Homologous recombination-deficient cancers: approaches to improve treatment and patient selection

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Summary, discussion and future perspectives

Chapter 8

Summary

A very toxic type of DNA lesion that needs to be repaired to maintain genomic integrity and cellular viability, is the DNA double-stranded break (DSB). Homologous recombination (HR) is a tightly regulated pathway that can faithfully repair DSBs in S- or G2-phase of the cell cycle. Mutations in HR genes cause a predisposition to cancer; yet, HR defects also result in increased sensitivity to poly (ADP-ribose) polymerase (PARP) inhibitors due to induced synthetic lethality[1,2].

Mutations in *BRCA1* or *BRCA2*, two core HR genes, lead to an increased risk to develop high-grade serous ovarian cancer (HGSOC) and breast cancer. For patients with ovarian or breast cancers with confirmed *BRCA1/2* mutations, PARP inhibitors have become available for treatment. Unfortunately, acquired or intrinsic resistance to PARP inhibitors occurs, underscoring the need to improve patient selection and improve PARP inhibitor sensitivity to prevent acquired resistance. To this end, it is necessary to further increase our knowledge of the exact working mechanisms of PARP inhibition. Furthermore, it is important to understand the cellular and molecular consequences of BRCA defects in cancer cells to improve tolerable and effective combination therapies.

Genomic instability, which is a hallmark of HR-deficient cancer cells, has increasingly been associated with anti-tumor immune responses, while it has also been coined as a cell-intrinsic mechanism to evade clearance by the immune system. A better understanding of the involvement of the immune system in HR-deficient cancers might further improve effective combination therapies including immune checkpoint inhibitors.

PARP inhibitors are currently approved to treat *BRCA1/2*-mutated ovarian and breast cancer, while HR deficiency can also be caused by mutations in other DNA repair genes. Patients with HR-defective cancers caused by a non-*BRCA1/2* mutation are currently not eligible for PARP inhibitor treatment. To increase the patient population that might benefit from PARP inhibitor treatment, a way to adequately select patients with HR-deficient tumors is needed. In this thesis, we aimed to dissect the molecular mechanisms and cellular consequences of HR-deficient cancer cells to improve and select patients for whom PARP inhibitor treatment may be beneficial.

To assess the relationship between genomic instability and the immune system, a literature study was performed in **chapter 2,** to describe the multiple ways by which genomic instability leads to cGAS/STING-mediated inflammatory signaling. We described that activation of cGAS/STING signaling has consequences on tumor development and leads to both tumor-promoting and anti-tumor responses in the microenvironment. Genomically unstable tumor cells may have evolved to escape immune surveillance mechanisms that are triggered by cGAS/STING signaling. Possible immune-evasion mechanisms involve the upregulation of immune-checkpoint components, expression of oncogenes, upregulation of autophagy, somatic copy number alterations (CNAs), and activation of cytoplasmic nucleases to lower the amount of cytoplasmic DNA and subsequent interferon (IFN) responses. Finally, cGAS/STING-mediated signaling might be an important determinant of anti-cancer therapy responses and this pathway could be therapeutically targeted, for example using STING agonists, to improve responses to immune-checkpoint blockade or DNA-damaging agents.

In **chapter 3**, we aimed to better understand the underlying mechanisms of cell death induced by PARP inhibitor treatment in HR-deficient cancer cells. We observed that DNA lesions induced by PARP inhibitor treatment in BRCA2-depleted cells were transmitted into mitosis. The observed replication lesions, as detected by FANCD2 foci, resulted in increased numbers of chromatin bridges and lagging chromosomes during anaphase and telophase. A similar observation was seen for several human and murine cell lines depleted for BRCA1 or RAD51 and treated with PARP inhibitor. Using live-cell imaging, we showed that

the unresolved chromatin bridges are associated with micronucleation and cell death. Mechanistically, the trapping of PARP during S-phase appeared required for the induction of mitotic chromosome bridges. Finally, we observed that the cytotoxicity of PARP inhibition could be rescued by the depletion of EME1, which led to a mitotic bypass. These data add to the knowledge of how PARP inhibition is cytotoxic in HR-deficient cancer cells. These insights could be further exploited to potentiate PARP inhibitor treatment with combination strategies.

Surprisingly, loss of HR genes such as BRCA2 is tolerated in cancer cells, whereas these genes are essential in normal cells. This phenomenon is called the 'BRCA paradox'. It was suggested that BRCA-deficient cells undergo specific alterations to be able to survive in the presence of genomic instability. In **chapter 4**, we performed a genome-wide genetic screen to identify genes of which a loss of function rescued BRCA2 inactivation. Among the most significant gene mutations, we identified components from the TNFα receptor complex, namely *TNFRSF1A* (encoding TNFR1) and *KHDRBS1* (encoding SAM68), to rescue cell death induced by BRCA2 inactivation in KBM-7 cells. We showed that loss of TNFR1 and SAM68 conferred a survival advantage in several human cancer cell lines depleted for BRCA2. The relation between BRCA2 inactivation and TNFα signaling was shown by the observation that BRCA2 depletion resulted in increased TNFα secretion, activation of downstream TNFR1 signaling kinases JNK and p38, and enhanced sensitivity towards TNFα treatment. The enhanced sensitivity towards TNFα treatment was rescued by depletion of TNFR1 or SAM68 and was not restricted to BRCA2 depletion, as BRCA1 or FANCD2 depletion or low-dose hydroxyurea treatment also sensitized cells to TNFα treatment. Proteomic and transcriptomic analysis revealed the upregulation of IFN-related pathways upon BRCA2 depletion. Finally, we showed that the observed IFN pathway activation upon BRCA2 inactivation is triggered by the formation of micronuclei, which instigates a cGAS/STING-dependent inflammatory response. In conclusion, our data revealed that micronuclei induced by loss of BRCA2 instigate a cGAS/STING-mediated IFN response, which resulted in re-wired TNFα signaling and enhanced TNFα sensitivity.

One of the mechanisms described in chapter 2 that could underlie the observation that genomic unstable tumor cells can escape immune surveillance triggered by cGAS/STING signaling, is oncogene overexpression. In **chapter 5**, we explored whether overexpression of MYC influenced immune responses in murine and human triple-negative breast cancer (TNBC) models in a *BRCA1/2* deficient context. Using two human TNBC cell lines, we found that several overexpressed oncogenes were capable of downregulating immune-related signatures based on RNA sequencing data. We focused on MYC, as MYC is the most frequently overexpressed oncogene in *BRCA*-mutant breast cancer and TNBC. Gene set enrichment analysis (GSEA) on The Cancer Genome Atlas (TCGA) data confirmed that TNBC samples with amplified *MYC* have downregulated immune-related mRNA expression signatures. In a *Brca1*-mutant TNBC mouse model, MYC overexpression resulted in a dramatic loss of lymphocytic infiltration and resulted in decreased tumor latency. Using tumor-derived organoids and BRCA1/2-depleted human TNBC cell lines, we showed that MYC overexpression altered several IFN-related responses, including decreased cytokine secretion, reduced expression of IFN-stimulated genes, and decreased phosphorylation of Interferon regulatory factor 3 (IRF3) and Signal transducer and activator of transcription 1 (STAT1). Furthermore, MYC overexpression directly decreased the migration and activation of lymphocytes *in vitro*. Finally, using chromatin immunoprecipitation (ChIP) followed by whole genome sequencing, we found that MYC directly controlled the expression of cytosolic nucleic acid-sensing factors as a possible mechanism to downregulate IFN signaling in a *Brca1*-depleted context. Combined, we uncovered a potential role of MYC overexpression in the immune evasion of BRCA1/2-defective TNBC through inhibition of STING-mediated IFN responses.

