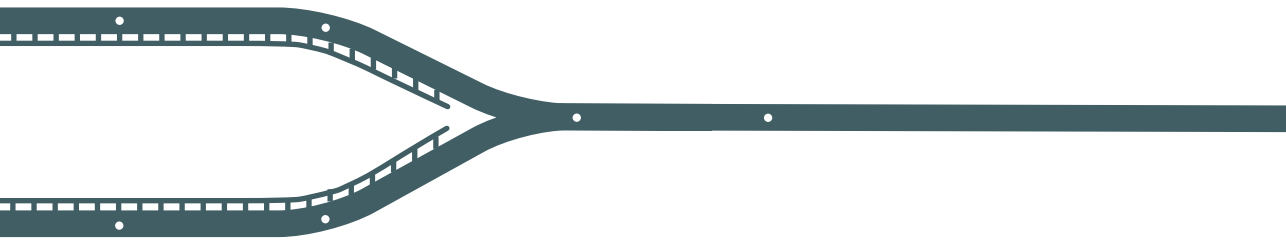
Using functional genetic screens to understand and overcome PARP inhibitor resistance

Paes Lobo Lopes Dias, M.



Chapter 7

General discussion and conclusions

The successful development of PARP inhibitors (PARPi) to target *BRCA1/2*-mutant cancers provides breakthrough evidence that exploiting synthetic lethal interactions is a promising therapeutic strategy that could be applicable to several cancers. Nonetheless, as with all targeted therapies that have entered the clinic, PARPi resistance is common and develops through multiple mechanisms. The major focus of this thesis is to understand the molecular mechanisms behind PARPi resistance and to find potential therapeutic targets that could be exploited to design combination treatment regimens that inhibit or delay resistance.

PARPi AS SINGLE-AGENT THERAPY – IS IT ENOUGH?

Over the past 14 years, multiple mechanisms of PARPi resistance have been identified and characterized. Even though the clinical relevance is yet to be confirmed for most mechanisms, they suggest that tumor cells can find several ways to escape PARPi therapy. As discussed in **Chapter 2**, most clinical trials testing PARPi have been initiated within the past few years and thus progression-free survival (PFS) is currently the most widely used primary outcome in these studies whereas overall survival (OS) data remain limited. One of the few pieces of evidence that PARPi can improve OS comes from a preplanned OS analysis on the phase III SOLO2 trial, which demonstrated that maintenance treatment with olaparib extends the median OS of patients with relapsed platinum-sensitive advanced-stage *BRCA1/2*-mutant ovarian cancer by 12.9 months in comparison to placebo[1]. Nevertheless, the OS benefit remains to be determined for other PARPi and for other indications. Although emerging data show that PARPi delay disease recurrence and prolong OS in certain settings, the lack of prolonged responses in patients receiving PARPi points towards acquired mechanisms of resistance to single-agent therapy, highlighting the need for rational combination treatments. The use of combination therapies aimed at further amplifying the antitumor efficacy of PARPi, such as indirect inhibition of homologous recombination (HR), abrogation of cell-cycle checkpoint signaling or immunotherapy, targeting acquired vulnerabilities of resistant cells (e.g., the increased sensitivity of 53BP1- and PARG-deficient cells to ionizing radiation), and suppressing the mutator phenotype of *BRCA1/2*-mutated tumors with inhibitors of POLQ are promising approaches to tackle PARPi resistance **(Chapter 2)**. Overall, PARPi as single-agents may not lead to prolonged responses and complete tumor eradication, but there are several approaches that can be explored to improve this promising targeted therapy in order to reduce the mortality rate in patients with *BRCA1/2*-mutated tumors.

PREDICTION OF PARPi RESPONSE AND DETECTING RESISTANCE

Currently, four different PARPi have been approved for the treatment of ovarian, breast,

prostate and pancreatic cancer. In these settings, biomarkers such as sensitivity to platinum-based chemotherapy, *BRCA1/2* mutations and HR deficiency (HRD) tests enable patient selection for PARPi therapy. Nevertheless, these biomarkers may fail to provide an accurate functional status of HR or predict the benefit from PARPi therapy. Recent studies have explored the clinical utility of RAD51 nuclear foci as a surrogate marker of HR activity and predictor of PARPi response, and found that RAD51 foci detection in tumor cells derived from established patient-derived xenograft models from breast, ovarian and pancreatic cancers recapitulated patient HRD status and treatment response[2–5]. Moreover, The RAD51 test showed higher accuracy than HR gene mutations and genomic HRD analysis for predicting PARPi response.

