Dissecting PARP inhibitor resistance with functional genomics

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Abstract

The Poly-(ADP-ribose) polymerase (PARP) inhibitor (PARPi) olaparib was the first licenced cancer drug that targeted an inherited form of cancer, namely ovarian cancers caused by germline *BRCA1* or *BRCA2* gene mutations. Multiple different PARPi have now been approved for use in a wider group of gynaecological cancers as well as for the treatment of *BRCA*-gene mutant breast cancer. Despite these advances, resistance to PARPi is a common clinical phenotype. Understanding, at the molecular level, how tumour cells respond to PARPi has the potential to inform how these drugs should be used clinically and since the discovery of this drug class, multiple different functional genomic strategies have been employed to dissect PARPi sensitivity and resistance. These have included genetic perturbation via classical gene targeting, gene silencing by siRNA or shRNA or transposon mutagenesis techniques. Recently, CRISPR-Cas9-based mutagenesis has greatly expanded the available range of relevant preclinical models and the precision of mutagenesis. Here, we review how these approaches have been used either in low-throughput, hypothesis-testing experiments or in the setting of large, hypothesis-generating, genetic screens aimed at understanding the molecular basis of PARPi sensitivity and resistance.

Introduction – PARP inhibitors in cancer treatment

Four different PARPi (olaparib, rucaparib, niraparib and talazoparib) are now FDA-approved [1]. These clinical PARPi primarily target PARP1 (Poly(ADP-ribose) polymerase 1) a DNA binding protein that is involved in sensing, signalling and mediating the repair of single or double stranded DNA breaks. Upon DNA binding via its N-terminal zinc finger domains, conformational changes in PARP1 structure activate PARP1's C-terminal catalytic domain which hydrolyses β-NAD+ to add successive ADP-ribose moieties onto target proteins, producing poly-(ADP-ribose) chains (PAR) [2,3]. Synthesis of PAR on PARP1 itself and other substrates recruits factors that mediate repair of damaged DNA, and ultimately leads to dissociation of PARP1 from DNA [2]. As well as inhibiting PARP1 catalytic activity, clinical PARPi block PARP1 dissociation, "trapping" PARP1 on damaged DNA [4-6]. Although the precise mechanism by which PARPi kill tumour cells remains to be elucidated, a working model suggests that either trapped PARP1 and/or the loss of DNA repair normally mediated by PARP1 causes a form of DNA damage, such as replication-associated double strand breaks, that is normally repaired by DNA repair processes such as homologous recombination (HR). HR is controlled by proteins including BRCA1 and BRCA2, leading to the hypothesis that tumours with mutations in the genes encoding these proteins are unable to effectively process the DNA damage that PARPi cause, leading to large scale genomic rearrangements and selective tumour cell death [7,8]. From a functional genomics perspective, isogenic cell lines with *BRCA1* or *BRCA2* gene targeting events were used to initially demonstrate the synthetic lethality between PARP inhibitors and BRCA-gene defects, as was the use of *BRCA1* or *BRCA2* gene silencing [7-9]. Following this, genetically engineered mice with either *Brca1* or *Brca2* mutant mammary tumours were used to confirm the synthetic lethality in an *in vivo* setting [10,11]. Subsequent studies have revealed other cancer gene defects that cause PARPi sensitivity, including those in additional genes that control HR, such as *PALB2, RAD51C, RAD51D* [12-17], *CDK12* [16] and Ewing's sarcoma gene fusions [18,19].

High grade serous ovarian cancers appear to be enriched for defects in HR, including tumours with mutations in either *BRCA1*, *BRCA2*, *CDK12, RAD51C* and *RAD51D*. These HR defects can often be seen clinically as sensitivity to platinum salts, drugs that also cause DNA damage that requires HR for repair. As such, olaparib, rucaparib, and niraparib are now approved for the treatment of ovarian cancers that are "platinum sensitive" [20]; here PARPi are used to prevent

recurrence of disease as a maintenance therapy after the initial use of a platinum salt. Furthermore, the PARPi talazoparib has recently been approved for the treatment of *BRCA1* or *BRCA2* mutant breast cancers [21]. Nevertheless, in many patients treated with PARPi, *de novo* or acquired resistance is observed. Understanding the causes of this resistance is thus very important. In this article we review recent studies that have used functional genomics approaches to identify PARP inhibitor resistance mechanisms.

Reversion mutations and 53BP1-mediated mechanisms identified by functional genomics

The application of functional genomics has also been used to identify a number of mechanisms of PARPi resistance. The first mechanism of PARPi resistance was identified by the genomic and proteomic analysis of PARPi-resistant BRCA2 mutant tumour cells generated by chronic *in vitro* exposure to olaparib [22]. These experiments identified secondary, reversion, mutations in *BRCA2* that restore the open reading frame of the gene and cause PARPi and also platinum salt resistance by restoring the ability of BRCA2 to mediate HR [22-24]. Similar reversion mutations in *BRCA1, BRCA2, RAD51C, RAD51D* or *PALB2* have now been seen in patients with either PARPi or platinum salt resistance, demonstrating that this process operates in the clinical disease and is not restricted to *BRCA2* [23,25-33]

Using a systematic approach to identify the genes that restore HR in cells with *BRCA*-gene defects, Bouwman and colleagues used an *in vitro* transposon mutagenesis screen to identify *Trp53bp1* (encoding p53 binding protein 1, 53BP1) disruption as a suppressor of cellular lethality caused by *Brca1* deletion [34]. Loss of wild type *Trp53bp1* restores HR in *Brca1* deleted cells and also causes PARPi resistance [34,35]. One role of BRCA1 in HR is to promote DNA resection at double strand breaks via recruitment of the MRN (MRE11, RAD50, NBN) complex, resulting in the generation of single stranded 3′ overhangs that are eventually converted into RAD51 nucleoprotein filaments, the substrate for the strand invasion step of HR. In the absence of BRCA1 this resection is lacking and 53BP1 promotes nonhomologous end joining at double strand breaks instead, leading to chromosomal rearrangements and eventual p53-dependent cell death. Loss of 53BP1 alleviates this repression of resection and allows HR to occur. Other studies have identified further genes encoding factors that counteract resection as PARPi resistance genes,

including *REV7* (*MAD2L2*), *RIF1* and *PTIP* [36-38] and a series of genes, discussed later, identified by CRISPR-Cas9 mutagenesis screens.

