Homologous recombination deficiency and ovarian cancer

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Abstract:

The discovery that PARP inhibitors block an essential pathway of DNA repair in cells harbouring a BRCA mutation has opened up a new therapeutic avenue for high-grade ovarian cancers. BRCA1 and BRCA2 proteins are essential for high-fidelity repair of double-strand breaks of DNA through the homologous recombination repair (HRR) pathway. Deficiency in HRR (HRD) is a target for PARP inhibitors. The first PARP inhibitor, olaparib, has now been licensed for BRCA–mutated ovarian cancers. Whilst mutated BRCA genes are individually most commonly associated with HRD other essential HRR proteins may be mutated or functionally deficient potentially widening the therapeutic opportunities for PARP inhibitors. HRD is the first phenotypically defined predictive marker for therapy with PARP inhibitors in ovarian cancer. Several different PARP inhibitors are being trialled in ovarian cancer and this class of drugs has been shown to be a new selective therapy for high-grade ovarian cancer. Around 20% of high-grade serous ovarian cancers harbour germline or somatic BRCA mutations and testing for BRCA mutations should be incorporated into routine clinical practice. The expanded use of PARP inhibitors in HRD deficient (non-BRCA mutant) tumours using a signature of HRD in clinical practice requires validation.

Introduction:

Until recently, the treatment of ovarian cancer has not been adapted to histological or biological variability in the tumour. Surgery and platinum-taxane chemotherapy remain the cornerstone of primary treatment followed at recurrence by further platinum-based chemotherapy until the tumour becomes ‘platinum-resistant’. It is now clear that epithelial ovarian cancer (EOC) comprises several different diseases [1, 2]. The collective term represents a distinct and diverse group of molecularly and aetiologically distinct pathologies with differing clinical behaviour (Figure 1). If the outcome of advanced ovarian cancer is to improve then our approach to treatment and the development of novel agents must target and exploit distinct subgroups within this heterogeneity.

Around 70% of EOC are high-grade serous adenocarcinomas. A defining feature of this subtype is the presence of mutations within the tumour suppressor gene p53 [3, 4]. In addition, molecular analysis of high-grade serous ovarian cancer (HGSOC) by The Cancer Genome Atlas (TCGA) has shown that around half have aberrations in homologous recombination repair (HRR), a critical DNA damage response pathway [5]. Repair of DNA damage following platinum-based therapy has long been considered an important determinant of tumour chemosensitivity. Several genetic lesions causing homologous recombination deficiency (HRD) include germline and somatic BRCA mutations as well as mutations of genes such as ATM, CHEK2, RAD51 and MRE11A (Table 1), and epigenetic silencing have been described in HGSOC. Exploitation of HRD by inhibitors of PARP (poly ADP ribose polymerase) a DNA repair enzyme involved in base-excision repair producing further disruption of DNA damage repair has formed the basis of a new molecularly targeted therapeutic strategy to treat ovarian cancer [6]. Over the last decade, studies with inhibitors of DNA repair, specifically PARP inhibitors in BRCA-mutated ovarian cancers have resulted in truly personalized medicine in ovarian cancer.

Here we review the central role of homologous recombination deficiency (HRD) in broadening the application of PARP inhibitors as monotherapy or in combination with other agents in ovarian cancer, and discuss how testing for HRD will become a key requirement for future trials and treatment decisions.

Biology:

DNA damage response and repair pathways**:**

The recognition and subsequent repair of DNA damage is essential for normal cellular function and maintenance of genomic stability. In humans acquired or inherited defects in DNA repair pathways result in an increased lifetime risk of cancer [7]. To date 450 genes are implicated in the DNA damage response

and repair [8] and these genes can be sub-grouped, by function, into five distinct pathways [9]. Each pathway, shown in figure 2a, has evolved to deal with a specific type of DNA damage, although there is some overlap in their functions. DNA double-strand breaks (DSB), the most lethal insult to the genome, left un-repaired result in genomic instability and cell death [10] . DSB can arise as a result of direct damage to both strands of DNA from exogenous anti-cancer treatments such as ionising radiation or the topoisomerase inhibitors [5] or as part of normal physiology e.g. to permit genetic recombination during meiosis [11]. DNA double-strand breaks can be repaired by multiple pathways. The classical non-homologous end-joining (C-NHEJ) pathway relies on the hetero-dimer Ku 70/Ku80, and ligates DSB ends without a template, it is active throughout the cell cycle. Homologous recombination repair (HRR), shown in figure 2 is an error-free pathway that uses a homologous DNA template to repair DSB and is initiated by end resection from the DNA break ends to generate a long stretch of single-strand DNA for strand invasion. HRR is used when cells enter S and G2 because cyclin-dependent kinases are needed for promoting end resection and to activate HRR. Microhomology-mediated end joining (MMEJ) is a back-up pathway and enables Ku independent alternative NHEJ [12]. It like HRR requires cyclin-dependent kinase activities and increases when cells enter S phase. MMEJ shares the initial end resection step with HRR but does not require S139-phosphorylated histone H2AX (γ-H2AX), suggesting that initial end resection likely occurs at DSB ends.