Close monitoring of treatment response and early detection of PARPi-resistant subclones are crucial to the success of a treatment regimen. Studies testing the clinical utility of RAD51 nuclear foci also found that this method captured dynamic changes in HR status upon acquisition of PARPi resistance[2]. However, RAD51 nuclear foci as tool for detecting acquired resistance also has potential limitations as pointed out by our work, where we show that PARPi resistance can be triggered independently of HR, in both *BRCA1*- and *BRCA2*-mutated tumors **(Chapter 3, Fig1)**. Moreover, standard tumor biopsy sampling, required for RAD51 nuclear foci detection, is typically invasive and thus often cannot be conducted on a regular basis. Therefore, non-invasive methods of assessing tumor genomics using blood or plasma samples, such analysis of circulating tumor DNA (ctDNA) in liquid biopsies, are being investigated, as these can be performed serially and might provide a better indication of tumor heterogeneity and emergence of therapy-resistant subclones **(Chapter 2, Box 3)**. Although further research is required, liquid biopsies and ctDNA based assays could provide a fast and cost-effective method for early detection of known alterations associated with PARPi resistance, eventually indicating the need for further treatment. Finally, monitoring of PARPi treatment response in patients will also generate molecular data that may be used to assess the clinical relevance of the various PARPi resistance mechanisms identified in preclinical studies.

HR-RESTORATION VIA LOSS OF 53BP1 EMERGES AS A CRUCIAL RESISTANCE MECHANISM IN BRCA1-DEFICIENT TUMORS

To date, the best clinically documented mechanism of resistance is (epi)genetic reactivation of BRCA1/2 function. This is most likely because most clinical data are from trials testing PARPi as second-line therapy following first-line treatment with platinum-based chemotherapies. Since BRCA1/2 reactivation has been shown to be the dominant mechanism of platinum resistance in *BRCA1/2*-mutated tumors, patients who received first-line platinum therapy may have already developed BRCA1/2-proficient tumor subclones, which are cross-resistant to PARPi. It is thus conceivable that the landscape of PARPi resistance mechanisms will be

different in patients who receive PARPi as first-line therapy. Evaluation of the relevance of previously reported BRCA1/2-independent PARPi resistance mechanisms in a realistic and experimentally tractable preclinical *in vivo* setting is therefore warranted. In **Chapter 3**, we used a collection of matched PARPi-naïve and PARPi-resistant mammary tumors derived from genetically engineered mouse models (GEMMs) of *BRCA1/2*-associated breast cancer. These panels of tumors were generated by treating tumor-bearing mice with PARPi until they spontaneously developed resistance. Analysis of these tumors showed that PARPi resistance via restoration of HR occurred in the majority of BRCA1-deficient tumors but in none of the BRCA2-deficient tumors (**Chapter 3, Fig. 1)**. Moreover, we found that nearly half of the HR-restored tumors could be explained by downregulation or loss-of-function mutations in 53BP1 whereas loss of other DNA double-strand break (DSB) end-protection factors was only sporadically observed (**Chapter 3, Fig. 2)**. In line with this, PARPi resistance induced by 53BP1 loss is much stronger than resistance induced by depletion of *Shld1/2* or *Ctc1* in BRCA1-deficient mouse mammary tumors[6,7]. Loss of 53BP1 has also been observed in patient-derived tumor xenografts with acquired resistance to PARPi, and mutations in *TP53BP1* have been reported in tumor biopsies from patients with metastatic BRCA1-associated breast cancer receiving PARPi, providing additional evidence that loss of 53BP1 may play an important role in driving resistance in the clinic[3,8,9]. Altogether, these data suggest BRCA1-deficient tumors treated with PARPi are under high selective pressure to restore HR and if they cannot reactivate BRCA1, HR restoration via 53BP1 loss will most likely be the prevalent mechanism of PARPi resistance, stressing the need to design treatment strategies to combat 53BP1 loss-mediated resistance. In this thesis, we discuss potential strategies to overcome 53BP1 loss-mediated resistance in **Chapter 2**, and used functional genetic screens to identify vulnerabilities of BRCA1/53BP1 double-deficient cells in **Chapters 4** and **5**.