PARP1-mediated mechanisms of resistance

The use of transposon mutagenesis also highlighted the potential for alterations in PARP1 itself being a cause of PARPi resistance. We used genome-wide transposon mutagenesis of mouse ES cells using the *piggyBac* transposon, followed by *in vitro* exposure of cells to a PARPi, to show that deletion of the *Parp1* gene, and complete loss of Parp1 expression, caused profound PARPi resistance [39]. This observation mirrored a similar observation made using a panel of DT40 chicken cells with different genetic defects generated by gene targeting [4]; this showed that deletion of *Parp1* caused PARPi resistance, whilst deletion of DNA polymerase β, or the nuclease FEN1 (flap structure-specific endonuclease 1) normally associated with processing of the 5' ends of Okazaki fragments in lagging strand DNA synthesis, caused PARPi sensitivity [4]. These observations supported the PARP trapping hypothesis, which proposes that PARP1/DNA nucleoprotein complexes are stabilised in the presence of PARPi and these are more likely to explain the cytotoxicity of PARPi rather than the impairment of single strand break repair that might be caused by inactivation of PARP1's catalytic activity [4,40].

We recently applied a CRISPR-Cas9 mutagenesis screening approach to further investigate the role of PARP1 in PARPi-induced cytotoxicity [41]. Using one of the first genome-wide CRISPR-Cas9 guide RNA libraries to be described [42], we mutagenised *Brca* wild-type mouse ES cells and used a strong selective pressure (Surviving Fraction (SF) ~ 0) of the PARPi talazoparib to select PARPi-resistant CRISPR-Cas9 mutagenised clones. Such an approach allows direct analysis of the resistant mutants, but is likely to miss weaker resistance phenotypes. Nine out of the 24 talazoparib-resistant clones bore a *Parp1* sgRNA vector and most of these had lost Parp1 protein expression. However we identified one talazoparib-resistant mutant clone that expressed Parp1 protein despite the presence of the *Parp1* sgRNA; this clone proved to have a CRISPR-Cas9-induced deletion of a methionine residue at position 43 in the first zinc finger domain of Parp1. The resulting Parp1 protein was unable to bind DNA or to be trapped by talazoparib [41].

The isolation of these zinc finger domain mutants from a genetic screen provided direct evidence that the DNA binding activity of PARP1 is important for the

cytotoxicity of PARP inhibitors, and is further evidence for the trapping hypothesis. Furthermore, these experiments demonstrated that inspection of the exact mutations generated in genome-wide CRISPR-Cas9 mutagenesis screens can provide further detail on the mechanisms in play. This has also been shown in other studies, including the identification of CRISPR-Cas9 generated *MAP2K1* mutant alleles that cause resistance to the MAPK kinase inhibitor selumetinib [43]. Isolation of such mutants can be extremely informative, but is somewhat serendipitous, relying on the presence of appropriate sgRNAs in the library and particular mutations being tolerated by cells.

To discover other non-truncating mutations that might affect PARPi cytotoxicity, we took a tiling mutagenesis approach to assess how a diverse set of *PARP1* mutations might alter PARPi resistance. Our strategy, which we termed “tag-mutate-enrich” (Figure 1a) involves first introducing a C-terminal GFP tag into the endogenous gene of interest (in this case *PARP1*) using the CRISPaint [44] approach. The method enriches for small, in-frame, mutations such as the p.43delM mutation isolated in the genome-wide screen described earlier.

Analysing the mutations in the PARPi resistant population provided a map of the regions of PARP1 that mediate PARP inhibitor cytotoxicity [41]. As expected, several clusters of mutations were identified in the DNA binding domains. Some contribution from relative guide RNA effectiveness might be expected to skew the results; however when mapping the DNA binding domains onto the zinc finger domain crystal structure, we found that mutations were closely associated with protein-DNA contacts, suggesting that their isolation was driven by function rather than mutagenesis efficiency (Figure 1b). Interestingly we identified a number of other mutation clusters outside the DNA binding domain associated with PARPi resistance, including a cluster of mutations in the WGR domain that we predict mediates DNA binding-driven conformational changes in PARP1. The map of PARP1 function generated in this way showed good correlation with PARP1 mutations associated with PARPi resistance identified *via* an ENU mutagenesis screen carried out in haploid HAP1 cells [45]. Furthermore, a PARP1 variant of unknown significance (p.R591C) that we identified in a *de novo* PARP inhibitor resistant patient mapped to one of the most frequently mutated residues in the WGR region.

Experiments in wild type cells can be useful for studying mechanisms of drug action; however questions about clinical resistance mechanisms are best studied in a

relevant cell type which for PARPi, based on current clinical guidance, is breast or ovarian cancer cells with a homologous recombination defect caused by either a *BRCA1* or *BRCA2* mutation. A number of groups, including ours, have recently carried out CRISPR-Cas9 mutagenesis screens in such backgrounds that have resulted in a more detailed understanding of how PARP inhibitors kill cells, and how resistance might develop, in these contexts.

Functional genomics of PARPi resistance identifies new DNA repair proteins

As discussed earlier, previous work identified defects in 53BP1 or its effectors RIF1 and REV7, as a cause of PARPi inhibitor resistance in *BRCA1* mutant cells via restoration of resection and thus HR activity. A number of recent studies have expanded the understanding of how 53BP1 defects mediate PARPi resistance by using CRISPR-Cas9 mutagenesis screens in *BRCA1*-deficient cells. These included screens in RPE1 (retinal pigment epithelial) cells with gene-targeted defects in *BRCA1* and *TP53* [46] but also screens in patient-derived cell lines, including those in basal-like breast cancer cell lines with *BRCA1* mutations, such as SUM149PT [46-49] and MDAMB436 [50], and the ovarian cancer cell lines UWB1.289 [50,51], JHOS-2 [50] and COV362 [50,52]. The Jonkers and Rottenberg groups have also made use of mouse embryonic stem cells and mouse mammary tumour cells with Cre-engineered *Brca1* and *Trp53* deletions [47].