In **chapter 6**, we reviewed the recent literature on how the HR pathway is mechanistically wired and described current treatment options for HR-deficient cancers with a focus on PARP inhibitors. Resistance to PARP inhibition in the clinic is common and we elaborated on the currently known resistance mechanisms, including secondary mutations within the *BRCA1/2* genes that restore their function, mutations in other repair genes such as *TP53BP1*, *REV7,* or *RIF1* to restore HR function, or alterations in *PAXIP* or *PARP1* to restore protection of replication forks. We elaborated on several patient selection methods, such as mutation analyses, genomic 'scar' analyses, or functional HR read-outs, to properly select patients eligible for PARP inhibitor treatment.

Currently, only patients with germline or somatic *BRCA1/2* mutations are eligible for PARP inhibitor treatment, while it is suggested that up to 50% of all HGSOCs are HR-deficient but do not harbor a *BRCA1/2* mutation. In **chapter 7,** we correlated genomic features and *ex vivo* assessed HR functionality and replication fork stability with *in vivo* olaparib responses in a cohort of HGSOC patient-derived xenograft (PDX) models. Based on CNVs profiles and *BRCA1/2* mutations, a subset of PDX models was selected for *in vivo* olaparib sensitivity and *ex vivo* assays. We found that *BRCA1/2* alterations or genomic instability profiles did not correlate significantly to *in vivo* olaparib response, because not all *BRCA1/2*-mutated or genomic unstable models responded to PARP inhibition. We assessed the capability of tumor cells to form RAD51 foci upon irradiation as a read-out for HR functionality using the *ex vivo* RECAP assay. As HR genes are also involved in the protection of replication forks, we additionally assessed the capability of tumor cells to protect stalled replication forks using an *ex vivo* fiber analysis. Replication fork protection or replication speed in *ex vivo* tumor tissue did not correlate to *in vivo* olaparib responses, whereas the RAD51-based RECAP assay identified all PDX models that responded to *in vivo* olaparib, and also detected PARP inhibitor-sensitive models lacking a *BRCA1/2* alteration. Genomic sequence analysis of a panel of DNA repair-associated genes revealed several mutations as a possible underlying cause of HR deficiency which needs further investigation.

Discussion and future perspectives

Mechanisms of cytotoxicity of PARP inhibitors

In chapter 3 we aimed to further elucidate the mechanism-of-action underlying PARP inhibitor cytotoxicity to possibly improve combined treatment strategies and overcome resistance. The initially proposed mechanism of PARP inhibitor sensitivity was based on the role of PARP in single-stranded break (SSB) repair and the increased formation of DSBs during replication, if SSBs are not properly repaired upon PARP inhibition. However, we found that levels of DSBs upon PARP inhibitor treatment in interphase as measured γH2AX foci were only marginal present. Surprisingly, we observed high levels of replication stress upon PARP inhibition and increased levels of unresolved DNA lesions in mitosis resulting in chromatin bridge formation and lagging chromosomes in HR deficient cancer cells. The observed mitotic aberrancies subsequently resulted in multinucleation or cell death. A few years earlier, it was demonstrated that the mechanism of action of PARP inhibitors also involved trapping of PARP onto damaged DNA, resulting in collapsed replication forks[3,4]. Indeed, we showed that genetic inactivation of *PARP1* was not as effective as chemical PARP inhibition, highlighting the importance of the presence of PARP itself. Additionally, we uncovered that progression through mitosis is important for the PARP inhibitor-induced cytotoxicity. Combined, our data highlight that drugs that promote mitotic entry might potentiate the cytotoxic effects of PARP inhibition. These combination strategies could improve PARP inhibitor responses and might also be used to address resistance to PARP inhibition, which is commonly observed in the clinic.

Potentiating PARP inhibitors

We showed that PARP inhibition leads to more mitotic aberrancies and longer mitosis. Furthermore, PARP inhibition slows down the G2 phase of the cell cycle. This cell cycle delay may give cells more time to repair the damage, which might contribute to PARP inhibitor resistance[5]. Therefore, potentially effective therapy may involve a combination of PARP inhibitors with drugs that accelerate mitotic entry, including cell cycle checkpoint inhibitors, such as WEE1, CHK1, or ATR inhibitors. Treatment with these inhibitors inactivates the G2/M checkpoint and thereby forces cells into mitosis while preventing repair of DNA lesions. Indeed, induced mitotic entry upon ATR inhibition increased the cytotoxicity of PARP inhibition in BRCA2-deficient cells[6]. Additionally, PARP inhibition combined with ATR or CHK1 inhibition resulted in premature mitotic entry and increased cell death in *BRCA1/2* mutant ovarian cancer cells[7]. Several clinical studies are currently ongoing that combine PARP inhibitors with ATR inhibitors, predominantly in patients with advanced prostate- and ovarian cancer.

These combination therapies could also be used to treat patients with tumors that are PARP inhibitor-resistant through mechanisms that are independent of accelerated mitotic entry. Indeed, PARP inhibitor resistance was reversed by ATR inhibition as inhibition of ATR disrupts BRCA1-independent loading of RAD51 onto DSBs and stalled replication forks[8,9]. In line with this model, data demonstrated that ATR inhibition suppressed HR and synergized with PARP inhibition in HR-proficient cells[10]. Indeed, ATR inhibition impaired the loading of RAD51, and DNA end resection, resulting in an HR-deficient phenotype[11]. These data suggest that combining ATR inhibitors with PARP inhibition might also be effective beyond *BRCA*-mutated cancers. The combination of PARP inhibitors and cell cycle checkpoint inhibitors could also be synergistic due to the roles of cell cycle checkpoint kinases in the protection of stalled replication forks. For example, ATR protects ssDNA at stalled replication forks by providing RPA[12] and phosphorylation of SMARCAL1[13], while WEE1 negatively regulates MUS81-mediated fork degradation[14]. Through these mechanisms, inhibition of cell cycle checkpoint inhibitors may target two possible PARP inhibitor resistance mechanisms at the same time; a cell cycle arrest in the G2 phase and repair/processing of stalled replication forks. In line with this notion, combination therapies of PARP inhibitors with inhibitors of the WEE1 or ATR kinases have shown efficacy in PARP inhibitor-resistant HR-deficient TNBC and ovarian PDX models. This combination therapy resulted in increased levels of replication stress, including pRPA32 and γ-H2AX[15]. Several clinical studies are currently investigating the combination of PARP inhibitors with ATR inhibitors. The combination treatment of PARP inhibitors with cell cycle checkpoint inhibitors might be effective in both HR-deficient and HR-proficient cancer cells.

PARP inhibitors are currently also being combined with other molecularly targeted drugs including inhibitors of mTOR and EGFR and anti-angiogenics[16,17]. The rationale behind combinations with molecular targeted drugs is to induce an HR deficiency phenotype (also called 'BRCAness') by suppression of HR genes. However, many of these targets are involved in MAPK signaling, which governs cell cycle control at the G1/S transition. If these agents result in a G1/S arrest[18], it could result in diminished effectiveness of PARP inhibition, especially as we have shown that cell cycle progression and mitosis are necessary for PARP inhibitor cytotoxicity (chapter 3).

Inflammatory signaling in HR-deficient cancer cells

It is still largely unknown how cancer cells can survive in the absence of functional HR. Most of the HR genes belong to the human 'essentialome', which lists genes that are essential for the viability of cells[19–21]. This observation also suggests that the previously described 'BRCA paradox' could be regarded as an 'HR paradox'. To address this paradox, in chapter 4 we performed a genome-wide genetic screen and found TNFα signaling as a determinant of cell viability upon BRCA2 depletion. Previously, inactivation of BRCA2 in

human cancer cells was shown to increase sensitivity towards death receptor-mediated apoptosis with compounds activating the TRAIL receptor, which activates pathways similar to those activated by TNFα[22]. Treatment with TRAIL receptor agonists did not result in a cell cycle arrest but induced an early apoptotic event, which supports our findings that BRCA2-depleted cells display intrinsic activation of TNFα-related apoptotic markers. Furthermore, the presence of germline *BRCA1* mutations was associated with decreased TNFα production and lower expression of TNFα-induced ICAM-1 expression on monocytes[23]. Combined with our data, this suggests that *BRCA1* or *BRCA2* mutant tumors are sensitive to autocrine or paracrine TNFα or TRAIL, and therefore need to downregulate TNFα to maintain viability. Also, these findings support the exploration of agents that activate TNFα or TRAIL receptors to induce apoptosis in *BRCA1* or *BRCA2* mutant tumor cells[22].