FUNCTIONAL GENETIC SCREENS AS TOOLS TO UNDERSTAND PARPi RESPONSE AND IDENTIFY CANDIDATE DRUG TARGETS

In this thesis, we performed several functional genetic screens with the purpose of finding novel PARPi resistance mechanisms and candidate therapeutic targets to improve PARPi response. In **Chapters 3, 4 and 5**, we carried out enrichment as well as dropout screens, using focused and genome-wide shRNA and CRISPR knockout (CRISPRko) libraries, in different mouse and human cell line models. Together, our work shows that functional genetic screens provide a powerful unbiased discovery tool for identifying genes that modulate PARPi response. Importantly, several considerations should be made regarding the choice of perturbations and the model systems, in order to improve chances of validation and the translational value of the identified factors.

Screens using CRISPRko libraries have been shown to outperform those using shRNA libraries, mostly due to their reduced off-target effects[10]. Moreover, screens using shRNA technology might miss potential hits that would be detected with CRISPRko technology, since gene downregulation may result in no or a weaker phenotype compared to gene deletion. Another advantage of CRISPR/Cas9 technology is the fast and cost-effective development of customized CRISPRko libraries for secondary validation screens, which can be very powerful to further prioritize hits from the initial screen (**Chapter 5**). Nevertheless, our work shows that shRNA library screens may also have some advantages because they can probe both non-essential and essential genes, allowing identification of essential genes that modulate PARPi response upon shRNA-mediated downregulation. For example, since complete loss of mitochondrial LIG3 activity is lethal, we wouldn't have identified LIG3 as a modulator of PARPi response if the screens were carried out using a CRISPRko library. This is not only the case for LIG3, but for several DNA damage response (DDR)-associated genes. Additionally, gene downregulation allows residual protein expression which more closely resembles the effect of a chemical inhibitor, and is therefore more suitable to look for therapeutic targets. The recent development of CRISPR interference (CRISPRi), which allows for sequence-specific repression of gene expression, combines the advantages of CRISPR technology with the benefits of gene downregulation. Lastly, gain-of-function screens, such as CRISPR activation (CRISPRa) screens, are still largely unexplored. CRISPRa screens have the potential to find gain-of-function factors driving PARPi resistance, which could potentially be therapeutically targeted to combat or prevent resistance.

In addition to the screening library, the results yielded by the screen will also depend on the model system in which the screen is performed. For example, in the screens carried out in **Chapter 4**, LIG3 was the only overlapping hit in embryonic stem cells and mammary tumoroids, even though both models were deficient for BRCA1, p53 and 53BP1. Similarly, the hits from the focused screens in **Chapters 3** and **5** showed limited overlap between the cell lines used. Thus, it is important to consider carrying out the same screen in several relevant model systems in order to identify common hits and avoid pursuing candidates that are model/cell line-specific.

Besides screening in multiple models, choosing *in vitro* models that better represent how cells grow *in vivo* also increases the chances of making clinically relevant discoveries. In **Chapter 6**, we described a detailed protocol for screening in three-dimensional (3D) mouse mammary tumor-derived organoids (tumoroids). Compared to 2D cell line models, 3D tumoroids retain crucial *in vivo* features, such as morphology and 3D structure, and are able to recapitulate drug response of the original tumor from which they were derided[11]. Nevertheless, screens in tumoroids still don't capture all the biological processes present in tumors in patients. For example, a drawback of screens in 3D tumoroid lines (and other *in vitro* culture systems) is the lack of stromal cells including immune cells. This prevents screening for factors involved in tumor-microenvironment interactions, which have been found to influence PARPi response[12–14]. Indeed, in **Chapter 3** we found increased immune