A significant number of DSB arise during DNA replication when a replication fork encounters an unrepaired SSB; the HRR pathway together with the nuclear enzyme, PARP-1, are particularly important to repairing these collapsed replication forks [13, 14]. The HRR pathway involves the function of both the BRCA1 and BRCA2 proteins. BRCA1 becomes part of a large complex associated genome surveillance complex (BASC). BASC is thought to act as a sensor for the DSB DNA damage and includes: the MRN complex, mismatch repair proteins (MSH2, MSH6 and MLH1), BLM syndrome helicase and ATM [15]. BRCA2 has a more direct role in repair through its regulation of the Rad51 recombinase. It has been suggested that the BRCA2-Rad51 complex binds to the exposed DNA, and this enables the loading of Rad51 on

to the break and the formation of the presynaptic filament [16]. Deficiency in BRCA1 or BRCA2 results in HRR deficiency (HRD), and this can therapeutically exploited by PARP inhibitors.

Development of PARP inhibitors and Synthetic lethality:

The first PARP enzyme of the 17 member nuclear super-family was discovered over 50 years ago [17] and the first PARP inhibitor (3-aminobenzamide) 20 years later [18]. Today's small molecule PARP inhibitors mediate their anti-cancer effects as catalytic inhibitors blocking repair of DNA single strand breaks by the BER/SSBR pathway. The initial clinical development of PARP inhibitors as cancer treatments focused on their role as chemo-sensitisers, and there was no scientific rationale that they would have single-agent activity. However in 2005, two articles published in Nature reported that cells deficient in BRCA1 and 2 were 100- to 1000-fold more sensitive to PARP inhibitors than BRCA1/2 heterozygote or wild-type cell lines [19, 20]. Bryant et al [19] used the PARP inhibitors NU1025 and AG14361, a forerunner to rucaparib (Clovis Oncology). Farmer et al [20] demonstrated the sensitivity of two small molecule inhibitors KU0058684 and KU0058948, forerunners to olaparib. Both research groups independently concluded that the BRCA-deficient cells were selectively sensitive to PARP inhibition by a mechanism of 'synthetic lethality'.

This is a process by which cancer cells are selectively targeted by the inactivation of two genes or pathways when inactivation of either gene or pathway alone is non-lethal. The term, originally used by geneticists in the 1940s was proposed as a possible anti-cancer strategy in the late 1990s [21]. In this case PARP inhibitors inhibit the repair of single strand DNA breaks by the BER/SSBR pathway and these accumulate and left unrepaired result in DNA DSB which must be repaired for cell survival. In the setting of HRD e.g. due to a BRCA mutation then these DNA DSB cannot be accurately and efficiently repaired and the PARP inhibition ultimately results in cell death. Recent reports have challenged this hypothesis and proposed alternative models of synthetic lethality [22]. For example, it is now known that PARP

inhibitors themselves can be directly toxic to cells by trapping PARP-1 and 2 at the site of the damaged DNA [23]. These trapped PARP-DNA complexes may in turn obstruct replication forks, which require BRCA-dependent HRR to be resolved. Interestingly, depleting PARP with PARP inhibitors has been shown to be more cytotoxic than depleting PARP through siRNA [24]. It is also known that PARP itself is critical to mediate Mre11-dependent replication restart at stalled replication forks and this is also relevant to the mechanism of synthetic lethality [14].
Clinical trials were initiated a decade ago to explore whether PARP inhibitors could through a model of synthetic lethality be therapeutically active in tumours defective in HRR.