Activation of TNFα signaling and secretion of TNFα in our cell lines appeared to be part of a broader interferon response triggered by cGAS/STING signaling upon micronuclei formation. cGAS/STING signaling has recently been found to be an important determinant of anti-tumor immune-responses which further supports the importance of whole-genome screening methods to find unexpected players upon DNA damage responses, including TNFα signaling. As a result of BRCA2 deficiency, mitotic aberrancies can result in the formation of micronuclei in the cytoplasm that are recognized by cytoplasmic DNA sensors such as cGAS that subsequently activate a STING-induced interferon response[24]. Both TNFα signaling and interferon signaling, as described in chapter 2, can have pro- and anti-tumor effects[25]. In general, activation of STING-induced interferon signaling serves as a cell-intrinsic innate immune response to trigger cell clearance[26]. Surprisingly, genomic instability induced by e.g. HR deficiency is a common feature of cancer which is associated with increased levels of cytoplasmic DNA. This notion is supported by our observations that the amounts of micronuclei were increased upon BRCA1 or BRCA2 depletion. In line with our data, chromosomally unstable cancer cells, derived from metastatic tumor models, were characterized by a high frequency of chromosome missegregation resulting in elevated levels of cytosolic DNA and an increased inflammatory phenotype[27]. Interestingly, cGAS/STING activation in response to cytoplasmic DNA in these chromosomally unstable cells did not result in previously described interferon signaling[24,28] but instead resulted in non-canonical NF-κB activation[27]. It remains unclear how genomically unstable cancer cells deal with the constant presence of cytoplasmic DNA. A shift towards non-canonical NF-kB signaling might be a mechanism to suppress the anti-tumor immune responses downstream of cGAS/STING activation in favor of a metastatic inflammatory phenotype. Additionally, breast cancers defined by a DNA-damage response deficient profile were associated with lymphocyte infiltration, increased cytosolic DNA, cGAS/STING pathway activation, and cytokine secretion[29]. However, the underlying S-phase damage also resulted in a STING-dependent upregulation of the immune-checkpoint PD-L1, which might explain the lack of immune-mediated cytotoxicity in these tumors[29]. Also, treatment with chemotherapeutic agents was shown to induce PD-L1 expression in ovarian cancer via NF-kB signaling[30]. In line with these data, we also observed the upregulation of PD-L1 expression upon BRCA2 depletion in our TNBC cell lines (unpublished data). Further research is warranted to investigate if the upregulation of non-canonical NF-kB signaling in *BRCA*-mutated cancer cells is responsible for immune evasion despite the continuous presence of cytoplasmic DNA and cGAS/STING activation.

Patient selection for PARP inhibitor treatment

Currently, patients are selected for PARP inhibitor therapy based on *BRCA1/2* mutational status and *BRCA1* promoter methylation. However, mutations in other HR genes have also been reported in multiple cancer types, including *PALB2*, *CHEK2,* or *ATM* mutations[31–33]. Although these mutations are less frequently observed in the clinic (≤2%), they might still

account for a significant patient population that might benefit from PARP inhibitor treatment. Therefore, identifying patients with HR-deficient tumors is important, as has been shown that PARP inhibitors can be effective in patients beyond *BRCA1/2* mutations[34–36].

A challenge in the clinic is that resistance to PARP inhibition is common, which further highlights the importance of patient selection. Several resistance mechanisms have been discovered that were described in chapter 6. For the majority of PARP inhibitor resistance mechanisms, there is a clear difference for *BRCA1* or *BRCA2* mutant cells as BRCA1 and BRCA2 serve distinct roles in HR. For *BRCA1* mutant cells, PARP inhibitor resistance occurs if factors are inactivated that inhibit end resection at DSBs and promote NHEJ, including 53BP1, REV7, Shieldin complex or CST complex genes. Inactivation of these genes results in the restoration of HR in *BRCA1* mutant cells[37–40]. However, BRCA2 functions downstream of end resection in the loading of RAD51, and thus HR cannot be restored in the absence of BRCA2 through modulation of end resection factors[41]. In *BRCA2* mutant cells, inactivation of EZH2/MUS81 and PTIP was shown to restore fork protection, which may underly PARP inhibitor resistance[42,43]. Interestingly, the protection of stalled replication forks from MRE11-mediated degradation was demonstrated to only play a minor role in the viability of BRCA2-deficient cells[44]. These data also suggested that the protection of replication forks is therefore not an important factor in PARP inhibition resistance. It could be that restoration of fork protection in BRCA2-deficient cancer cells, is sufficient to deal with replication stress induced by PARP inhibitors, but is not sufficient to explain the 'BRCA2 paradox'. Furthermore, in chapter 7 we demonstrated that PARP inhibitor sensitivity in HGSOC PDX models was not associated with the ability of tumor cells to protect stalled replication forks.

Current knowledge points towards (independent) functions in the protection of replication forks by many of the HR genes. Up to now, PARP inhibitor resistance in the clinic mainly occurs through the restoration of HR by reverse mutations that restore the reading frame of the mutant allele, suggesting that the function of HR is dominant in determining PARP inhibitor sensitivity[45,46].

Many of the PARP inhibitor resistance mechanisms in a BRCA1/2-deficient context have been discovered in cell line or mouse models to explain the biology underlying possible resistance mechanisms, but have not yet been frequently assessed in the clinic. In metastatic breast cancer tissue used for PDX experiments, *TP53BP1* mutations and RAD51 amplification were found to cause PARP inhibitor resistance in *BRCA1*-mutant tumors[47]. Furthermore, decreased 53BP1 levels were associated with decreased anti-tumor efficacy of the PARP inhibitor ABT-767 in HR-deficient ovarian cancer biopsies[48]. As the patient population that is being treated with PARP inhibitors is increasing, resistance will more often occur and will lead to increased insight in which resistance mechanisms are clinically relevant. In parallel, organoids and primary cultures derived from PARP inhibitor-resistant patients followed in time could serve to gain further insight into the sensitivity and resistance mechanisms to PARP inhibitors.

As described previously, secondary mutations within the mutant HR gene or in other additional genes can restore HR function or replication fork stability. Additionally, there are large numbers of 'variants of unknown significance' (VUS) within HR genes for which it is currently unclear if they have pathogenic potential[49]. Therefore, genetic testing is complicated by the large numbers of VUS alleles and possible mutational combinations that restore HR function need to be included. A proper patient selection tool should aim to identify tumors that capture HR deficiency on a genomic or functional level, which is also known as the 'BRCAness' phenotype.

Various HR deficiency tests have been developed that mainly focus on genomic tumor features. The myChoice HRD test was unable to predict responses to the PARP inhibitor Niraparib in ovarian cancer[35]. HRDetect, based on whole-genome sequencing profiles from

BRCA1/2-mutated breast cancers, was able to detect HR deficient tumors that responded to platinum-based chemotherapy[50]. However, studies that use HRDetect to predict responses to PARP inhibitors are currently lacking. Importantly, these genomic analyses do not reflect the current HR functionality, which is required at the time of treatment decision making.