infiltration in PARPi-resistant BRCA1-deficient tumors that did not restore HR **(Chapter 3, Fig. 3)**. Moreover, tumoroids derived from one of our PARPi-resistant BRCA1-deficient tumors did not recapitulate PARPi resistance *in vitro* but upheld PARPi resistance *in vivo*, suggesting PARPi resistance in this tumor may be driven via cell-extrinsic mechanisms[11]. Together, these results underscore the importance of screening in model systems that adequately recapitulate the complexities of the tumor microenvironment, such as co-culture systems with stromal elements (e.g., fibroblasts, immune cells[14–18]). Another disadvantage of *in vitro* models, including 3D tumoroids, is the non-physiological medium composition, which make it difficult to screen for metabolic pathways, such as oxidative phosphorylation and fatty acid metabolism, found to be upregulated in PARPi-resistant BRCA2-deficient tumors **(Chapter 3, Fig. 4).** In line with this, the genome-wide screen carried out in **Chapter 5** identified several subunits from mitochondrial Complex I as modulators of PARPi response, but these results could not be reproduced in the secondary screens with focused libraries, nor in individual validation experiments, most likely due to variations in cell density and medium conditions across experiments. Hence, while 3D model systems better mimic *in vivo* conditions and offer certain advantages for functional genetic screens, current technologies still fail to fully recapitulate the *in vivo* physiological environment.

The need to perform functional genetic screens in cancer models that more accurately recapitulate human disease, including the tumor microenvironment and the interactions between different cell types, makes *in vivo* screens particularly attractive. To date, the majority of *in vivo* CRISPR screens have aimed to investigate cancer phenotypes (e.g., tumor initiation, metastasis) and the use of *in vivo* screens to study modulators of therapy response remains largely unexplored. *In vivo* screens can be performed by directly delivering the CRISPR library to the animal or by transplanting cancer cell lines or organoids that were modified with the CRISPR library *in vitro*. Direct *in vivo* gene editing in somatic cells enables screens in tumor cells that originated *de novo* from endogenous cells. For example, *in vivo* CRISPR knockout screens have been performed in genetically engineered mouse models (GEMMs) of $Braf^{V600E}$- or $Kras^{G12D}$-driven lung cancer to identify modulators of response to immunotherapy and carboplatin[19,20]. These studies used pooled libraries in which the constructs harbor a sgRNA for CRISPR-mediated gene editing as well as cre-recombinase for tumor initiation. A similar approach could involve intraductal injection of a lentiviral sgRNA-Cre library in GEMMS of BRCA1/2-deficient breast cancer to study PARPi response *in vivo*[21–24].

A limitation of direct *in vivo* approaches is the difficulty in predicting the number of tumor-initiating cells that are being targeted, which makes it challenging to calculate the multiplicity of infection (MOI) required to maintain adequate representation of the library *in vivo*. Such technical parameters are more easily achievable in transplant-based screens using tumor-derived cell lines or organoids. Another advantage of *in vivo* CRISPR screens using transplantable models is that it allows screening in diverse genetic contexts without the need of a pre-existing GEMM. There are a few studies that used *in vivo* CRISPR screens

to study drug response by transplanting mouse cancer cell lines into immunocompetent animals. For example, one study carried out an *in vivo* CRISPRko screen by transplanting the murine B16 melanoma cell line into syngeneic immunocompetent mice to identify genes that modulate response to immunotherapy[25]. Another report used CRISPRa *in vivo* screens to study resistance to temozolomide by transplanting a murine acute B-cell lymphoblastic leukemia cell line into syngeneic immunocompetent animals[26]. GEMM-derived mammary tumoroids retain key characteristics of the original tumor (e.g., morphology, treatment response) and can be easily manipulated *in vitro* and subsequently orthotopically engrafted with high efficiency[11]. They are therefore promising tools to perform *in vivo* CRISPR screens to identify modulators of PARPi response **(Chapter 6)**. Importantly, library size remains a limiting factor in both direct and transplant-based *in vivo* CRISPR screening approaches, as high-complexity screens can require large numbers of recipient mice in order to maintain the desired library coverage (which is usually higher in dropout screens than in enrichment screens).