These screens have revealed several new components of the 53BP1 pathway that act to suppress resection at DSBs in *BRCA1*-deficient cells. Most of these screens identified sgRNAs targeting *C20orf196* (now known as *SHLD1*), as well another poorly characterised gene *FAM35A* (now known as *SHLD2*) as causing PARPi resistance. Further study of these genes, and parallel proteomic profiling [49,53,54] identified interactions between these proteins and REV7 and RIF1 as well as a further uncharacterised protein (CTC534A2.2) encoded by an uncharacterised gene (now named *SHLD3*) present in an intron of *TRAPPC13*. The complex of these proteins, along with REV7, is now referred to as Shieldin. Shieldin is recruited to DSBs in a 53BP1- and RIF1-dependent manner, and SHLD mutant cells also display sensitivity to ionising radiation (IR) and defects in class switch recombination, both characteristics of defective NHEJ. SHLD2 contains OB fold ssDNA binding domains in its C terminus and is proposed to bind and protect exposed ssDNA at DNA ends from further resection [46,49,53,55].

Combining screen results identifies robust effects

An ever-present problem with genome-wide screens is how to prioritise hits for further analysis. Effect size is one way to do this, but may suffer from sensitivity issues – for example, in the screens described above *C20orf196* and *FAM35A* were not the top hits in any single screen judged purely by effect size, but were clearly reproducible across the different cell types, CRISPR gRNA screening libraries, PARP inhibitors (both olaparib and talazoparib were used in this set of screens) and laboratories. Pathway and/or complex analysis of hits is another useful principle where these are known – for example Barazas *et al*. identified *CTC1, STN1* (*OBFC1*) and *TEN1* among the highly ranked genes in a set of PARPi resistance screens [47]. These are known to interact as the CST complex that acts to counteract resection at telomeres [54]. Further validation of the screen results demonstrated that sgRNA targeting *Ctc1* can restore IR-induced Rad51 focus formation in *Brca1*-mutant cells, suggesting that this complex may also play a role in counteracting resection at non-telomeric DSBs.

The principle of screening across multiple cell types has also been used to reveal a role for the dynein light chain protein DYNLL1 in PARP inhibitor resistance [50]. He *et al* screened a panel of *BRCA1* mutant ovarian cancer cell lines for olaparib resistance and identified *DYNLL1* as a highly ranked gene across all three lines, and the top ranked hit in COV362 cells. DYNLL1 knockout restored resection in *BRCA1* mutant cells, which the authors suggest is due to DYNLL1 binding MRE11 and thus inhibiting DNA resection. DYNLL1 also interacts with 53BP1 [56] and another recent study has suggested that effects on 53BP1 recruitment and oligomerisation may also play a role [57].

These studies clearly implicate the 53BP1-mediated suppression of resection as being central to PARP inhibitor sensitivity in *BRCA1* mutant cells, and identify a number of nodes that can influence this process. However many of these screens also identified PARP1 loss as a mediator of resistance. This is somewhat unexpected at first glance, given the well-established synthetic lethal relationship between *PARP1* and *BRCA1* [7-9], which might imply that *PARP1* loss of function mutations are not tolerated in *BRCA1* mutant cells. PARP1 mutations were identified in the SUM149PT and COV362 screens, but not in RPE1-*BRCA1*$^{-/-}$ or mouse *Brca1*$^{\Delta/\Delta}$ lines. We additionally found that MDA-MB-436 cells did not appear to tolerate complete loss of PARP1. SUM149PT and COV362 cells both have

frameshift mutations in exon 11 of *BRCA1* [48,52], which can be bypassed by a splice variant of *BRCA1* that skips exon 11 [58]. The BRCA1 protein variant encoded by this variant retains some HR activity. It has recently been shown that overexpression of this variant can rescue PARP inhibitor sensitivity in SUM149PT cells, and that knocking out the remaining BRCA1 activity by mutation of the BRCA1 BRCT domain or siRNA knockdown results in even greater PARP inhibitor sensitivity [59] and a lack of *PARP1* mutation tolerance [41]. This may explain why *PARP1* mutants are not recovered in cell lines with BRCT domain mutations (such as MDA-MB-436) or large engineered deletions. In a similar vein, isolation of PARP inhibitor resistant patient-derived xenografts has demonstrated fusion of C-terminal *BRCA1* sequence to promoters of other genes as a potential resistance mechanism [60], events that would be difficult to capture in a CRISPR-Cas9 screen.

PARPi resistance in BRCA2 mutant cells

PARPi resistance in *BRCA2* mutant cells has not been so extensively investigated as mechanisms that operate in *BRCA1* mutant cells, in part because of a paucity of relevant *BRCA2* mutant tumour cell lines that are suitable for screens. Previous studies have suggested a role for replication fork stabilisation in promoting PARPi resistance in BRCA2 mutant cells [36]. Gogola *et al* recently published an shRNA screen for PARPi resistance using a DNA repair focused shRNA library in mouse *Brca2* mutant mammary tumour cell lines and organoids [61]. This identified shRNA targeting *Parg* (poly-(ADP-ribose) glycohydrase) as the top resistance-causing hit in the library. PARG is one of a number of enzymes that can remove PAR chains from modified proteins; thus loss of PARG would be expected to maintain PARylation for longer. PARP inhibitors might be expected to act upstream of this step, blocking formation of the PAR polymer. However, PARG knockdown or inhibition resulted in persistent PARylation even in the presence of olaparib, suggesting that losing PARG activity can somewhat circumvent the effects of PARP inhibitors in a dominant fashion. PARG knockdown or inhibition did not obviously affect PARP1 trapping kinetics, but it seems possible that the residual PARylation under conditions of PARG inhibition results in a less toxic trapped PARP1 lesion. *Parp1* shRNAs did not cause resistance in the *Brca2* mutant cells; whether PARP1 loss might be a relevant resistance mechanism in the context of patient derived *BRCA2* mutations (as for *BRCA1* exon 11 mutations, above) remains to be investigated.

Perspective

As well as uncovering potential mechanisms of clinical PARP inhibitor resistance, screening for PARP inhibitor resistant mutants also answers specific biological questions. This is particularly evident for screens in *BRCA1* mutant cells, which have enabled discovery of novel components of the 53BP1 pathway, including the Shieldin complex. Genome-wide CRISPR-Cas9 genetic screens clearly allow much more detail than previous technologies – for example, the transposon screen that originally identified *Trp53bp1* as a PARPi resistance factor did not uncover other components of the pathway [34]. A good case can be made for widening the definition of "genes" in CRISPR libraries, as there may be more to find – illustrated by the lack of guides targeting *SHLD3* in the libraries screened thus far.