The importance of functional HR testing at the time of treatment initiation was demonstrated in *BRCA1/2*-mutated breast cancer PDX models in which restoration of HR occurred and caused PARP inhibitor resistance[47]. Specifically, secondary mutations that cause PARP inhibitor resistance, can arise during treatment with DNA damaging agents such as chemotherapy or PARP inhibitor treatment. Indeed, a majority of the PARP inhibitor-resistant models in this manuscript were originally derived from metastatic *BRCA1*-mutant breast cancer patients that were pre-treated with chemotherapy regimens or olaparib, but in which the *BRCA1* mutation was still present in the harvested tumor tissue for PDX development[47]. Two of the resistant models were shown to have somatic mutations in *TP53BP1* resulting in 53BP1 loss, which was previously been described to be an important PARP inhibitor resistance mechanism in a *BRCA1*-mutated context[39,51,52]. In a different study, three metastatic breast cancer patients with a germline *BRCA1* or *BRCA2* mutation were identified as HR deficient initially but became HR-proficient after treatment with several DSB-inducing agents, including carboplatin with or without PARP inhibition[53]. In the described studies, restoration of HR could be detected with a functional RAD51-based assay[47,53]. Patients with TNBC or HGSOC are, both in the current setting and in clinical trials, often pre-treated with (platinum-based) chemotherapy prior to PARP inhibitor treatment, possibly inducing resistance. As platinum-based chemotherapy and PARP inhibitor sensitivity often co-exist due to similar underlying DNA repair deficiencies, a functional HR test will probably predict platinum response initially and subsequent PARP inhibitor response in consecutive treatment regimes. However, it should be noted that only 53.8% of a cohort of patients with HR-deficient ovarian cancer responded to platinum chemotherapy[54]. Also, PARP inhibitor resistance can occur in platinum-sensitive tumors and vice versa[40,55]. Of note in this context, *TP53BP1* mutations were shown to cause resistance to PARP inhibition but not cisplatin resistance in *BRCA1* mutant mouse models[39]. It is important to develop patient selection tools for both PARP inhibition and platinum chemotherapy separately.

Many studies that report on the effectiveness of functional testing of HR were performed in breast cancer and studies to correlate functional HR to *in vivo* PARP inhibitor response are lacking in ovarian cancer. In chapter 7, we performed a functional RAD51-based assay called the RECAP (REpair CAPacity) assay in ovarian PDX models. From several functional and genomic features, the RECAP assay effectively predicted *in vivo* olaparib response and identified a subset of PARP inhibitor-sensitive, HR-deficient PDX tumors lacking a *BRCA1/2* alteration. Within our consortium, the RECAP test was compared to two genomic scar based HR deficiency tests, a BRCA1/2-like classifier[56] and Classifier of HOmologous Recombination Deficiency (CHORD)[57], in a cohort of 71 breast tumors[58]. These different tests could not identify the same population of breast cancer patients as HR deficient (60-70% concordance). Using the BRCA1-classifier in our panel of PDX models, we identified 6 models of which 5 responded to *in vivo* olaparib and were also HR deficient based on the RECAP assay. More importantly, additional PDX models were identified by the RECAP assay in our PDX cohort that responded to *in vivo* olaparib but were not identified by the BRCA1-classifier (false negatives using the BRCA1-classifier). Multiple HR deficiency tests, both functional and genomic, should be included in clinical trials to determine which one predicts best for *in vivo* PARP inhibitor response. Until now, RAD51-based assays have shown promising results in identifying HR-deficient tumors and in predicting *in vivo* response in several models for breast cancer or ovarian cancer[59–62]. Surprisingly, one report suggested that diminished RAD51 foci failed to predict response to the PARP inhibitor Niraparib in a few HGSOC PDX models[63]. However, in

this specific study, the RAD51 foci assay was conducted in a completely different set-up, in which dissociated PDX cells were used, at different time points post-irradiation and using a higher irradiation dose. Also, proper controls were lacking to discriminate HR-proficient from HR-deficient cells.

The next step in the development of PARP inhibitor patient selection tools is to design proper prospective clinical trials to validate the predictive value of the RECAP assay in patients treated with PARP inhibitors. Although the RECAP assay appears a very effective test in predicting response, it comes with some logistic and functional challenges. Firstly, the RECAP test requires the processing of fresh tumor tissue, the availability of a radiation source, and three days of physical labor. Also, the assay requires the use of an antibody, which is considered an unstable reagent. Furthermore, the use of the RECAP assay in ovarian tissue seems challenging as only 40% of patient samples resulted in a successful RECAP result (manuscript in preparation). In the UMCG, we encountered similar challenges in performing the RECAP assay in ovarian tissue due to pre-treated biopsies with chemotherapy and the use of eosin during the process for pathology diagnosis, which interferes with immunofluorescence analysis (unpublished data). Also, a relatively low frequency of HR-deficient samples was detected in the ovarian cancer patient cohort (20%), which might be explained by the high percentage of these patients receiving neoadjuvant treatment, whereby patients who respond to platinum due to an HR deficiency were not included for further analysis (in preparation, LUMC). Eventually, a good functional test should be designed to be applicable in all hospitals and on several tumor types. Transforming the RECAP assay to a RAD51 foci staining on paraffin-embedded material with immunohistochemistry on unirradiated tissue should be of great value and has already been demonstrated to be effective in archived breast tumor samples[61].

Besides the challenges that have to be overcome in performing the RECAP test, the RECAP test is restricted to a selected feature (RAD51 recruitment) of one specific pathway. The test thereby excludes possible unknown functions of HR genes that might also be of importance in PARP inhibitor sensitivity. Namely, impaired ribonucleotide excision repair caused by mutations in ribonuclease H2 was shown to induce PARP inhibitor sensitivity, which is independent of HR function[64]. It is currently unknown how relevant ribonucleotide excision repair deficiency is in cancer.

It is still not entirely clear if the ability of cells to protect replication forks is of importance for PARP inhibitor sensitivity and resistance. Interestingly, organoids derived from a germline *BRCA2* mutant ovarian cancer patients and positive for a genomic HR-deficient signature, appeared to PARP inhibitor-resistant but sensitive to carboplatin and ATR inhibition, suggesting an underlying fork protection defect and not an HR defect[65]. Unfortunately, functional RAD51 foci formation could not be assessed in these organoids to determine HR status. This observation underscores the need to determine how important functional pathways, including replication fork protection, are for PARP inhibitor sensitivity in clinical samples. However, using separation-of-function mutations, replication fork protection was shown to be of minor importance for PARP inhibitor sensitivity in BRCA2 deficient cells[44]. In our HGSOC PDX cohort, replication fork protection also did not correlate to *in vivo* olaparib response as shown in chapter 7.

Silencing inflammatory signaling by oncogene expression

Many cancer types, including TNBC and HGSOC, are characterized by high levels of genomic instability as well as overexpression of a variety of oncogenes. In chapter 5 we aimed to unravel the role of oncogene overexpression in the tumor-cell intrinsic inflammatory response and found that overexpression of several oncogenes in breast cancer is associated with silenced immune-related signatures. We demonstrated that MYC overexpression diminished anti-tumor

immune responses in BRCA-deficient TNBC models. MYC was previously shown to regulate immune signaling, through modulation of the expression of immune checkpoint proteins CD47 and PD-L1[66,67]. Furthermore, preliminary data suggested that MYC, in complex with MIZ1, prevents activation of type I interferon response induced by genomic instability[68]. Recently, and in line with our data, MYC-MIZ1 complexes were shown to bind directly to promoters of IFN regulators, including IRFs and STAT1. Thereby, MYC suppressed IFN signaling and NK cell-mediated immune responses in pancreatic cancer models[69]. Important to note in this context is that overexpression of MYC did not rescue cell death induced by depletion of BRCA1 or BRCA2 in our panel of TNBC cell line models. The possible role of MYC in altering immune responses, therefore, does not appear to be related to directly resolving the 'BRCA paradox'.