Altogether, *in vivo* CRISPR screens are a powerful tool that enables the identification of factors modulating therapy response in physiological conditions. Developing and optimizing technologies for *in vivo* screens in immunocompetent animals is therefore important to further unravel the biological processes underlying PARPi response and to enhance the translational relevance of our findings.

POTENTIAL USE OF LIG3 INHIBITORS TO IMPROVE PARPi RESPONSE

In **chapter 4**, functional genetic screens performed in cells and tumoroids identified depletion of LIG3 as an enhancer of PARPi toxicity in BRCA1-deficient cells. The improved response to PARPi mediated by LIG3 depletion was dependent on BRCA1 deficiency but independent of the loss of 53BP1-RIF1-shieldin pathway. In line with these observations, LIG3 depletion enhanced the efficacy of PARPi against BRCA1-deficient and BRCA1/53BP1 double-deficient mammary tumors in mice. Together, these results highlight LIG3 as a potential therapeutic target to improve response to PARPi and possibly inhibit or delay emergence of resistance. Our work also showed that LIG3 loss promotes formation of MRE11-mediated post-replicative single-stranded DNA (ssDNA) gaps in PARPi-exposed BRCA1-deficient and BRCA1/53BP1 double-deficient cells, ultimately leading to an accumulation of chromosomal abnormalities. These observations, together with work from Cong and colleagues, reveal ssDNA gap exposure as a vulnerability in BRCA1/2-deficient cells and as a predictor of PARPi sensitivity, and propose targeting LIG3 (and ssDNA gap suppression) as a novel approach to improve response to PARPi[27]. In support of the latter, we found LIG3 to be overexpressed in a portion of triple-negative breast cancers and in serous ovarian cancers, suggesting that LIG3 could possibly be targeted in these cancer types, which are often HR-

deficient and therefore treated with PARPi in the clinic.

The *LIG3* gene encodes both mitochondrial and nuclear protein isoforms[28]. Mitochondrial LIG3 is essential for cellular viability as it ensures mtDNA integrity, and consequently, complete loss of *Lig3* results in cell death and early embryonic lethality in mice[29]. In contrast, nuclear LIG3 has been shown to be dispensable for cell viability[30]. Targeting both nuclear and mitochondrial isoforms of LIG3 with small-molecule inhibitors might therefore result in undesirable toxicity. Importantly, our work shows that loss of the nuclear LIG3 isoform is sufficient to improve response of BRCA1/53BP1 double-deficient cells to PARPi, indicating that PARPi-resistance in these cells is mediated by nuclear LIG3. Moreover, shRNA-mediated depletion of LIG3 (which targets both mitochondrial and nuclear LIG3) had no or minor effects on cell growth and did not affect tumor growth in mice (**Chapter 4, Fig. 2, 6 and supplementary Fig. 4)**, indicating that low levels of mitochondrial LIG3 are sufficient for cell survival. Hence, while complete loss of LIG3 is detrimental for normal cells, it is conceivable that reducing LIG3 activity with selective inhibitors might result in a clinically useful therapeutic window. Indeed, several inhibitors for other DDR targets that are essential for normal cell viability, such as ATR and WEE1 inhibitors, have been developed as anti-cancer drugs and are currently evaluated in clinical trials[31]. Lastly, toxicity derived from loss of mitochondrial LIG3 can be mitigated by targeting the BRCT domain, which is crucial for nuclear LIG3-mediated DNA repair and required for LIG3-mediated PARPi resistance in BRCA1/53BP1 double-deficient cells **(Chapter 4, Fig. 2),** but dispensable for mitochondrial DNA repair. Further experiments are ongoing to assess the effect of mutations in the catalytic and BRCT domain of *LIG3* in the response to PARP inhibition, *in vitro* and *in vivo*. Altogether, our work shows that pharmacological inhibition of LIG3 activity, or other strategies that increase exposure to ssDNA gaps, may constitute a novel approach to counteract PARPi resistance and thereby improve progression-free survival of patients.