Clinical experience of PARP inhibitors in ovarian cancer:

The first phase I trial was performed in a group of patients enriched for a BRCA mutation using AZD2281, an oral PARP inhibitor acquired from Kudos by AstraZeneca and later called olaparib. The activity of olaparib soon became clear as almost half the heavily pre-treated ovarian cancer patients responded, and the drug was associated with only minor side effects [25] . An expanded phase I trial was then performed in 48 patients with BRCA-mutated ovarian cancer, one patient with a variant BRCA mutation of unknown significance and another with a strong family history of breast/ovarian cancer. A tumour response, measured by RECIST or CA125 fall, was seen in 20 (40%) patients and the median duration of response was 28 weeks. The study included 24 patients with ‘platinum-resistant’ and 13 with ‘platinum-refractory’ disease [26]. The clinical benefit rate (response and disease stabilization) was greatest among patients with ‘platinum-sensitive’ disease. The activity of olaparib in BRCA-related ovarian cancer was confirmed in a sequential two-dose cohort study of 400mg and 100 mg bd. An overall response rate of 33% was seen in patients receiving the higher dose, and 13% with 100mg bd [27].

The initial development strategy of olaparib compared the activity of olaparib with pegylated liposomal doxorubicin (PLD) in a group of women with BRCA-mutated recurrent ovarian cancer (Study 12). In this small three-arm

randomized trial (with 200 and 400 mg dose-levels of olaparib) the progression-free survival (PFS) with olaparib and PLD were similar. The expected response rate to olaparib was confirmed, but the progression-free survival to PLD in women with BRCA-mutated ovarian cancer was higher than expected [28].

The integrity of BRCA proteins is key to effective HRR, although other proteins are also important for this process [29]. Data began to emerge demonstrating that somatic mutations or methylation of BRCA as well as dysfunction of other HR-related proteins could be associated with a 'platinum-sensitive' phenotype in high grade ovarian cancer potentially selecting for sensitivity to PARP inhibitors [3, 30]. These data suggested HRD could result from genetic lesions other than germ line BRCA mutations. This observation also strongly implied that PARP inhibitors might have clinical utility in a larger group of women with ovarian cancer. To explore this hypothesis further, a large randomized placebo-controlled trial was designed to include patients who were likely to have an 'HRD phenotype', by selecting a population of women with high-grade serous cancer and 'platinum-sensitivity' following repeated platinum therapies (study 19). This was a maintenance study in which olaparib was given following a response to platinum-based therapy [31]. The presence of a BRCA mutation was not required but it was anticipated that the population would be enriched for a BRCA mutation because of the above entry criteria. During the trial, data emerged that olaparib was active in 24% of 46 patients with high grade serous ovarian cancer without a germ line BRCA mutation[32]. Study 19 demonstrated a clear benefit of maintenance olaparib, with an extension in the progression-free survival from the start of maintenance treatment from 4.8 to 8.4 months (hazard ratio: 0.35; 95% CI, 0.25 to 0.49; P<0.001). About 22% of the patients had a BRCA mutation and 14 % were known to be BRCA negative. An early analysis of overall survival showed no benefit, which led to a more detailed study of BRCA status of the entire population. Information on BRCA status in both germ line and tumour became available in 96% of patients. Overall, 51 % had either a germ line or somatic mutation of BRCA. In the BRCA-mutated group the PFS was 11.2 months compared to 4.3 months in patients on placebo (hazard ratio: HR

0·18; 95% CI, 0·10–0·31; p<0·0001). In the 118 patients in the BRCA-wild type group there was still a significant benefit in favour of olaparib, although less marked (hazard ratio: 0·54; 95% CI 0·34–0·85; p=0·0075) [33]. Survival data are still immature for both the BRCA-mutated and overall population. There is a non-significant OS trend in favour of olaparib, but the results are confounded by cross-over to a PARP inhibitor in subsequent studies in 23% of the population. Nevertheless, the beneficial value of olaparib was further supported by secondary endpoint analyses, such as time to first subsequent therapy after progression and time to second subsequent therapy and led in December 2014 to approval of the drug by the European Medicines Agency for maintenance therapy in relapsed HGSOC following a response to platinum-based therapy. Additional supportive data has also come from a study in 'platinum-sensitive' ovarian cancer in which olaparib was given with carboplatin and paclitaxel and then as maintenance. In study 41 no additive benefit was seen with chemotherapy, but the PFS of the group receiving olaparib was significantly longer than in those receiving chemotherapy alone (HR 0·51 [95% CI 0·34–0·77]; p=0·0012) and the effect was even greater in the small number of patients with a known BRCA mutation[34]. In the USA the FDA licensed olaparib as monotherapy therapy in patients with BRCA-mutated ovarian cancer who have received three or more lines of treatment. The evidence for this was derived mainly from 'study 42' [35], a trial of monotherapy with additional data from earlier trials. The licence was based on the data of 137 patients and the response rate was 34% with an average response duration of 7.9 months [36].