In vivo, we demonstrated that MYC overexpression completely abolished the presence of immune cells in the tumor microenvironment. Interestingly, TNBC and HGSOC patients are often characterized by high amounts of tumor-infiltrating lymphocytes (TILs)[59] and it has been shown that responses to immune checkpoint inhibitors strongly depend on the composition and activity of immune cells in the tumor[70]. Interestingly, improved immunogenicity was observed in *BRCA1* mutant ovarian cancer, as judged by infiltration of T cells, which was triggered by interferon signaling and could be augmented by PARP inhibitor treatment (unpublished data, Coukos et al.). Unfortunately, clinical responses to immune checkpoint inhibitor monotherapies have been disappointing in patients with TNBC and HGSOC[71,72]. In contrast to some literature, the decreased amounts of TILs observed in our *Brca1*-mutant mouse model might be caused by the high levels of amplified MYC in our models (~8-fold) compared to patient tumors, in which MYC expression is generally considered an amplification with an average of >2,46 copy number amplification. Furthermore, MYC overexpression is the only altered factor in our mouse model, while a patient tumor harbors many more alterations besides an MYC amplification making the mouse model a very clean but also somewhat extreme situation. In line with our data, elevated expression of MYC in *KRAS* mutant lung and pancreatic cancer models resulted in immune-suppressive tumor microenvironments by altered CCL9/ IL-23 signaling and repression of IFN regulators[67,69]. These data demonstrated that overexpression of MYC also altered the immune micro-environment of the tumor cells extrinsically. We additionally estimated immune cell type fractions in complex TCGA breast cancer samples using CIBERSORT analyses and showed that high MYC expression correlated to lower fractions of $CD8^+$ T cells. However, caution should be taken when drawing conclusions based on TCGA data, as these samples contain complex biopsies that are often taken at the edge of the tumor in which cell populations might not exactly reflect the situation within the tumor. It would be interesting to perform retrospective sub-analyses on breast cancer samples to find correlations between high MYC expression and TILs using immunohistochemistry. A key experiment to confirm the role of MYC in the infiltration of immune cells *in vivo* is the use of mice without an adaptive immune system to verify if the effects of MYC on earlier tumor development depend on the suppression of an adaptive immune response.

We demonstrated that MYC overexpression not only diminished immune cell recruitment but also lowered the activity of T cells *in vitro*. Interestingly, high amounts of CNAs were also associated with decreased $CD8^+$ T cell activity in the tumor microenvironment[73,74] whereas high MYC levels were associated with decreased CNAs in the *Brca1*-mutant mouse model that we used[75]. Future research should aim to uncover if overexpression of MYC not only diminishes the presence and activation of immune cells *in vivo* but also if MYC amplification is associated to the level of CNAs and are together responsible for shaping the tumor microenvironment.

MYC was previously described as a master regulator that targets a large part of the transcriptome[76]. Recent literature suggests that MYC targets multiple regulators of the IFN pathway simultaneously[69]. We ultimately identified an immune-related co-functionality

network consisting of genes that were downregulated upon direct binding and expression of MYC and could explain in the diminished inflammatory responses. One of the identified downregulated genes was *DDX58*, the gene encoding RIG-I, which was shown to be a cytoplasmic sensor of RNA, instead of DNA[77]. Recently, mitochondrial DNA damage was demonstrated to result in elevated levels of cytoplasmic RNA that triggered an interferon response via RIG-I and was independent of cGAS[78]. However, it remains unclear if the depletion of BRCA1/2 also results in cytoplasmic RNA release. Also, it has been suggested that RIG-I indirectly can trigger interferon signaling in response to cytoplasmic DNA[79]. The role of mitochondrial damage and the release of RNA in the cytoplasm should be further studied in the context of cancer. Importantly, it is not excluded that there is overlap in the recognition of DNA and RNA by several pattern recognition receptors, including cGAS and RIG-I, to trigger interferon signaling in response to cytoplasmic nucleic acids. Interestingly, systemic RIG-I activation enhanced the sensitivity to anti-PD-1 checkpoint inhibition in an acute myeloid leukemia model via interferon signaling and increased numbers of T cells[80].

Technically, for future research regarding MYC overexpression, it is advised that experiments should be performed using multiple monoclonal cell lines, as the selection of clones from a heterogeneous population will probably result in clones that have significant biological differences due to different levels of MYC expression. Additionally, the use of an inducible plasmid might also be an alternative to the use of multiple clones per cell line and thereby resembling a more polyclonal *in vivo* situation. Also, the cell line models that we used were not completely compatible with the *in vivo* models, as tumor development cannot be followed in cell lines. Yet, these cell line models reflect relatively clean genetic models, which we used to demonstrate that MYC overexpression diminished the interferon response upon acute BRCA depletion *in vitro*. In an *in vivo* setting, this could allow BRCA1/2-depleted cells to elongate their viability and remain under the radar of the immune system longer, and thereby obtain enough time to acquire properties to survive BRCA depletion.

Targeting oncogene expression and interferon signaling in genomic unstable cancer

The recruitment and activity of immune cells, together with NF-κB and interferon-γ signaling, determine responses to immunotherapy[81,82]. In chapters 2, 4, and 5 we demonstrated that these pathways are increased upon BRCA1/2 inactivation and are potentially decreased by oncogene overexpression. These observations highlight the importance of understanding the exact consequences of HR deficiency on the tumor environment to improve treatment regimes. Important follow-up questions in this context are: Can we further trigger interferon signaling to increase sensitivity towards immune therapies? And: Can we target the upstream mechanism that is responsible for the downregulation of interferon signaling in HR-deficient tumors, such as MYC overexpression?

MYC has been studied extensively in the past, but it never resulted in direct targeting of MYC using specific inhibitors[83]. Recently, inhibition of cyclin-dependent kinase (CDK) 7 and CDK12/13 by THZ1 was demonstrated to target transcriptional addiction in cancer cells[84], including cellular addiction to MYC. Specifically, THZ1 treatment decreased MYC expression and was effective in ovarian cancer PDX models[85]. Furthermore, CDK7 inhibition caused DNA damage, micronuclei formation, and interferon signaling which was suggested to be independent of cGAS/STING signaling, although an alternative mechanism is lacking[86]. Dinaciclib, a CDK1/2/5/9 inhibitor, was synthetic lethal with MYC expression in TNBC[87,88]. Interestingly, combined treatment of dinaciclib with niraparib, a PARP inhibitor, increased DNA damage levels and downregulated HR resulting in synthetic lethality in TNBC models[87]. Additionally, the expression of MYC determined the sensitivity to combined treatment of olaparib with Palbociclib (CDK4/6 inhibitor) in ovarian cancer[89]. These data suggest possible combination strategies with CDK inhibitors to target tumors with overexpressed MYC.

Furthermore, Bromo- and Extra-Terminal domain (BET) bromodomain inhibitors, often used in the setting of acute leukemia and multiple myeloma, were demonstrated to downregulate transcription and expression of MYC and MYC-dependent target genes[90,91]. Several studies already reported the effective combination of bromodomain inhibitors with PARP inhibitors in HR-proficient cancer cells, based on the downregulation of genes involved in HR by bromodomain inhibitors[92,93]. Future research is needed to investigate if bromodomain inhibitors might also be effective in *BRCA*-mutant cancer cells, that depend on overexpression of MYC for evading clearance by the immune system.