ssDNA GAPS AS KEY DETERMINANTS OF PARPi RESPONSE

Until recently, the synthetic lethality observed between PARPi and *BRCA1/2* mutations has been attributed to two key functions of BRCA1 and BRCA2 during DNA replication: (i) their requirement for error-free repair of DSBs via HR and (ii) their role in the protection of stalled replication forks from degradation by nucleases[32]. By showing that depletion of LIG3 resensitizes HR-proficient BRCA1/53BP1 double-deficient cells to PARPi by exposing ssDNA gaps behind replication forks, our work **(Chapter 4 and Chapter 4 addendum)** and that of others revealed a third role of BRCA1 (and BRCA2) in post-replicative ssDNA gap suppression[27]. Importantly, the induction of PARPi-mediated ssDNA gap exposure in LIG3-depleted BRCA1/53BP1 double-deficient cells was not a result of decreased HR, indicating that HRD does not fully explain PARPi response. Moreover, depletion of BRIP1,

which phenocopies loss of BRCA1 in regards to its HR and fork protection (FP) functions, does not result in exposure to PARPi-induced ssDNA gaps, which goes in line with the (unexpected) lack of response of BRIP1-deficient cells to PARPi[27]. Further evidence that PARPi sensitivity cannot be explained by HRD and loss of FP alone arises from observations in Fanconi anemia (FA) patient fibroblasts that carry a dominant RAD51 mutant allele, RAD51-T131P, which was reported to destabilize RAD51 nucleofilaments, leading to unscheduled nucleolytic processing of replicating DNA[33–35]. Unexpectedly, RAD51-T131P cells are HR proficient but sensitive to PARPi, suggesting that the FP defect underlies the sensitivity[27,33]. However, restoration of fork protection by depletion of RADX did not render cells resistant to PARPi[27,36,37]. Instead, PARPi-exposed RAD51-T131P cells displayed increased ssDNA gap exposure which was not reduced upon depletion of RADX, hinting that PARPi-sensitivity of these cells was due to their inability to suppress PARPi-induced ssDNA gaps[27]. Altogether, these data indicate that PARPi-mediated ssDNA gap exposure can explain PARPi response in models in which HR and FP cannot.

It remains unclear, however, if PARPi toxicity stems only from DNA replication-associated ssDNA gaps and if HR and FP can be uncoupled from PARPi response. To understand if ssDNA gaps are better predictors of PARPi response than HR or FP, one should measure PARPi-mediated ssDNA gap formation in all available HR-deficient models of PARPi resistance, as well as in BRCA1/2- and/or HR-proficient PARPi-sensitive cells (e.g., cells deficient for PBRM1, ARID1A or PTEN, which have been reported to be sensitive to PARPi)[32,38–40]. Finally, while the ssDNA gap model remains to be validated in the clinic, one could test the potential clinical relevance of using ssDNA gaps as PARPi response predictors by analyzing PARPi-mediated ssDNA exposure in tumoroids derived from matched PARPi-naïve and PARPi-resistant PDX tumors or the mouse mammary tumors described in **Chapter 3**. If loss of ssDNA gap suppression stands as an ideal PARPi response biomarker, it could improve patient stratification and broaden the range of cancers eligible for PARPi therapy.

CONCLUSIONS

Understanding the biology of PARPi response and resistance in HR-deficient cancers will enable the development of rational treatment strategies to prevent and/or delay the onset of resistance and will ultimately lead to improved outcomes for patients. This thesis contributed to these efforts by providing an unbiased and effective assessment of the contribution of previously reported BRCA1/2-independent resistance mechanisms in an experimentally tractable preclinical *in vivo* setting; and by identifying several candidate genes and pathways that could potentially be exploited in the clinic to improve PARPi response, such as inhibition of LIG3 and ssDNA gap suppression. Moreover, we show that functional genetic screens are key to advance our knowledge on PARPi response and resistance, and that, in order to improve translational value of the results from these screens, it is important to perform

screens in multiple models and to invest in the optimization of *in vivo* screens. Finally, emerging data show that PARPi delay disease recurrence and can prolong OS in certain settings; however, most patients receiving PARPi will ultimately develop progressive disease. It will therefore be important to determine which mechanisms drive PARPi resistance in patients, and whether improvements in PFS and OS can be achieved by giving PARPi earlier in the course of treatment.