Olaparib, ten years after entering clinical trials is now established as a treatment for BRCA-mutated ovarian cancer. Side effects from treatment are generally mild, with fatigue, nausea and anaemia being the most commonly reported adverse events. Diarrhoea, altered taste, headache and 'cold-like' symptoms have also been reported. Two rare but serious side effects are pneumonitis and myelodysplastic syndrome/ AML. The latter has been reported in patients with a BRCA mutation and it is not clear by how much the risk is increased with olaparib. No significant detrimental effects have been seen in Quality of Life measurements [37] and only 7 (5%) patients on study

19 discontinued olaparib due to adverse events. Some patients have remained on treatment for several years. Careful monitoring for long-term toxicities continues but the number of cases of myelodysplasia or acute leukaemia remains very low, less than 1% of more than 22,000 patients. Olaparib is currently formulated as a capsule and the recommended dose of 400 mg twice daily requires that women take 16 capsules per day. A new tablet formulation, 300mg twice daily is being evaluated in new, ongoing studies including SOLO-2, a randomized maintenance trial in high-grade carcinomas with a BRCA mutation (NCT01874353).

Overcoming resistance to PARP inhibitors

Despite reports of prolonged clinical responses to the PARP inhibitor olaparib [38] the majority of ovarian cancers become resistant to treatment. There are several mechanisms by which resistance to PARP inhibitors can occur. Primary resistance often occurs in tumours that do not have HRD, which is likely to be in around 50% of high-grade serous ovarian cancer cases. Induction of HRD in ovarian cancer to overcome resistance by altering the tumour microenvironment through hypoxia [39] or by combining PARP inhibitors with agents that might down-regulate HRR, such as PI3Kinase inhibitors [40] might render HRR competent cells sensitive to PARP inhibition. This concept, known as 'contextual' synthetic lethality, could have some effect on primary resistance and possibly broaden the application of this class of drugs in the treatment of cancer, and is the rationale behind other ongoing combination studies. Acquired resistance to PARP inhibitors in BRCA-mutated tumours may be more difficult to overcome; mechanisms have been demonstrated in preclinical models and patients, which include secondary molecular defects that partly restore BRCA function, increasing drug efflux mediated by overexpression of P-glycoprotein and most recently reduced or absent 53BP1 [41-43] (figure 3).

The majority of PARP inhibitor studies have excluded patients with prior PARP inhibitor exposure and so there are as yet no clinical reports of repeated therapy, or using a different PARP inhibitor in previously treated tumours. It is also unknown whether re-treatment with other PARP inhibitors

that are either more potent (in vitro), such as BMN673 (talazoparib) [44] or have distinct inhibitory profiles such as rucaparib (inhibits tankyrases) will overcome resistance seen with another PARP inhibitor. What is clear is that patients who develop resistance do respond to subsequent therapies [45] allaying some of the concern that prolonged therapy with a PARP inhibitor may induce platinum and other drug resistance.

Defining the HRD population:

Maintenance trials with other PARP inhibitors, such as niraparib and rucaparib, two PARP inhibitors that are active as monotherapy in BRCA-mutated ovarian cancer [46, 47] are in progress (NOVA; NCT01847274 and ARIEL3; NCT01968213). Importantly these trials include patients without a BRCA-mutation, testing the hypothesis that PARP inhibitor therapy could be useful in a wider group of patients. Defining HRD is not straightforward as it represents a phenotypic behaviour of tumour cells resulting from one or more abnormalities in the many proteins responsible for HRR. In addition to germ line BRCA mutations, other less common germ line and/or somatic mutations involved in HRD and predictive of a response to platinum may be present in almost one third of ovarian tumours [48]. Gene expression analysis has been explored to identify ‘BRCAness’, a phenotype that correlates with ‘platinum-sensitivity’ [49] but a clinically useful prospective test for HRD is needed for decision-making. Simple tests, such as immunochemistry to identify Rad51 foci, involved in the HRR process have been difficult to set up, and it is unlikely that these will be easily applicable to clinical practice [50]. The Clovis Oncology Group in combination with Foundation Medicine are exploring genomic scarring as a marker for a ‘BRCA-like’ state. The first results of ARIEL2, a trial of rucaparib in predominantly BRCA-wild type ‘platinum sensitive’ tumours was able to dichotomise patients into ‘BRCA-like’ and biomarker negative tumours with response rates (RECIST/CA125) to rucaparib in 45% and 21 % respectively [51]. It is clear that if the indication for PARP inhibitors is to expand into a BRCA-wild type population robust tests with a high probability of determining HRD status are needed.

