# Resistance to PARP inhibition by DNA damage response alterations in BRCA1/2-deficient tumors

Gogola, E.

# CHAPTER 1

Scope of the thesis

The discovery of *BRCA1* ("BReast CAncer gene 1") more than 20 years ago provided the first evidence that the risk of cancer could be affected by mutations in a single gene, and triggered the identification of other high-penetrance susceptibility genes by linkage analysis[1,2]. According to current data, about 5-10% of all breast cancer (BC) cases and up to 25% of familial BC cases are attributed to pathogenic mutations in *BRCA1* or *BRCA2* ("BReast CAncer gene 2")[3]. Deleterious mutations in either gene greatly increase the risk of developing BC: it is estimated that about 85% of women with a family history of BC and disruptive mutations in *BRCA1*/2 will develop cancer by the age 70[3] (12%, general population risk[4]).

Although *BRCA1* and *BRCA2* were first described as BC genes and subsequently associated with the hereditary breast and ovarian cancer (HBOC) syndrome, *BRCA* mutations also contribute to the prevalence of other types of cancers, including prostate and pancreatic malignancies[5]. In fact, depending on the population investigated, between 1 in 40 and 1 in 400 people are *BRCA* mutation carriers, and the rates are similar between the sexes[6]. Extending *BRCA* genetic testing beyond HBOC should therefore aid cancer prevention in a broader population of cancer patients. Moreover, these patients should benefit from therapeutic strategies specifically designed to target tumors deficient in BRCA1/2.

Inhibition of poly(ADP-ribose) polymerase 1/2 (PARP1/2) is the prime example of a tailored approach that exploits the BRCA1/2 deficiency. BRCA1 and BRCA2 are tumor suppressor proteins involved in the maintenance of genomic stability, largely due to their role in the repair of DNA double-strand breaks (DSBs) by homologous recombination (HR)[7]. *BRCA1/2* inactivation results in a HR defect and makes cells more reliant on alternative, often error-prone pathways that prevent the generation of DSBs or mediate their repair. The HR deficiency clearly explains the hypersensitivity of *BRCA*-depleted cells to anti-cancer agents that induce DSB formation, including PARP inhibitors (PARPi)[8,9]. Clinical PARPi block the catalytic activity of PARP1/2, which act as DNA sensors and contribute to several DNA repair processes[10]. Depletion of *PARP1*, although well-tolerated in normal cells, is synthetically lethal with inactivating mutations in *BRCA1/2*, indicating that PARP1 function becomes essential in the absence of BRCA1/2[11]. In addition to their inhibitory properties, several PARPi exhibit the ability to trap PARP1/2 onto the DNA. Resulting bulky PARP1-DNA adducts interfere with chromatin transactions and lead to the formation of collapsed replication forks (RFs) which are normally repaired by HR[12,13]. Thus, PARPi act in a dual mode to induce aberrant DNA intermediates that are highly cytotoxic in the context of dysfunctional HR.

The demonstration of single-agent anti-cancer efficacy and the wide therapeutic index of PARPi in *BRCA*-mutation carriers confirmed the validity of the synthetic lethality approach and provided a strong rationale for the clinical application of PARPi therapy[11,14]. Nowadays, four different PARPi have been granted regulatory approval to treat BRCA-associated malignancies[15]. However, beneficial responses to PARPi are often not durable

due to emerging drug resistance[11]. Understanding the molecular basis of resistance will have significant impact on several aspects of PARPi utility in the clinic, including patient stratification, early detection of a recurrent disease and improvement of combination regimens that could prevent or target resistant clones. Ultimately, a better understanding of the resistance mechanisms should translate into clinical benefit of patients receiving PARPi therapy.

To understand the precise molecular mechanisms of PARPi resistance is the major focus of my thesis. **Chapter 2** provides a general introduction to this topic and a comprehensive summary of hitherto identified mechanisms of PARPi resistance[16]. The majority of them were discovered in pre-clinical settings. In contrast to the analysis of complex, heterogeneous patient-derived tumor samples, well-defined cellular or animal genetic models are often more useful for the identification of the basic underlying mechanisms. Also for my PhD project the *in vitro* and *in vivo* animal models of BRCA1/2-deficient cancer were instrumental to dissect different mechanisms of PARPi resistance. In particular, I benefited from the genetically engineered mouse models (GEMMs) of BRCA1/2-associated breast cancer, generated in our laboratory[17,18]. In **Chapter 3** we extend the utility of our GEMMs to study drug resistance by establishing tumor-derived organoid cultures. We demonstrate that organoids can be efficiently derived and rapidly expanded *in vitro*, while faithfully recapitulating the morphology, gene expression and drug response of the original tumor. Ease of genetic modification and efficient engraftment into recipient mice render cancer organoids a powerful approach for robust testing of putative PARPi resistance factors. Tumor-derived organoids became a routinely used tool in our laboratory, and contributed to several collaborative PARPi resistance studies[19–21], some of which are included in the latter chapters.

In **Chapter 4** we describe a new role of the REV7/MAD2L2 protein, previously known for its function in translesion synthesis (TLS), in regulating DSB repair pathway choice between HR and non-homologous end joining (NHEJ)[22]. REV7 acts downstream of the BRCA1 antagonist 53BP1, and loss of REV7, similarly to a depletion of 53BP1[23–25], results in reactivation of functional HR and drives PARPi resistance in *Brca1*-deficient cells. As we demonstrate in **Chapter 5**, HR restoration is a frequent, but not the only mechanism of PARPi resistance in BRCA1-deficient cells. Importantly, our data strongly suggest that HR cannot be restored in the absence of BRCA2. These results indicate that BRCA2, unlike BRCA1 is indispensable for the HR-mediated repair. In contrast, the requirement of BRCA2 in protecting stalled RF can be bypassed. As shown in **Chapter 6**, loss of PTIP blocks the recruitment of the MRE11 nuclease to stalled RFs, thereby protecting nascent DNA strands from extensive degradation. Because collapsed forks require HR for their repair, depletion of PTIP alleviates PARPi-mediated toxicity in BRCA1/2-deficient cells[21].

To better understand HR-independent mechanisms of PARPi resistance, we combined the multi-omics analysis of GEMM-derived BRCA2-deficient tumors that acquired PARPi-resistance *in vivo* with genetic screens in tumor-derived 2D cell lines and 3D organoids[19]. This approach, described in **Chapter 7**, yielded poly(ADP-ribose) glycohydrolase (PARG), an enzyme responsible for PAR catabolism, as a top hit. Our data suggest that PARPi do not completely inhibit the enzymatic function and allow residual PARP1/2 activity, which is normally counteracted by PARG. In the absence of PARG, however, PAR degradation is prevented leading to partial restoration of PARP1 signaling and PARPi resistance.
In summary, the research described in this thesis has advanced our understanding of PARPi resistance and yielded new ideas and questions that should be addressed in future studies. Extending on the discussions included in each of the chapters, I highlight some of these outstanding questions in the final section of this work (**Chapter 8**).

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