REFERENCES

1. Poveda, A. *et al.* Olaparib tablets as maintenance therapy in patients with platinum-sensitive relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a final analysis of a double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Oncol.* **2045**, 1–12 (2021).
2. Pellegrino, B. *et al.* Preclinical In Vivo Validation of the RAD51 Test for Identification of Homologous Recombination-Deficient Tumors and Patient Stratification. *Cancer Res.* **82**, 1646–1657 (2022).
3. Cruz, C. *et al.* RAD51 foci as a functional biomarker of homologous recombination repair and PARP inhibitor resistance in germline BRCA-mutated breast cancer. *Ann. Oncol.* **29**, 1203–1210 (2018).
4. Castroviejo-Bermejo, M. *et al.* A RAD51 assay feasible in routine tumor samples calls PARP inhibitor response beyond BRCA mutation. *EMBO Mol. Med.* **10**, e9172 (2018).
5. Llop-Guevara, A. *et al.* Identification of homologous recombination deficiency (HRD) by RAD51 in a tumor molecular profiling program for precision medicine.
6. Noordermeer, S. M. *et al.* The shieldin complex mediates 53BP1-dependent DNA repair. *Nature* **560**, 117–121 (2018).
7. Barazas, M. *et al.* The CST Complex Mediates End Protection at Double-Strand Breaks and Promotes PARP Inhibitor Sensitivity in BRCA1-Deficient Cells. *Cell Rep.* **23**, 2107–2118 (2018).
8. Dev, H. *et al.* Shieldin complex promotes DNA end-joining and counters homologous recombination in BRCA1-null cells. *Nat. Cell Biol.* **20**, 954–965 (2018).
9. Waks, A. G. *et al.* Reversion and non-reversion mechanisms of resistance to PARP inhibitor or platinum chemotherapy in BRCA1/2-mutant metastatic breast cancer. *Ann. Oncol.* **31**, 590–598 (2020).
10. Evers, B. *et al.* CRISPR knockout screening outperforms shRNA and CRISPRi in identifying essential genes. (2016).
11. Duarte, A. A. *et al.* BRCA-deficient mouse mammary tumor organoids to study cancer-drug resistance. *Nat. Methods* **15**, 134–140 (2018).
12. Pantelidou, C. *et al.* PARP Inhibitor Efficacy Depends on CD8 + T-cell Recruitment via Intratumoral STING Pathway Activation in BRCA-Deficient Models of Triple-Negative Breast Cancer. *Cancer Discov.* **9**, 722–737 (2019).
13. Pellegrino, B. *et al.* PARP inhibition increases immune infiltration in homologous recombination repair (HRR)-deficient tumors. *Ann. Oncol.* **30**, v760 (2019).
14. Mehibel, M. *et al.* Modulating the tumor microenvironment to enhance efficacy of PARP inhibitors. *J. Clin. Oncol.* **37**, e14715–e14715 (2019).
15. Dijkstra, K. K. *et al.* Generation of Tumor-Reactive T Cells by Co-culture of Peripheral Blood Lymphocytes and Tumor Organoids. *Cell* **174**, 1586-1598.e12 (2018).
16. Cattaneo, C. M. *et al.* Tumor organoid–T-cell coculture systems. *Nat. Protoc. 2019 151* **15**, 15–39 (2019).
17. Liu, J. *et al.* Cancer-Associated Fibroblasts Provide a Stromal Niche for Liver Cancer Organoids That Confers Trophic Effects and Therapy Resistance. *CMGH* **11**, 407–431 (2021).
18. Joung, J. *et al.* CRISPR activation screen identifies BCL-2 proteins and B3GNT2 as drivers of cancer resistance to T cell-mediated cytotoxicity. *Nat. Commun.* **13**, (2022).
19. Li, C. *et al.* Quantitative in vivo analyses reveal a complex pharmacogenomic landscape in lung adenocarcinoma. *Cancer Res.* **81**, 4570–4580 (2021).
20. Dervovic, D. *et al.* In vivo CRISPR screens reveal Serpinb9 and Adam2 as regulators of immune therapy response in lung cancer. *bioRxiv* 2022.03.13.484176 (2022).
21. Liu, X. *et al.* Somatic loss of BRCA1 and p53 in mice induces mammary tumors with features of human BRCA1-mutated basal-like breast cancer. *Proc. Natl. Acad. Sci.* **104**, 12111–12116 (2007).
22. Jonkers, J. *et al.* Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. *Nat. Genet.* **29**, 418–425 (2001).
23. Annunziato, S. *et al.* Comparative oncogenomics identifies combinations of driver genes and drug targets in BRCA1-mutated breast cancer. *Nat. Commun. 2019 101* **10**, 1–12 (2019).
24. Annunziato, S. *et al.* Modeling invasive lobular breast carcinoma by CRISPR/Cas9-mediated somatic genome editing of the mammary gland. *Genes Dev.* **30**, 1470–1480 (2016).
25. Manguso, R. T. *et al.* In vivo CRISPR screening identifies Ptpn2 as a cancer immunotherapy target. *Nature* **547**, 413–418 (2017).
26. Brauna, C. J. *et al.* Versatile in vivo regulation of tumor phenotypes by dCas9-mediated transcriptional perturbation. *Proc. Natl. Acad. Sci. U. S. A.* **113**, E3892–E3900 (2016).
27. Cong, K. *et al.* Replication gaps are a key determinant of PARP inhibitor synthetic lethality with BRCA deficiency. *Mol. Cell* (2021).
28. Lakshmipathy, U. & Campbell, C. The human DNA ligase III gene encodes nuclear and mitochondrial proteins. *Mol. Cell. Biol.* **19**, 3869–76 (1999).
29. Puebla-Osorio, N., Lacey, D. B., Alt, F. W. & Zhu, C. Early Embryonic Lethality Due to Targeted Inactivation of

