2.5 weeks after the final plpC injection. Mice received vehicle or olaparib (50 mg/kg) i.p. injections every other day.

Bone Marrow Transplantation

Bone marrow transplantations were done as previously described (17). Adult recipient mice (CD45.1) were administered a minimum lethal dose of radiation using an XRAD 320 X-ray irradiator (Precision X-Ray) to deliver two doses of 1540 rad (1,080 rad in total) at least 3 hr apart. Cells were injected into the retro-orbital venous sinus of anesthetized recipients. Blood was obtained from the submandibular plexus of recipient
mice at the indicated time points after transplantation. Red blood cells were lysed with ammonium chloride potassium buffer.

**Statistical Analysis**

Data are displayed as mean ± SEM. Statistical significance between two groups was assessed using the two-tailed Student’s t test (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001). Statistical significance between multiple groups was determined by one-way ANOVA followed by Bonferroni corrected post-hoc comparisons. To perform Bonferroni correction, statistical comparisons were made using unpaired 2-tailed Student’s t tests and the critical P value (α) was divided by the number of comparisons being made. *P < 0.0167, **P < 0.0033; ***P < 0.0003, etc. Log-rank test was used for survival analysis. Chi square analysis was assessed with the Fisher exact test. P values less than 0.05 were considered significant.

**Study approval**

The present studies in mice were reviewed and approval by the University of Texas (UT) Southwestern Institutional Animal Care and Use Committee, UT Southwestern Medical Center, Dallas, Texas (APN 2017-102119).
AUTHOR CONTRIBUTIONS

TSR and VEM initiated the study and GLP, KM, SRH, RW, and TSR designed/interpreted the experiments. GLP, KM, SRH, and RW performed experiments and collected the data.

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REFERENCES


Figure 1. Brca1/Trp53 double deficient Mx1-Cre;Brca1^{F/F};Trp53^{+/-} mice develop a rapid and robust hematological disease. (A) Schematic of experimental design. Control, Mx1-Cre;Brca1^{F/F} and Mx1-Cre;Brca1^{F/F};Trp53^{+/-} were treated with polyinosinic:polycytidylic acid (pIpC) and monitored for up to 12 weeks post initial pIpC treatment. (B-C) Average peripheral blood white blood cell (WBC) reads* (B) and red blood cell (RBC) (C) counts before (prebleed) and after initial pIpC treatment. *Indicative of non-leukocyte, nucleated cells. Numbers of mice: Control (n=13), Mx1-Cre;Brca1^{F/F} (n=11), and Mx1-Cre;Brca1^{F/F};Trp53^{+/-} (n=9). (D-E) Wright-Giemsa stained peripheral blood smears from age-matched control and Mx1-Cre;Brca1^{F/F};Trp53^{+/-} mice. (F-G) Spleen and liver weights at moribund/terminal stage or 12 weeks post initial pIpC treatment. Numbers of mice: Control (n=11), Mx1-Cre;Brca1^{F/F} (n=9), and Mx1-Cre;Brca1^{F/F};Trp53^{+/-} (n=9). (H-K) Representative H&E stained sections of effaced spleens (I) and infiltrated livers (K) of Mx1-Cre;Brca1^{F/F};Trp53^{+/-} mice compared to control (H,J) mice. Values represent mean ±SEM. Statistical significance was assessed using one-way ANOVA followed by Bonferroni correction (*p < 0.0167, **p < 0.003, ***p < 0.0003, ****p < 0.00003). Controls were without Mx1-Cre, all other mice carry Mx1-Cre.
Figure 2. Increased immature erythroblasts in hematopoietic tissue of diseased *Mx1-Cre;Brca1^{F/F};Trp53^{+/−}* mice. (A) Hematopoietic stem cell (HSC), multipotent progenitor (MPP), common myeloid progenitor (CMP), granulocyte monocyte progenitor (GMP), and megakaryocyte/erythrocyte progenitor (MEP) frequencies in the bone marrow of control (n=9), *Mx1-Cre;Brca1^{F/F}* (n=8), and *Mx1-Cre;Brca1^{F/F};Trp53^{+/−}* (n=5) mice. (B-E) Flow cytometric analysis of peripheral blood (top), spleen (middle), and bone marrow (bottom) for CD45−SSClo erythroblasts (A), early CD71+/Ter119− (B), mid CD71+/Ter119+ (C), and late CD71+/Ter119+ (D) erythroid progenitors in control (n=9), *Mx1-Cre;Brca1^{F/F}* (n=8), and *Mx1-Cre;Brca1^{F/F};Trp53^{+/−}* (n=7) mice. (F) Percentage of CD45+ cells in spleen B cells (B), T cells (T), granulocytes (Mac1+), c-kit+ cells, and erythroid progenitors (early CD71+ Ter119−, mid CD71+Ter119+, and late CD71−Ter119+) in control (n=9), *Mx1-Cre;Brca1^{F/F}* (n=8), and *Mx1-Cre;Brca1^{F/F};Trp53^{+/−}* (n=5) mice. Values represent mean ±SEM. Statistical significance was assessed using one-way ANOVA followed by Bonferroni correction (*p < 0.0167, **p < 0.003, ***p < 0.0003, ****p < 0.00003). Controls were without *Mx1-Cre*, all other mice carry *Mx1-Cre*. 
Figure 3