Of note, it was demonstrated that activation of the STING pathway is required for the olaparib response in BRCA1-deficient ovarian tumors[94]. In contrast to acute inactivation of BRCA in cell line models, a constitutive *BRCA1* defect in itself did not result in an increased interferon response in this *in vivo* model[94] as these cancer cells might already be adapted to evade the immune system. However, PARP inhibition might trigger interferon signaling in these tumors as these signaling cascades are probably suppressed in established tumors. The combination of olaparib with a STING inhibitor or blocking antibody against Interferon Alpha And Beta Receptor Subunit 1 (IFNAR1), both attenuated the antitumor activity of olaparib indicating that caution should be taken by combining PARP inhibitors with agents that inhibit STING or IFN responses[94]. Additionally, tumor cells lacking IFNAR failed to respond to chemotherapy, highlighting the importance of interferon signaling in tumor cells towards DNA damaging agents[95].

One of the approaches to trigger interferon signaling in tumors is through the administration of STING agonists, including 2′3′-Cyclic GMP-AMP (cGAMP). cGAMP is a second messenger that is produced by cGAS in response to cytosolic double-stranded DNA, which subsequently activates STING. Notably, cGAMP can also be excreted and taken up by neighboring cells, to activate e.g. NK cells[96–98]. STING agonists were shown to be able to promote radiation-induced anti-cancer immunity and showed promising effects *in vivo*[99–101]. However, poor results in clinical studies were obtained so far due to instability and high polarity of the drugs or due to poor STING agonist capacity[102]. cGAMP treatment in combination with immune checkpoint therapies or DNA damaging agents is suggested to be more effective than cGAMP treatment alone. Specifically, activation of STING by cGAMP alone resulted in immune cells with low cross-priming activity[103]. Currently, several new synthetic cGAMP compounds are being investigated in clinical trials in combination with immune checkpoint inhibitors (e.g. NCT03010176, NCT02675439, NCT03172936). However, caution should be taken regarding cGAMP treatment in tumors that are not chromosomally unstable, as it has been shown that cGAMP increases invasion and migration of cells with low chromosomal instability, probably due to the tumor-promoting effects of non-canonical NF-κB activation[27,104].

The above-mentioned STING agonists are currently not being tested in *BRCA* mutant cancers specifically. However, the described effects of cGAS/STING pathway activation in BRCA-deficient cells on innate immune responses suggest a prominent role for immune checkpoint inhibition in genomically unstable tumors. Indeed, cGAS and STING protein levels correlated to PD-L1 expression in ovarian cancer cell lines that could be further enhanced by cGAMP treatment[105]. Furthermore, PD-L1 levels are increased upon induction of DSBs and upon loss of DNA repair proteins[106], thereby supporting the rationale of combining DNA damaging agents, including PARP inhibitors, with immune checkpoint inhibitors[107,108]. A phase I study combining pamiparib with tislelizumab in solid tumors showed promising results[109]. Also, combining niraparib with pembrolizumab in patients with recurrent ovarian carcinoma was tolerable and showed better responses than monotherapy of both agents, also in patients without a BRCA mutation[110]. Currently, approximately 10 clinical trials are ongoing in which PARP inhibitors are combined with immune checkpoint inhibitors (e.g. NCT02734004, NCT02571725) illustrating the high expectations of targeting DNA damage responses and immune responses simultaneously.

Concluding remarks

Unraveling the mechanisms and consequences of HR deficiency in cancer cells has led to important new insights into the links between DNA damage and immune responses. Future research should aim to investigate how genomic unstable cancer types can suppress the anti-tumor immune responses and investigate how these mechanisms could be targeted to improve the outcome of immune checkpoint inhibitor therapies. It appears of key importance to target HR-deficient tumors at three levels, specifically; induction of DNA damage, triggering cGAS/STING signaling and interferon signaling, and targeting immune checkpoints. For the selection of patients for PARP inhibitor treatment, it is important to perform prospective clinical studies to compare several functional and genomic HR deficiency tests.

References

1. Farmer, H. et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 434, 917–921 (2005).
2. Bryant, H. E. et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature 434, 913–917 (2005).
3. Pommier, Y., O'Connor, M. J. & De Bono, J. Laying a trap to kill cancer cells: PARP inhibitors and their mechanisms of action. Science Translational Medicine vol. 8 362ps17 (2016).
4. Murai, J. et al. Trapping of PARP1 and PARP2 by clinical PARP inhibitors. Cancer Res. 72, 5588–5599 (2012).
5. Rein, I. D., Landsverk, K. S., Micci, F., Patzke, S. & Stokke, T. Replication-induced DNA damage after PARP inhibition causes G2 delay, and cell line-dependent apoptosis, necrosis and multinucleation. Cell Cycle 14, 3248–3260 (2015).
6. Schoonen, P. M. et al. Premature mitotic entry induced by ATR inhibition potentiates olaparib inhibition-mediated genomic instability, inflammatory signaling, and cytotoxicity in BRCA2-deficient cancer cells. Mol. Oncol. 13, 2422–2440 (2019).
7. Kim, H. et al. Targeting the ATR/CHK1 axis with PARP inhibition results in tumor regression in BRCA-mutant ovarian cancer models. Clin. Cancer Res. 23, 3097–3108 (2017).
8. Yazinski, S. A. et al. ATR inhibition disrupts rewired homologous recombination and fork protection pathways in PARP inhibitor-resistant BRCA-deficient cancer cells. Genes Dev. 31, 318–332 (2017).
9. Haynes, B., Murai, J. & Lee, J. M. Restored replication fork stabilization, a mechanism of PARP inhibitor resistance, can be overcome by cell cycle checkpoint inhibition. Cancer Treatment Reviews vol. 71 1–7 (2018).
10. Ning, J. F. et al. Myc targeted CDK18 promotes ATR and homologous recombination to mediate PARP inhibitor resistance in glioblastoma. Nat. Commun. 10, 1–18 (2019).
11. Dibitetto, D. et al. ATR Inhibitors as Potent Modulators of DNA End Resection Capacity. doi:10.1101/2020.01.13.905059.
12. Toledo, L. I. et al. XATR prohibits replication catastrophe by preventing global exhaustion of RPA. Cell 155, 1088 (2013).
13. Couch, F. B. et al. ATR phosphorylates SMARCAL1 to prevent replication fork collapse. Genes Dev. 27, 1610–1623 (2013).
14. Domínguez-Kelly, R. et al. Wee1 controls genomic stability during replication by regulating the Mus81-Eme1 endonuclease. J. Cell Biol. 194, 567–579 (2011).
15. O'Connor, M. J. et al. Abstract 932: Reversing PARP inhibitor resistance by targeting the replication stress response. in Cancer Research vol. 79 932–932 (American Association for Cancer Research (AACR), 2019).
16. Ibrahim, Y. H. et al. PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient triple-negative breast cancer to PARP inhibition. Cancer Discov. 2, 1036–1047 (2012).
17. 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 vol. 16 81–104 (2019).
18. Russo, M. et al. Adaptive mutability of colorectal cancers in response to targeted therapies. Science (80-.). 366, 1473–1480 (2019).
19. Blomen, V. A. et al. Gene essentiality and synthetic lethality in haploid human cells. Science (80-.