DNA Ligase III. *Mol. Cell. Biol.* **26**, 3935–3941 (2006).
30. Simsek, D. *et al.* Crucial role for DNA ligase III in mitochondria but not in Xrcc1-dependent repair. *Nature* **471**, 245–8 (2011).
31. Pilié, P. G., Tang, C., Mills, G. B. & Yap, T. A. State-of-the-art strategies for targeting the DNA damage response in cancer. *Nature Reviews Clinical Oncology* **16**, 81–104 (2019).
32. Paes Dias, M., Moser, S. C., Ganesan, S. & Jonkers, J. Understanding and overcoming resistance to PARP inhibitors in cancer therapy. *Nature Reviews Clinical Oncology* 1–19 (2021).
33. Wang, A. T. *et al.* A Dominant Mutation in Human RAD51 Reveals Its Function in DNA Interstrand Crosslink Repair Independent of Homologous Recombination. *Mol. Cell* **59**, 478–490 (2015).
34. Zadorozhny, K. *et al.* Fanconi-Anemia-Associated Mutations Destabilize RAD51 Filaments and Impair Replication Fork Protection. *Cell Rep.* **21**, 333–340 (2017).
35. Mijic, S. *et al.* Replication fork reversal triggers fork degradation in BRCA2-defective cells. *Nat. Commun.* **8**, 859 (2017).
36. Bhat, K. P. *et al.* RADX Modulates RAD51 Activity to Control Replication Fork Protection. *Cell Rep.* **24**, 538–545 (2018).
37. Dungrawala, H. *et al.* RADX Promotes Genome Stability and Modulates Chemosensitivity by Regulating RAD51 at Replication Forks. *Mol. Cell* **67**, 374-386.e5 (2017).
38. Chabanon, R. M. *et al.* PBRM1 Deficiency Confers Synthetic Lethality to DNA Repair Inhibitors in Cancer. *Cancer Res.* **81**, 2888–2902 (2021).
39. Shen, J. *et al.* ARID1A Deficiency Impairs the DNA Damage Checkpoint and Sensitizes Cells to PARP Inhibitors. *Cancer Discov.* **5**, 752–767 (2015).
40. Mendes-Pereira, A. M. *et al.* Synthetic lethal targeting of PTEN mutant cells with PARP inhibitors.