A

B

C

D

E

F

G

H
Figure 3. Oncogenic BRCA1 5382insC mutation accelerates the onset of Brca1/Trp53 deficiency-associated hematological disease. (A-B) Kaplan-Meier curves of overall survival after initial pIpC treatment. Control (dotted black, n=8), Mx1-Cre;Brca1^{F/F} (dashed red, n=19), Mx1-Cre;Brca1^{F/F};Trp53^{+/−} (solid red, n=17), Mx1-Cre;Brca1^{F/insC} (dashed black, n=15), and Mx1-Cre;Brca1^{F/insC};Trp53^{+/−} (solid black, n=24). (C-D) Peripheral blood white blood cell (WBC) reads* (C) and red blood cell (RBC) (D) counts before (prebleed) and after initial pIpC treatment. *Indicative of erythroid blast cells. Numbers of mice: control (n=8), Mx1-Cre;Brca1^{F/insC};Trp53^{+/−} (n=12), Mx1-Cre;Brca1^{F/insC} (n=9), Mx1-Cre;Brca1^{F/F};Trp53^{+/−} (n=16) and Mx1-Cre;Brca1^{F/F} (n=5). (E) Wright-Giemsa stained peripheral blood smears from age-matched control, Mx1-Cre;Brca1^{F/insC};Trp53^{+/−} and Mx1-Cre;Brca1^{F/F};Trp53^{+/−} mice (Grey arrow=normal lymphocyte and black arrow head=erythroid progenitor). (F-H) Flow cytometric analysis of bone marrow MEPs and spleen early (CD71^{+}Ter119^{−}) and mid (CD71^{+}Ter119^{+}) erythroid progenitors in control (n=3-6), Mx1-Cre;Brca1^{F/insC};Trp53^{+/−} (n=4), and Mx1-Cre;Brca1^{F/F};Trp53^{+/−} (n=7) mice. Statistical significance was assessed using log rank tests and one-way ANOVA followed by Bonferroni correction (*p < 0.0167, **p < 0.003, ***p < 0.0003, ****p < 0.00003). All mice carry Mx1-Cre except for controls that were without Mx1-Cre.
Figure 4. Leukemia of Brca1 and Trp53 double deficiency is transplantable through bone marrow and spleen. (A) Kaplan-Meier curves of overall survival of primary (solid line, n=7) and secondary (bold solid line, n=9) recipients of unfractionated (UF) Mx1-Cre;Brca1<sup>F/F</sup>;Trp53<sup>+/−</sup> bone marrow (BM). Recipients of UF control BM (dotted line, n=4) did not show lethality. (B) Kaplan-Meier survival curves of primary recipients of CD71<sup>+</sup> (dashed line, n=8), CD71<sup>+</sup>/c-kit<sup>+</sup> (solid line, n=13), c-kit<sup>+</sup> (dotted line, n=1) and secondary recipients of CD71<sup>+</sup>/c-kit<sup>+</sup> (bold solid line, n=5) Mx1-Cre;Brca1<sup>F/F</sup>;Trp53<sup>+/−</sup> BM.
cells. (C) Kaplan-Meier curves of overall survival of recipients of unfractionated (UF) (bold solid line, n=6), CD71^+/c-kit^+ (solid line, n=3), CD71^+ (dashed line, n=5), and c-kit^+ (dotted line, n=2) Mx1-Cre;Brcat^{E/F};Trp53^{+/-} spleen cells. (D) Terminal spleen weights of above recipient mice. Spleen weights of BM CD71^+ recipient mice (B) that showed prolonged survival are marked by open circles. Values represent mean ± SEM.
Figure 5

A

Vehicle/Olaparib

B

Vehicle-treated

C

Olaparib-treated

D

Terminal WBC reads K/uL

E

Spleen

Liver

F

Spleen

c-kit

CD71^Ter119^***

CD71^Ter119^-***

CD71 Ter119^**

G

Bone marrow

MEP

Control

Brca1^{1/2}; Trp53^{1/2}

Control

Brca1^{1/2}; Trp53^{1/2}

Control

Brca1^{1/2}; Trp53^{1/2}

Control

Brca1^{1/2}; Trp53^{1/2}

Control

Brca1^{1/2}; Trp53^{1/2}
Figure 5. PARP inhibitor, olaparib attenuates Brca1/Trp53 deficiency-associated hematopoietic phenotypes. (A) Schematic representation of the olaparib treatment schedule. (B-C) Average white blood cell (WBC) reads* of vehicle- or olaparib-treated control and Mx1-Cre;Brca1F/F;Trp53±/± mice. Vehicle, Control (n=8); vehicle, Mx1-Cre;Brca1F/F;Trp53±/± (n=13); olaparib, control (n=9); olaparib, Mx1-Cre;Brca1F/F;Trp53±/± (n=15). (D) Terminal WBC reads* in vehicle- (n=13) or olaparib- (n=15) treated Mx1-Cre;Brca1F/F;Trp53±/± mice. (E) Terminal spleen and liver weights of vehicle- or olaparib-treated control and Mx1-Cre;Brca1F/F;Trp53±/± mice. Control, vehicle- (n=4); Control, olaparib (n=5); Mx1-Cre;Brca1F/F;Trp53±/±/Vehicle (n=10); Mx1-Cre;Brca1F/F;Trp53±/±, olaparib (n=15). (F-G) Flow cytometric analysis of spleen (F) and bone marrow (G) show decreased c-kit+, CD71+ (early Ter119+ and mid Ter119+) erythrocyte and megakaryocyte/erythroid progenitor (MEP) frequencies in olaparib-treated Mx1-Cre;Brca1F/F;Trp53±/± mice compared to those vehicle-treated. No change in CD71+Ter119+ late erythrocytes. *Indicate erythroid blast cells. (Control=4-5, Mx1-Cre;Brca1F/F;Trp53±/±=6-7). Values represent mean ± SEM. Statistical significance was assessed using a two-tailed Student’s t test (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001) or one-way ANOVA followed by Bonferroni correction (*p<0.0125, **p<0.0025, ***p<0.0002, ****p<0.0002. Controls were without Mx1-Cre, all other mice carry Mx1-Cre.