CRISPR technology can be applied in other ways related to screens – not least in validation of screen results. Generation of individual clones for validation experiments is ultimately necessary, but time consuming to do for a list of candidates. Competition assays using synthetic CRISPR gRNAs and TIDE analysis [62] to look at enrichment of frameshift variants in mixed populations of CRISPR mutants under selection can help to address this bottleneck. Pooled synthesis of focused CRISPR libraries is now relatively cheap and conducting secondary CRISPR screens with higher guide coverage library for a triaged gene set across multiple cell lines may also be a good validation strategy. Alternatively, customised libraries can be synthesised based on mass spectrometry data or other methods that might implicate a set of genes in a particular phenotype.

Single-gene tiling mutagenesis screens are the ultimate conclusion of this approach, allowing a detailed functional analysis of a diverse allelic series to be conducted. Tiling mutagenesis is still subject to restrictions on PAM site positions and biases in mutagenesis outcome. Use of alternative Cas9 enzymes could help to further improve coverage. Base editing ("CRISPRx") enzymes, in which catalytic-dead Cas9 is fused to deaminases or other base modifying enzymes, cause point mutations directly and are a further option to increase the diversity of tiling pools – these also have the advantage that mutations are most likely to be in-frame, removing the need to enrich for in-frame mutations by way of a GFP tag.

The major question remaining is what are the clinical causes of acquired PARP inhibitor resistance? A full discussion of clinical resistance is outside the scope of this

article, but many of the PARPi resistance genes identified in the studies described above have been implicated clinically in some way. This is most often by correlation of low expression levels with poor outcome in ovarian cancer, where many patients will receive platinum treatment, resistance to which is also likely to be mediated by mechanisms that restore functional HR [20,63].

However, the major clinically described mechanism of acquired PARP inhibitor resistance to date is secondary mutation of the HR gene, restoring the open reading frame and thus HR competency. Although the calculation of the true rate is complicated by the extensive platinum pre-treatment in most patients that receive PARP inhibitors, secondary mutations clearly do not explain every case of resistance [29]. In addition, most of the candidate resistance genes mentioned in this article are not present on standard cancer gene sequencing panels, as they are not oncogenes or tumour suppressor genes – with the exception of *BRCA1* and *BRCA2* – which may lead to ascertainment bias in the reporting of clinical resistance mechanisms. More extensive profiling of resistant tumours using whole exome or specialised PARPi resistance panels will be required to establish whether mutations in any of these genes cause clinical resistance. Biopsies from tumours with acquired resistance have been difficult to access, but circulating DNA sequencing approaches coupled with the use of PARP inhibitors at earlier clinical stages may result in more evaluable resistant tumours in future. Knowing the resistance mechanism is likely to be important in the clinical management of the disease, as different mechanisms of PARP inhibitor resistance may induce different secondary sensitivities[41] – for example, *REV7* mutation causes extreme cisplatin sensitivity, whereas BRCA secondary mutations will likely cause cross-resistance to cisplatin [29,41]. Loss of *TP53BP1* has been previously shown to cause cisplatin resistance [34,41], whereas knockout of SHLD components in RPE1;*BRCA1*$^{-/-}$ cells resulted in increased cisplatin sensitivity relative to cells with *BRCA1* mutations but wild type SHLD [49]. These experiments suggest that clinical REV7, SHLD1 or SHLD2 mutations may result in a targetable vulnerability to cisplatin. Radio-sensitivity of *Trp53bp1* and *Rev7* mutant mouse tumours has also been demonstrated [64]. Profiling of drug sensitivities and other dependencies using further screens in resistant mutants will be key to answering the question of how to manage resistance clinically.

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Figures

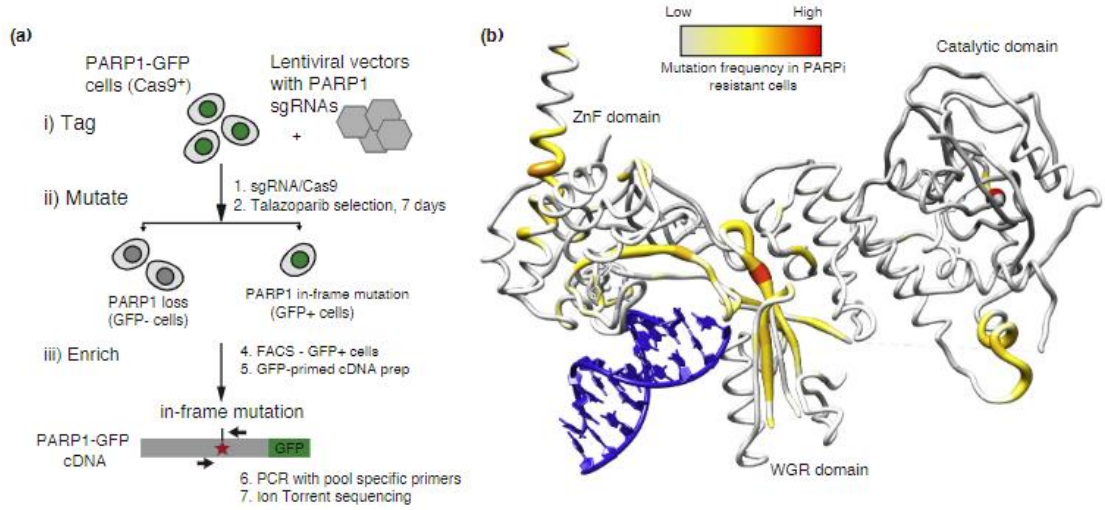

Figure 1. Focused CRISPR screens. A. Tag-mutate-enrich approach for focused mutagenesis of a single gene. (i) a C-terminal GFP tag is introduced to the gene of interest, for example via CRISPaint. (ii) A lentiviral library of all possible sgRNAs targeting the gene is introduced and the drug resistant population recovered. (iii) Isolation of GFP-positive cells using FACS enriches for mutations that preserve the reading frame. Complementary DNA is reverse transcribed from the GFP coding sequence and sequenced using overlapping PCR amplicons. **B.** Example of mutation frequency data from a tag-mutate-enrich screen. Residues in the 3D structure of PARP1 bound to a double stranded DNA break (PDB: 4OQB) are coloured by their mutation frequency in the talazoparib-resistant, GFP-positive, population. Figure adapted from [41].