PARP inhibitors in a wider population of ovarian cancer:

Data are emerging to show that tumour activity may be increased by combining olaparib with cediranib, an anti-VEGFR tyrosine kinase inhibitor [52]. A randomized trial of 'platinum-sensitive' ovarian cancer, demonstrated an additive effect of the combination resulting in a median progression free survival of 17.7 months compared to 9 months for olaparib alone. A subset analysis of outcome by BRCA status suggested that the greater benefit of the combination was seen in patients without a BRCA mutation. A hypothesis to consider is that the enhancing effect was due to an increase in HRD with cediranib due to tumour hypoxia [53, 54]. Two other studies with cediranib and olaparib are being planned, one comparing the combination with chemotherapy being conducted by the NCI-CTEP, and the other, the UK-led ICON9 trial adding olaparib to cediranib as maintenance treatment after chemotherapy with cediranib. A randomised first-line trial adding olaparib maintenance to chemotherapy and bevacizumab is has just opened (PAOLA1). Variation in the amount of HRD present in the tumours of these patients is likely to be an important factor that affects outcome, but until validated prospective tests are available, stratification on the basis of a BRCA mutation is all that can be reliably achieved.

Selecting OC Patients for PARP Inhibitor Therapy

The significant positive impact of PARP inhibition in the management of ovarian cancer will require a change in practice. A single approach to therapy for ovarian cancer is no longer valid. Identification of patients with a BRCA mutation is already an important consideration in routine clinical practice. Hitherto, testing of patients with a BRCA mutation has been focussed on risk-identification and risk-reduction in unaffected family members of women [55]. However, selecting patients for testing on the basis of family history alone is insufficient; studies testing cohorts of women with ovarian cancer have consistently shown that around 30% of women with a germ line BRCA mutation do not have a known family history of breast or ovarian cancer [56,

57]. The estimated frequency of a germ line BRCA mutation in high-grade ovarian cancer is around 15%[58], although probably higher when restricted to high-grade serous cancers, and about 10 fold higher among the Israeli Ashkenazi Jewish population where it accounts for 40% of ovarian cancer [59]. The activity of PARP inhibitors in patients with somatic BRCA mutations appears to be similar to those with germ line mutations [33], and somatic mutations are present in around 6-8% of cases [60]. Updated results from the ARIEL2 trial of rucaparib in 152 patients with 'platinum-sensitive' ovarian cancer and wild-type BRCA reported a response rate of 36% in patients with a '*BRCA*-like' signature, in contrast to 16% in patients that were biomarker negative [61]. It is likely on the basis of these results that a strategy will need to be developed to routinely test tumours for the presence of HRD.

Identification of patients who harbour a BRCA mutation has therapeutic implications for the individual and possible consequences for non-affected mutation carriers. As many ovarian cancer patients with a BRCA mutation do not have a family history, all patients with high-grade cancers should be counselled for a germ line mutation testing. The presence of a BRCA mutation has wider implications for women with ovarian cancer that has led some to recommend that all patients should be referred to genetics departments for counselling and testing. However, for many departments, the number of referrals would be overwhelming. This has led to the concept of genetic mainstreaming, an approach where counselling and testing take place within the Gynaecological Oncology environment with referral of positive cases to genetic clinics for family counselling and testing. Pilot studies have shown this to be feasible [62] and as the cost of testing falls, the prospect of routine testing of patients for germ line mutations is less daunting for funders. Nevertheless, the diagnosis of a germ line mutation in patients with ovarian cancer carries with it an extra burden; what to do about breast cancer prevention and how to deal with the often complex family dynamics surrounding the diagnosis in one family member. Guidelines for testing will need to be adapted but even with the current approach genetic counselling and testing remains under-utilised[63]. The introduction of olaparib into clinical practice has increased the number of centres and regions now establishing

routine testing for most women with high-grade serous ovarian cancer. However, the funding for olaparib is not uniform throughout Europe and this may affect the speed with which routine testing is implemented. In reality, not all ovarian cancer patients with a BRCA mutation will respond to a PARP inhibitor, and for some the duration of response is short. Little is known about the underlying factors affecting the duration of response. Also, some of the mutations detected may not be known to be pathogenic, and are called 'variants of unknown significance' (VUS). Sharing of data amongst geneticists and genetics laboratories is needed to improve the interpretation of these VUS [64].