). 350, 1092–1096 (2015).
20. Wang, T. et al. Identification and characterization of essential genes in the human genome. Science (80-.). 350, 1096–1101 (2015).
21. Hart, T. et al. High-Resolution CRISPR Screens Reveal Fitness Genes and Genotype-Specific Cancer Liabilities. Cell 163, 1515–1526 (2015).
22. De Toni, E. N. et al. Inactivation of BRCA2 in human cancer cells identifies a subset of tumors with enhanced sensitivity towards death receptormediated apoptosis. Oncotarget 7, 9477–9490 (2016).
23. Zielinski, C. C. et al. Defect of tumour necrosis factor-alpha (TNF-alpha) production and TNF-alpha-induced ICAM-1-expression in BRCA1 mutations carriers. Breast Cancer Res. Treat. 81, 99–105 (2003).
24. Harding, S. M. et al. Mitotic progression following DNA damage enables pattern recognition within micronuclei. Nature 548, 466–470 (2017).
25. Montfort, A. et al. The TNF paradox in cancer progression and immunotherapy. Frontiers in Immunology vol. 10 (2019).
26. Ishikawa, H., Ma, Z. & Barber, G. N. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. Nature 461, 788–92 (2009).
27. Bakhoum, S. F. et al. Chromosomal instability drives metastasis through a cytosolic DNA response. Nature 553, 467–472 (2018).
28. Dou, Z. et al. Cytoplasmic chromatin triggers inflammation in senescence and cancer. Nature 550, 402–406 (2017).
29. Parkes, E. E. et al. Activation of STING-Dependent Innate Immune Signaling By S-Phase-Specific DNA Damage in Breast Cancer. J. Natl. Cancer Inst. 109, djw199 (2017).
30. Peng, J. et al. Microenvironment and Immunology Chemotherapy Induces Programmed Cell Death-Ligand 1 Overexpression via the Nuclear Factor-kB to Foster an Immunosuppressive Tumor Microenvironment in Ovarian Cancer. (2015) doi:10.1158/0008-5472.CAN-14-3098.
31. Heeke, A. L. et al. Prevalence of Homologous Recombination–Related Gene Mutations Across Multiple Cancer Types. JCO Precis. Oncol. 2018, 1–13 (2018).
32. Minion, L. E. et al. Hereditary predisposition to ovarian cancer, looking beyond BRCA1/BRCA2. Gynecol. Oncol. 137, 86–92 (2015).
33. Buys, S. S. et al. A study of over 35,000 women with breast cancer tested with a 25-gene panel of hereditary cancer genes. Cancer 123, 1721–1730 (2017).
34. Coleman, R. L. et al. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet (London, England) 390, 1949–1961 (2017).
35. Mirza, M. R. et al. Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. N. Engl. J. Med. 375, 2154–2164 (2016).
36. Mateo, J. et al. DNA-repair defects and olaparib in metastatic prostate cancer. N. Engl. J. Med. 373, 1697–1708 (2015).
37. 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).
38. 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).
39. Bouwman, P. et al. 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. Nat. Struct. Mol. Biol. 17, 688–695 (2010).
40. Xu, G. et al. REV7 counteracts DNA double-strand break resection and affects PARP inhibition. Nature 521, 541–544 (2015).
41. Gogola, E., Rottenberg, S. & Jonkers, J. Resistance to PARP Inhibitors: Lessons from Preclinical Models of BRCA-Associated Cancer. Annu. Rev. Cancer Biol. 3, 235–254 (2019).
42. Rondinelli, B. et al. EZH2 promotes degradation of stalled replication forks by recruiting MUS81 through histone H3 trimethylation. Nat. Cell Biol. 19, 1371–1378 (2017).
43. Chaudhuri, A. R. et al. Replication fork stability confers chemoresistance in BRCA-deficient cells. Nature 535, 382–387 (2016).
44. Feng, W. & Jasin, M. BRCA2 suppresses replication stress-induced mitotic and G1 abnormalities through homologous recombination. Nat. Commun. 8, (2017).

45. Lord, C. J. & Ashworth, A. PARP inhibitors: Synthetic lethality in the clinic. Science (80-.). 355, 1152–1158 (2017).
46. Kondrashova, O. et al. Secondary Somatic Mutations Restoring RAD51C and RAD51D Associated with Acquired Resistance to the PARP Inhibitor Rucaparib in High-Grade Ovarian Carcinoma. Cancer Discov. 7, 984–998 (2017).
47. 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).
48. Hurley, R. M. et al. 53BP1 as a potential predictor of response in PARP inhibitor-treated homologous recombination-deficient ovarian cancer. Gynecol. Oncol. 153, 127–134 (2019).
49. Easton, D. F. et al. A systematic genetic assessment of 1,433 sequence variants of unknown clinical significance in the BRCA1 and BRCA2 breast cancer-predisposition genes. Am. J. Hum. Genet. 81, 873–883 (2007).
50. Davies, H. et al. HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. Nat. Med. 23, 517–525 (2017).
51. Bunting, S. F. et al. 53BP1 inhibits homologous recombination in brca1-deficient cells by blocking resection of DNA breaks. Cell 141, 243–254 (2010).
52. Noordermeer, S. M. et al. The shieldin complex mediates 53BP1-dependent DNA repair. Nature vol. 560 117–121 (2018).
53. Meijer, T. G. et al. Direct Ex Vivo Observation of Homologous Recombination Defect Reversal After DNA-Damaging Chemotherapy in Patients With Metastatic Breast Cancer. JCO Precis. Oncol. 1–12 (2019) doi:10.1200/po.18.00268.
54. Mukhopadhyay, A. et al. Clinicopathological features of homologous recombination-deficient epithelial ovarian cancers: Sensitivity to PARP inhibitors, platinum, and survival. Cancer Res. 72, 5675–5682 (2012).
55. Ceccaldi, R. et al. A unique subset of epithelial ovarian cancers with platinum sensitivity and PARP inhibitor resistance. Cancer Res. 75, 628–634 (2015).
56. Schouten, P. C. et al. Robust BRCA1-like classification of copy number profiles of samples repeated across different datasets and platforms. Mol. Oncol. 9, 1274–1286 (2015).
57. Nguyen, L., Martens, J., Hoeck, A. van & Cuppen, E. Pan-cancer landscape of homologous recombination deficiency. bioRxiv 2020.01.13.905026 (2020) doi:10.1101/2020.01.13.905026.
58. Meijer, T. G. et al. RECAP identifies BRCAness samples undetected by DNA-based homologous recombination deficiency tests. (Erasmus MC, 2020).
59. Meijer, T. G. et al. Functional ex vivo assay reveals homologous recombination deficiency in breast cancer beyond BRCA gene defects. Clin. Cancer Res. 24, 6277–6287 (2018).
60. Naipal, K. A. T. et al. Functional Ex vivo assay to select homologous recombination-deficient breast tumors for PARP inhibitor treatment. Clin. Cancer Res. 20, 4816–4826 (2014).
61. Castroviejo-Bermejo, M. et al. A RAD 51 assay feasible in routine tumor samples calls PARP inhibitor response beyond BRCA mutation . EMBO Mol. Med. 10, (2018).
62. Mukhopadhyay, A. et al. Development of a functional assay for homologous recombination status in primary cultures of epithelial ovarian tumor and correlation with sensitivity to poly(ADP-ribose) polymerase inhibitors. Clin. Cancer Res. 16, 2344–2351 (2010).
63. AlHilli, M. M. et al. In vivo anti-tumor activity of the PARP inhibitor niraparib in homologous recombination deficient and proficient ovarian carcinoma. Gynecol. Oncol. 143, 379–388 (2016).
64. Zimmermann, M. et al. CRISPR screens identify genomic ribonucleotides as a source of PARP-trapping lesions. Nature 559, 285–289 (2018).
65. Hill, S. J. et al. Prediction of DNA repair inhibitor response in short-term patient-derived ovarian cancer organoids. Cancer Discov. 8, 1404–1421 (2018).
66. Casey, S. C. et al. MYC regulates the antitumor immune response through CD47 and PD-L1. Science 352, 227–31 (2016).
67. Kortlever, R. M. et al. Myc Cooperates with Ras by Programming Inflammation and Immune Suppression. Cell 171, 1301-1315.e14 (2017).
68. Krenz, B. et al. MYC suppresses genomic-instability-induced innate immune signalling. (2019).
69. Muthalagu, N. et al. Repression of the Type I Interferon pathway underlies MYC & KRAS-dependent evasion of NK & B cells in Pancreatic Ductal Adenocarcinoma. Cancer Discov. CD-19-0620 (2020) doi:10.1158/2159-8290.cd-19-0620.