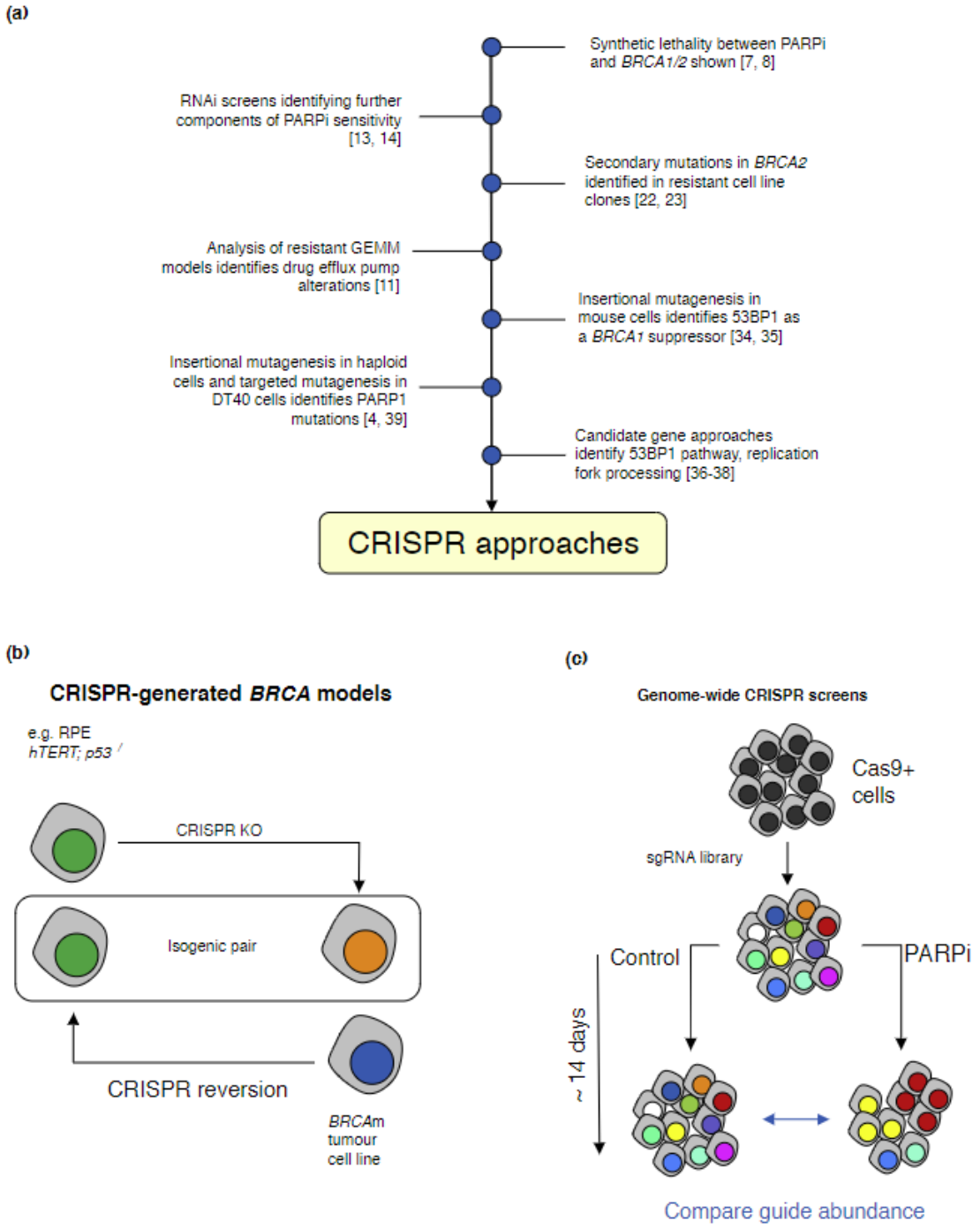

Figure 2. Progress in functional dissection of PARP inhibitor resistance . **A.** Approximate timeline and key studies for the discovery of genetic determinants of PARP inhibitor cytotoxicity, prior to the recent proliferation of CRISPR-based approaches. CRISPR-Cas9 mutagenesis has been used to make new specialised models (**B**) as well as to carry out forward genetic screens (a typical protocol is illustrated in **C**).

References

**** Of outstanding interest:**

Noordermeer *et al,* (2018); Dev *et al.* (2018): Forward genetic screens for PARP inhibitor resistance using CRISPR-Cas9 in *BRCA1* mutant cells, identifying the Shieldin complex.

Gogola *et al.* (2018): Identification of *Parg* loss as a suppressor of PARP inhibitor cytotoxicity in *Brca2* mutant cells.

*** Of special interest**:

Lin *et al.* (2018): A comprehensive study of secondary mutations in clinical PARPi resistance, demonstrating that presence of detectable secondary mutations after platinum treatment is predictive of poor response to rucaparib;

Pettitt *et al.* (2018); Barazas *et al.* (Cancer Res 2018): Studies using new CRISPR-based methods for rapid validation of hits from screens – including tiling screens, TIDE or deep sequencing analysis of frameshift mutation frequencies under selective pressure and rapid *in vivo* model generation.