However, restricting tests to measurement of germ line BRCA mutations will miss somatic mutations and other causes of HRD. There are at least 12 inherited genes associated with the HRD phenotype [65] (Table 1) in addition to somatic BRCA mutations. Mutations in these non-BRCA HRD genes are uncommon. It has been suggested that all patients should have tumour testing for at least a somatic BRCA mutation. However, interpretation of results may not always be straightforward, and there is uncertainty about how many of the HRD-related genes should be tested. Other measurements of genomic instability, such as the degree of genomic scarring (loss of heterozygosity (LOH)) may provide valuable supplementary information to mutational analysis [66]. This will become more important if current trials with niraparib or rucaparib maintenance therapy lead to licensing of the drugs in the non-BRCA group. There are arguments for testing tumour first and only referring patients with a BRCA mutation to geneticists for counselling and germ line testing.

Companion diagnostic HRD tests are being developed alongside the maintenance trials of PARP inhibitors in NOVA (niraparib) and ARIEL3 (rucaparib). One is in collaboration with Myriad Genetics [67] which uses three combined measures to provide an HRD score - loss of heterozygosity, telomeric allelic imbalance and large-scale state transitions in cancer cells [68]. A novel genetic biomarker test developed in collaboration with Foundation Medicine, based on the assessment of genomic instability by

measuring loss of heterozygosity (LOH) across the whole genome, expressed as a measure of ‘genomic scarring’ [69], reported with the updated results of ARIEL2 [61] will be used in ARIEL3.

Research that started by exploring that activity of PARP inhibitors in tumours harbouring a germ line BRCA mutation has identified a new molecularly defined genetic predictive marker for response. In parallel, work on DNA damage response repair and more complex genomic analysis of tumours has demonstrated the complexity of the HRD phenotype. At the same time these simultaneous advances have provided an opportunity to expand therapeutic research with PARP inhibitors. The interaction of PARP inhibitors with other molecularly targeted therapies such as inhibitors of angiogenesis further widens the potential clinical benefit of these drugs. A new paradigm for treatment is rapidly emerging, underpinned by a better understanding of the molecular processes that are defective in ovarian cancer. This is allowing clinicians to offer a molecularly selected patient population an effective treatment with low toxicity and durable benefit.

Table

HR-pathway gene	Observed Frequency All Epithelial Ovarian Cancer (%)	Observed frequency High Grade Ovarian Cancer (%)	Reference
RAD51C	0.41 – 2.9	1.9	[48, 70-72]
RAD51D	0.35 – 1.1	0.95	[3, 48, 72]
RAD51B	0.06	0.95	[3, 72]
RAD50	0.2	-	[70]
RAD54L	-	0.5	[61]
ATM	0.8 - 0.86	0.32 - 1.0	[3, 48, 70]
BRIP1	0.9 – 1.72	0.32 - 1.0	[3, 48, 73]
CHEK2	0.4 – 1.6	0.32 - 1.0	[3, 48, 70]
FANCA	-	0.5	[61]
FANCI	-	0.5	[61]
NBN	0.2 – 0.25	0.63 - 1.0	[3, 48, 70, 73]
PALB2	0.2 – 0.5	0.63	[3, 48, 73]