70. Binnewies, M. et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. Nat. Med. 24, 541–550 (2018).
71. Voorwerk, L. et al. Immune induction strategies in metastatic triple-negative breast cancer to enhance the sensitivity to PD-1 blockade: the TONIC trial. Nature Medicine vol. 25 920–928 (2019).
72. Hamanishi, J., Mandai, M. & Konishi, I. Immune checkpoint inhibition in ovarian cancer. doi:10.1093/intimm/dxw020.
73. Davoli, T., Uno, H., Wooten, E. C. & Elledge, S. J. Tumor aneuploidy correlates with markers of immune evasion and with reduced response to immunotherapy. Science (80-.). 355, (2017).
74. Rooney, M. S., Shukla, S. A., Wu, C. J., Getz, G. & Hacohen, N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. Cell 160, 48–61 (2015).
75. Annunziato, S., Barazas, M., Rottenberg, S. & Jonkers, J. Genetic Dissection of Cancer Development, Therapy Response, and Resistance in Mouse Models of Breast Cancer. doi:10.1101/sqb.2016.81.030924.
76. Kress, T. R., Sabò, A. & Amati, B. MYC: Connecting selective transcriptional control to global RNA production. Nature Reviews Cancer vol. 15 593–607 (2015).
77. Zhao, Y., Ye, X., Dunker, W., Song, Y. & Karijolich, J. RIG-I like receptor sensing of host RNAs facilitates the cell-intrinsic immune response to KSHV infection. Nat. Commun. 9, 4841 (2018).
78. Tigano, M., Vargas, D. C., Fu, Y., Tremblay-Belzile, S. & Sfeir, A. Nuclear sensing of mitochondrial DNA breaks enhances immune surveillance. bioRxiv 2020.01.31.929075 (2020) doi:10.1101/2020.01.31.929075.
79. Chiu, Y. H., MacMillan, J. B. & Chen, Z. J. RNA Polymerase III Detects Cytosolic DNA and Induces Type I Interferons through the RIG-I Pathway. Cell 138, 576–591 (2009).
80. Ruzicka, M. et al. RIG-I-based immunotherapy enhances survival in preclinical AML models and sensitizes AML cells to checkpoint blockade. Leukemia 34, 1017–1026 (2020).
81. Patel, S. J. et al. Identification of essential genes for cancer immunotherapy. Nature 548, 537–542 (2017).
82. Manguso, R. T. et al. In vivo CRISPR screening identifies Ptpn2 as a cancer immunotherapy target. Nature 547, 413–418 (2017).
83. Chen, H., Liu, H. & Qing, G. Targeting oncogenic Myc as a strategy for cancer treatment. Signal Transduction and Targeted Therapy vol. 3 (2018).
84. Christensen, C. L. et al. Targeting Transcriptional Addictions in Small Cell Lung Cancer with a Covalent CDK7 Inhibitor. Cancer Cell 26, 909–922 (2014).
85. Zeng, M. et al. Targeting MYC dependency in ovarian cancer through inhibition of CDK7 and CDK12/13. Elife 7, 1–20 (2018).
86. Zhang, H. et al. CDK7 Inhibition Potentiates Genome Instability Triggering Anti-tumor Immunity in Small Cell Lung Cancer. Cancer Cell 37, 37-54.e9 (2020).
87. Carey, J. P. W. et al. Synthetic lethality of PARP inhibitors in combination with MYC blockade is independent of BRCA status in triple-negative breast cancer. Cancer Res. 78, 742–757 (2018).
88. Horiuchi, D. et al. MYC pathway activation in triple-negative breast cancer is synthetic lethal with CDK inhibition. J. Exp. Med. 209, 679–96 (2012).
89. Yi, J. et al. MYC status as a determinant of synergistic response to Olaparib and Palbociclib in ovarian cancer. EBioMedicine 43, 225–237 (2019).
90. Delmore, J. E. et al. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell 146, 904–917 (2011).
91. Mertz, J. A. et al. Targeting MYC dependence in cancer by inhibiting BET bromodomains. Proc. Natl. Acad. Sci. U. S. A. 108, 16669–16674 (2011).
92. Sun, C. et al. BRD4 Inhibition Is Synthetic Lethal with PARP Inhibitors through the Induction of Homologous Recombination Deficiency. Cancer Cell 33, 401-416.e8 (2018).
93. Karakashev, S. et al. BET Bromodomain Inhibition Synergizes with PARP Inhibitor in Epithelial Ovarian Cancer. Cell Rep. 21, 3398–3405 (2017).
94. Ding, L. et al. PARP Inhibition Elicits STING-Dependent Antitumor Immunity in Brca1-Deficient Ovarian Cancer. Cell Rep. 25, 2972-2980.e5 (2018).
95. Sistigu, A. et al. Cancer cell–autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. Nat. Med. 20, 1301–1309 (2014).
96. Wu, J. et al. Cyclic GMP-AMP Is an Endogenous Second Messenger in Innate Immune Signaling by

Cytosolic DNA. Science (80-.). 339, 826–830 (2013).
97. Ablasser, A. et al. Cell intrinsic immunity spreads to bystander cells via the intercellular transfer of cGAMP. Nature 503, 530–4 (2013).
98. Marcus, A. et al. Tumor-Derived cGAMP Triggers a STING-Mediated Interferon Response in Non-tumor Cells to Activate the NK Cell Response. Immunity 49, 754-763.e4 (2018).
99. Carozza, J. A. et al. Extracellular cGAMP is a cancer-cell-produced immunotransmitter involved in radiation-induced anticancer immunity. Nat. Cancer 1, 184–196 (2020).
100. Cheng, N. et al. A nanoparticle-incorporated STING activator enhances antitumor immunity in PD-L1–insensitive models of triple-negative breast cancer. JCI Insight 3, (2018).
101. Ohkuri, T. et al. Intratumoral administration of cGAMP transiently accumulates potent macrophages for anti-tumor immunity at a mouse tumor site. Cancer Immunol. Immunother. 66, 705–716 (2017).
102. Marloye, M., Lawler, S. E. & Berger, G. Current patent and clinical status of stimulator of interferon genes (STING) agonists for cancer immunotherapy. Pharm. Pat. Anal. 8, 87–90 (2019).
103. Deng, L. et al. STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors. Immunity 41, 843–852 (2014).
104. Wang, J., Yi, S., Zhou, J., Zhang, Y. & Guo, F. The NF-κB subunit RelB regulates the migration and invasion abilities and the radio-sensitivity of prostate cancer cells. Int. J. Oncol. 49, 381–92 (2016).
105. Grabosch, S. et al. Cisplatin-induced immune modulation in ovarian cancer mouse models with distinct inflammation profiles. Oncogene 1 (2018) doi:10.1038/s41388-018-0581-9.
106. Sato, H. et al. DNA double-strand break repair pathway regulates PD-L1 expression in cancer cells. Nat. Commun. 8, 1751 (2017).
107. Higuchi, T. et al. CTLA-4 Blockade Synergizes Therapeutically with PARP Inhibition in BRCA1-Deficient Ovarian Cancer. Cancer Immunol. Res. 3, 1257–1268 (2015).
108. Jiao, S. et al. PARP Inhibitor Upregulates PD-L1 Expression and Enhances Cancer-Associated Immunosuppression. Clin. Cancer Res. 23, 3711–3720 (2017).
109. Friedlander, M. et al. Pamiparib in combination with tislelizumab in patients with advanced solid tumours: results from the dose-escalation stage of a multicentre, open-label, phase 1a/b trial. Lancet Oncol. 20, 1306–1315 (2019).
110. Konstantinopoulos, P. A. et al. Single-Arm Phases 1 and 2 Trial of Niraparib in Combination with Pembrolizumab in Patients with Recurrent Platinum-Resistant Ovarian Carcinoma. JAMA Oncol. 5, 1141–1149 (2019).