1. Lord CJ, Ashworth A: **PARP inhibitors: Synthetic lethality in the clinic**. *Science* 2017, **355**:1152-1158.
2. Langelier M-F, Eisemann T, Riccio AA, Pascal JM: **PARP family enzymes: regulation and catalysis of the poly(ADP-ribose) posttranslational modification**. *Curr. Opin. Struct. Biol.* 2018, **53**:187-198.
3. Caldecott KW: **Protein ADP-ribosylation and the cellular response to DNA strand breaks**. *DNA Repair* 2014, **19**:108-113.
4. Murai J, Huang S-YN, Das BB, Renaud A, Zhang Y, Doroshow JH, Ji J, Takeda S, Pommier Y: **Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors**. *Cancer Research* 2012, **72**:5588-5599.
5. Murai J, Huang S-YN, Renaud A, Zhang Y, Ji J, Takeda S, Morris J, Teicher B, Doroshow JH, Pommier Y: **Stereospecific PARP trapping by BMN 673 and comparison with olaparib and rucaparib**. *Mol. Cancer Ther.* 2014, **13**:433-443.
6. Murai J, Zhang Y, Morris J, Ji J, Takeda S, Doroshow JH, Pommier Y: **Rationale for poly(ADP-ribose) polymerase (PARP) inhibitors in combination therapy with camptothecins or temozolomide based on PARP trapping versus catalytic inhibition**. *J. Pharmacol. Exp. Ther.* 2014, **349**:408-416.
7. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ, Helleday T: **Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase**. *Nature* 2005, **434**:913-917.
8. Farmer H, McCabe N, Lord CJ, Tutt ANJ, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C, et al.: **Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy**. *Nature* 2005, **434**:917-921.
9. Patel AG, Sarkaria JN, Kaufmann SH: **Nonhomologous end joining drives poly(ADP-ribose) polymerase (PARP) inhibitor lethality in homologous recombination-deficient cells**. *Proc Natl Acad Sci U S A* 2011, **108**:3406-3411.
10. Evers B, Drost R, Schut E, de Bruin M, van der Burg E, Derksen PWB, Holstege H, Liu X, van Drunen E, Beverloo HB, et al.: **Selective inhibition of BRCA2-deficient mammary tumor cell growth by AZD2281 and cisplatin**. *Clin. Cancer Res.* 2008, **14**:3916-3925.

11. Rottenberg S, Jaspers JE, Kersbergen A, van der Burg E, Nygren AOH, Zander SAL, Derksen PWB, de Bruin M, Zevenhoven J, Lau A, et al.: **High sensitivity of BRCA1-deficient mammary tumors to the PARP inhibitor AZD2281 alone and in combination with platinum drugs**. *Proc Natl Acad Sci U S A* 2008, **105**:17079-17084.
12. McCabe N, Turner NC, Lord CJ, Kluzek K, Bialkowska A, Swift S, Giavara S, O'Connor MJ, Tutt AN, Zdzienicka MZ, et al.: **Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition**. *Cancer Research* 2006, **66**:8109-8115.
13. Lord CJ, McDonald S, Swift S, Turner NC, Ashworth A: **A high-throughput RNA interference screen for DNA repair determinants of PARP inhibitor sensitivity**. *DNA Repair* 2008, **7**:2010-2019.
14. Turner NC, Lord CJ, Iorns E, Brough R, Swift S, Elliott R, Rayter S, Tutt AN, Ashworth A: **A synthetic lethal siRNA screen identifying genes mediating sensitivity to a PARP inhibitor**. *The EMBO Journal* 2008, **27**:1368-1377.
15. Loveday C, Turnbull C, Ramsay E, Hughes D, Ruark E, Frankum JR, Bowden G, Kalmyrzaev B, Warren-Perry M, Snape K, et al.: **Germline mutations in RAD51D confer susceptibility to ovarian cancer**. *Nat Genet* 2011, **43**:879-882.
16. Bajrami I, Frankum JR, Konde A, Miller RE, Rehman FL, Brough R, Campbell J, Sims D, Rafiq R, Hooper S, et al.: **Genome-wide profiling of genetic synthetic lethality identifies CDK12 as a novel determinant of PARP1/2 inhibitor sensitivity**. *Cancer Research* 2014, **74**:287-297.
17. Min A, Im S-A, Yoon Y-K, Song S-H, Nam H-J, Hur H-S, Kim H-P, Lee K-H, Han S-W, Oh D-Y, et al.: **RAD51C-deficient cancer cells are highly sensitive to the PARP inhibitor olaparib**. *Mol. Cancer Ther.* 2013, **12**:865-877.
18. Brenner JC, Ateeq B, Li Y, Yocum AK, Cao Q, Asangani IA, Patel S, Wang X, Liang H, Yu J, et al.: **Mechanistic rationale for inhibition of poly(ADP-ribose) polymerase in ETS gene fusion-positive prostate cancer**. *Cancer Cell* 2011, **19**:664-678.
19. Garnett MJ, Edelman EJ, Heidorn SJ, Greenman CD, Dastur A, Lau KW, Greninger P, Thompson IR, Luo X, Soares J, et al.: **Systematic identification of genomic markers of drug sensitivity in cancer cells**. *Nature* 2012, **483**:570-575.
20. Pettitt SJ, Lord CJ: **PARP inhibitors and breast cancer: highlights and hang-ups**. *Expert Review of Precision Medicine and Drug Development* 2018, **00**:1-12.
21. Litton JK, Rugo HS, Ettl J, Hurvitz SA, Gonçalves A, Lee K-H, Fehrenbacher L, Yerushalmi R, Mina LA, Martin M, et al.: **Talazoparib in Patients with Advanced Breast Cancer and a Germline BRCA Mutation**. *N Engl J Med* 2018, **379**:753-763.
22. Edwards SL, Brough R, Lord CJ, Natrajan R, Vatcheva R, Levine DA, Boyd J, Reis-Filho JS, Ashworth A: **Resistance to therapy caused by intragenic deletion in BRCA2**. *Nature* 2008, **451**:1111-1115.
23. Sakai W, Swisher EM, Jacquemont C, Chandramohan KV, Couch FJ, Langdon SP, Wurz K, Higgins J, Villegas E, Taniguchi T: **Functional restoration of BRCA2 protein by secondary BRCA2 mutations in BRCA2-mutated ovarian carcinoma**. *Cancer Res* 2009, **69**:6381-6386.
24. Sakai W, Swisher EM, Karlan BY, Agarwal MK, Higgins J, Friedman C, Villegas E, Jacquemont C, Farrugia DJ, Couch FJ, et al.: **Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers**. *Nature* 2008, **451**:1116-1120.
25. Barber LJ, Sandhu S, Chen L, Campbell J, Kozarewa I, Fenwick K, Assiotis I, Rodrigues DN, Reis-Filho JS, Moreno V, et al.: **Secondary mutations in BRCA2 associated with clinical resistance to a PARP inhibitor**. *J. Pathol.* 2013, **229**:422-429.
26. Carneiro BA, Collier KA, Nagy RJ, Pamarthy S, Sagar V, Fairclough S, Odegaard J, Lanman RB, Costa R, Taxter T, et al.: **Acquired Resistance to Poly (ADP-ribose) Polymerase Inhibitor Olaparib in BRCA2-Associated Prostate Cancer Resulting From Biallelic BRCA2 Reversion Mutations Restores Both Germline and Somatic Loss-of-Function Mutations**. *JCO Precision Oncology* 2018:1-8.
27. Christie EL, Fereday S, Doig K, Pattnaik S, Dawson S-J, Bowtell DDL: **Reversion of BRCA1/2Germline Mutations Detected in Circulating Tumor DNA From Patients With High-Grade Serous Ovarian Cancer**. *Journal of Clinical Oncology* 2017, **35**:1274-1280.

28. Gornstein EL, Sandefur S, Chung JH, Gay LM, Holmes O, Erlich RL, Soman S, Martin LK, Rose AV, Stephens PJ, et al.: **BRCA2 Reversion Mutation Associated With Acquired Resistance to Olaparib in Estrogen Receptor-positive Breast Cancer Detected by Genomic Profiling of Tissue and Liquid Biopsy**. *Clinical Breast Cancer* 2018, **18**:184-188.
29. Lin KK, Harrell MI, Oza AM, Oaknin A, Ray-Coquard I, Tinker AV, Helman E, Radke MR, Say C, Vo L-T, et al.: **BRCA Reversion Mutations in Circulating Tumor DNA Predict Primary and Acquired Resistance to the PARP Inhibitor Rucaparib in High-Grade Ovarian Carcinoma**. *Cancer Discov* 2018:CD-18-0715.
30. Mayor P, Gay LM, Lele S, Elvin JA: **BRCA1 reversion mutation acquired after treatment identified by liquid biopsy**. *Gynecol Oncol Rep* 2017, **21**:57-60.
31. Quigley D, Alumkal JJ, Wyatt AW, Kothari V, Foye A, Lloyd P, Aggarwal R, Kim W, Lu E, Schwartzman J, et al.: **Analysis of Circulating Cell-Free DNA Identifies Multiclonal Heterogeneity of BRCA2 Reversion Mutations Associated with Resistance to PARP Inhibitors**. *Cancer Discov* 2017, **7**:999-1005.
32. Weigelt B, Comino-Méndez I, de Bruijn I, Tian L, Meisel JL, Garcia-Murillas I, Fribbens C, Cutts R, Martelotto LG, Ng CKY, et al.: **Diverse BRCA1and BRCA2 Reversion Mutations in Circulating Cell-Free DNA of Therapy-Resistant Breast or Ovarian Cancer**. *Clin. Cancer Res.* 2017, **23**:6708-6720.
33. Kondrashova O, Nguyen M, Shield-Artin K, Tinker AV, Teng NNH, Harrell MI, Kuiper MJ, Ho G-Y, Barker H, Jasin M, 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* 2017, **7**:984-998.
34. Bouwman P, Aly A, Escandell JM, Pieterse M, Bartkova J, van der Gulden H, Hiddingh S, Thanasoula M, Kulkarni A, Yang Q, et al.: **53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers**. *Nat Struct Mol Biol* 2010, **17**:688-695.
35. Bunting SF, Callen E, Wong N, Chen H-T, Polato F, Gunn A, Bothmer A, Feldhahn N, Fernandez-Capetillo O, Cao L, et al.: **53BP1 Inhibits Homologous Recombination in Brca1-Deficient Cells by Blocking Resection of DNA Breaks**. *Cell* 2010, **141**:243-254.
36. Ray Chaudhuri A, Callen E, Ding X, Gogola E, Duarte AA, Lee J-E, Wong N, Lafarga V, Calvo JA, Panzarino NJ, et al.: **Replication fork stability confers chemoresistance in BRCA-deficient cells**. *Nature* 2016, **535**:382-387.
37. Boersma V, Moatti N, Segura-Bayona S, Peuscher MH, van der Torre J, Wevers BA, Orthwein A, Durocher D, Jacobs JJL: **MAD2L2 controls DNA repair at telomeres and DNA breaks by inhibiting 5' end resection**. *Nature* 2015, **521**:537-540.
38. Xu G, Chapman JR, Brandsma I, Yuan J, Mistrik M, Bouwman P, Bartkova J, Gogola E, Warmerdam D, Barazas M, et al.: **REV7 counteracts DNA double-strand break resection and affects PARP inhibition**. *Nature* 2015, **521**:541-544.
39. Pettitt SJ, Rehman FL, Bajrami I, Brough R, Wallberg F, Kozarewa I, Fenwick K, Assiotis I, Chen L, Campbell J, et al.: **A Genetic Screen Using the PiggyBac Transposon in Haploid Cells Identifies Parp1 as a Mediator of Olaparib Toxicity**. *PLoS ONE* 2013, **8**:e61520.
40. Helleday T: **The underlying mechanism for the PARP and BRCA synthetic lethality: clearing up the misunderstandings**. *Mol Oncol* 2011, **5**:387-393.
41. Pettitt SJ, Krastev DB, Brandsma I, Dréan A, Song F, Aleksandrov R, Harrell MI, Menon M, Brough R, Campbell J, et al.: **Genome-wide and high-density CRISPR-Cas9 screens identify point mutations in PARP1 causing PARP inhibitor resistance**. *Nat Commun* 2018, **9**:1849.
42. Koike-Yusa H, Li Y, Tan EP, Velasco-Herrera MDC, Yusa K: **Genome-wide recessive genetic screening in mammalian cells with a lentiviral CRISPR-guide RNA library**. *Nat Biotechnol* 2014, **32**:267-273.
43. Donovan KF, Hegde M, Sullender M, Vaimberg EW, Johannessen CM, Root DE, Doench JG: **Creation of Novel Protein Variants with CRISPR/Cas9-Mediated Mutagenesis: Turning a Screening By-Product into a Discovery Tool**. *PLoS ONE* 2017, **12**:e0170445.

44. Schmid-Burgk JL, Höning K, Ebert TS, Hornung V: **CRISPaint allows modular base-specific gene tagging using a ligase-4-dependent mechanism**. *Nat Commun* 2016, **7**:12338.
45. Herzog M, Puddu F, Coates J, Geisler N, Forment JV, Jackson SP: **Detection of functional protein domains by unbiased genome-wide forward genetic screening**. *Sci Rep* 2018, **8**:6161.
46. Noordermeer SM, Adam S, Setiaputra D, Barazas M, Pettitt SJ, Ling AK, Olivieri M, Álvarez-Quilón A, Moatti N, Zimmermann M, et al.: **The shieldin complex mediates 53BP1-dependent DNA repair**. *Nature* 2018, **560**:117-121.
47. Barazas M, Annunziato S, Pettitt SJ, de Krijger I, Ghezraoui H, Roobol SJ, Lutz C, Frankum J, Song FF, Brough R, et al.: **The CST Complex Mediates End Protection at Double-Strand Breaks and Promotes PARP Inhibitor Sensitivity in BRCA1-Deficient Cells**. *Cell Reports* 2018, **23**:2107-2118.
48. Elstrodt F, Hollestelle A, Nagel JHA, Gorin M, Wasielewski M, van den Ouweland A, Merajver SD, Ethier SP, Schutte M: **BRCA1 mutation analysis of 41 human breast cancer cell lines reveals three new deleterious mutants**. *Cancer Research* 2006, **66**:41-45.
49. Dev H, Chiang T-WW, Lescale C, de Krijger I, Martin AG, Pilger D, Coates J, Sczaniecka-Clift M, Wei W, Ostermaier M, et al.: **Shieldin complex promotes DNA end-joining and counters homologous recombination in BRCA1-null cells**. *Nature Cell Biology* 2018, **20**:954-965.
50. He YJ, Meghani K, Caron M-C, Yang C, Ronato DA, Bian J, Sharma A, Moore J, Niraj J, Detappe A, et al.: **DYNLL1 binds to MRE11 to limit DNA end resection in BRCA1-deficient cells**. *Nature* 2018, **563**:522-526.
51. DelloRusso C, Welcsh PL, Wang W, Garcia RL, King M-C, Swisher EM: **Functional characterization of a novel BRCA1-null ovarian cancer cell line in response to ionizing radiation**. *Mol. Cancer Res.* 2007, **5**:35-45.
52. Mitra AK, Davis DA, Tomar S, Roy L, Gurler H, Xie J, Lantvit DD, Cardenas H, Fang F, Liu Y, et al.: **In vivo tumor growth of high-grade serous ovarian cancer cell lines**. *Gynecol. Oncol.* 2015, **138**:372-377.
53. Ghezraoui H, Oliveira C, Becker JR, Bilham K, Moralli D, Anzilotti C, Fischer R, Deobagkar-Lele M, Sanchiz-Calvo M, Fueyo-Marcos E, et al.: **53BP1 cooperation with the REV7-shieldin complex underpins DNA structure-specific NHEJ**. *Nature* 2018, **560**:122-127.
54. Mirman Z, Lottersberger F, Takai H, Kibe T, Gong Y, Takai K, Bianchi A, Zimmermann M, Durocher D, de Lange T: **53BP1-RIF1-shieldin counteracts DSB resection through CST- and Polα-dependent fill-in**. *Nature* 2018, **560**:112-116.
55. Gupta R, Somyajit K, Narita T, Maskey E, Stanlie A, Kremer M, Typas D, Lammers M, Mailand N, Nussenzweig A, et al.: **DNA Repair Network Analysis Reveals Shieldin as a Key Regulator of NHEJ and PARP Inhibitor Sensitivity**. *Cell* 2018:1-41.
56. Lo KWH, Kan H-M, Chan L-N, Xu W-G, Wang K-P, Wu Z, Sheng M, Zhang M: **The 8-kDa dynein light chain binds to p53-binding protein 1 and mediates DNA damage-induced p53 nuclear accumulation**. *J Biol Chem* 2005, **280**:8172-8179.
57. Becker JR, Cuella-Martin R, Barazas M, Liu R, Oliveira C, Oliver AW, Bilham K, Holt AB, Blackford AN, Heierhorst J, et al.: **The ASCIZ-DYNLL1 axis promotes 53BP1-dependent non-homologous end joining and PARP inhibitor sensitivity**. *Nat Commun* 2018, **9**:1-12.
58. Tammaro C, Raponi M, Wilson DI, Baralle D: **BRCA1 exon 11 alternative splicing, multiple functions and the association with cancer**. *Biochem. Soc. Trans* 2012, **40**:768-772.
59. Wang Y, Bernhardy AJ, Cruz C, Krais JJ, Nacson J, Nicolas E, Peri S, van der Gulden H, van der Heijden I, O'Brien SW, et al.: **The BRCA1-Δ11q Alternative Splice Isoform Bypasses Germline Mutations and Promotes Therapeutic Resistance to PARP Inhibition and Cisplatin**. *Cancer Research* 2016, **76**:2778-2790.
60. Ter Brugge P, Kristel P, van der Burg E, Boon U, de Maaker M, Lips E, Mulder L, de Ruiter J, Moutinho C, Gevensleben H, et al.: **Mechanisms of Therapy Resistance in Patient-Derived Xenograft Models of BRCA1-Deficient Breast Cancer**. *J. Natl. Cancer Inst.* 2016, **108**:djw148.
61. Gogola E, Duarte AA, de Ruiter JR, Wiegant WW, Schmid JA, de Bruijn R, James DI, Guerrero Llobet S, Vis DJ, Annunziato S, et al.: **Selective Loss of PARG Restores**

PARylation and Counteracts PARP Inhibitor-Mediated Synthetic Lethality. *Cancer Cell* 2018, **33**:1078-1093.e1012.

62. Brinkman EK, Chen T, Amendola M, van Steensel B: **Easy quantitative assessment of genome editing by sequence trace decomposition**. *Nucleic Acids Res.* 2014, **42**:e168.
63. Evans T, Matulonis U: **PARP inhibitors in ovarian cancer: evidence, experience and clinical potential**. *Ther Adv Med Oncol* 2017, **9**:253-267.
64. Barazas M, Gasparini A, Huang Y, Küçükosmanoğlu A, Annunziato S, Bouwman P, Sol W, Kersbergen A, Proost N, de Korte-Grimmerink R, et al.: **Radiosensitivity is an acquired vulnerability of PARPi-resistant BRCA1-deficient tumors**. *Cancer Research* 2018:canres.2077.2018.