Figure 1: Histological and molecular sub-types of EOC. g=germline, t= tumour

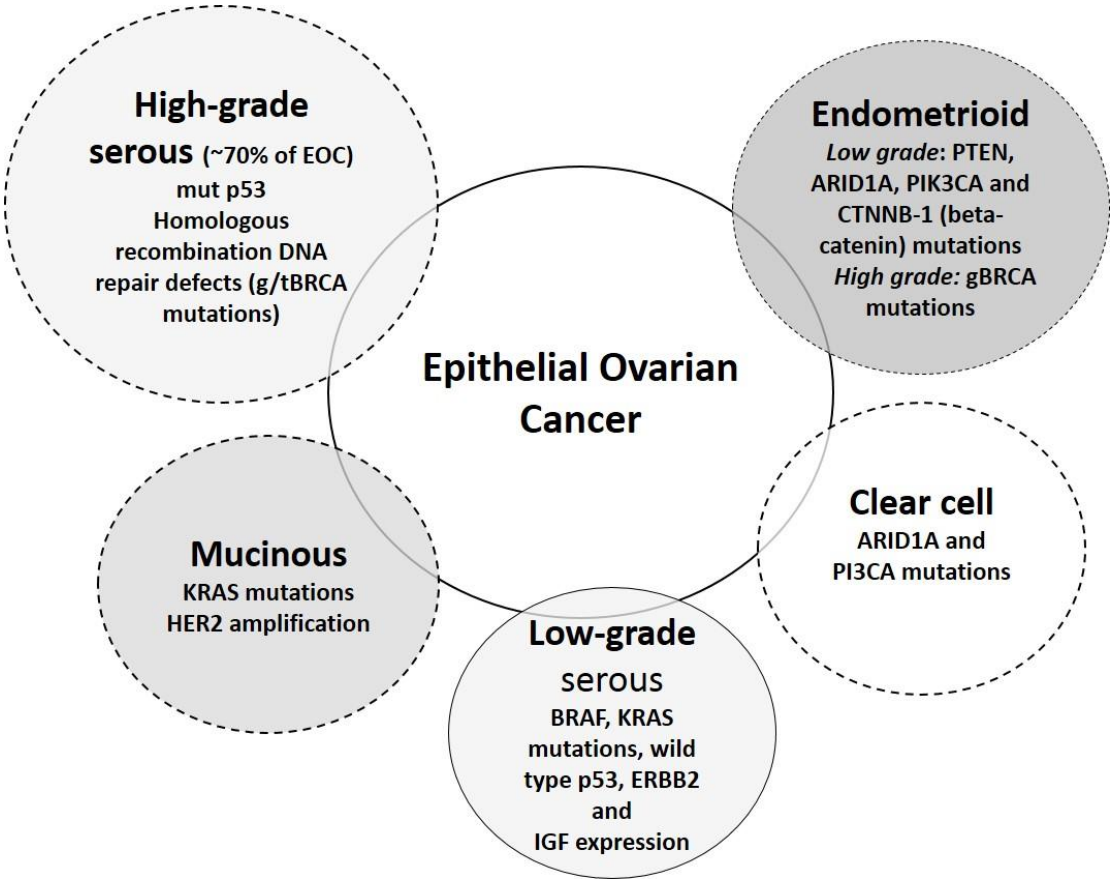


Figure 2

Figure 2a: The five DNA repair pathways in man

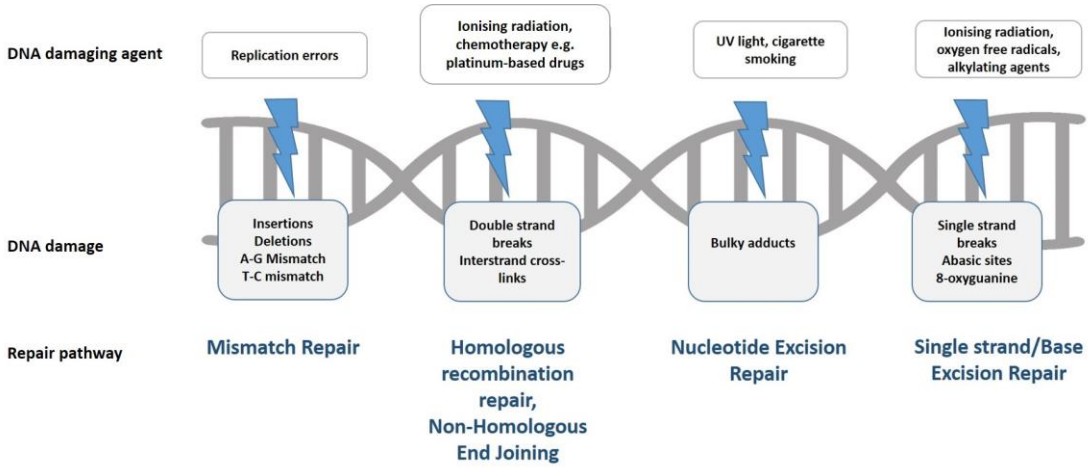


Figure 2b: DNA double strand break repair by Homologous recombination repair (HRR)

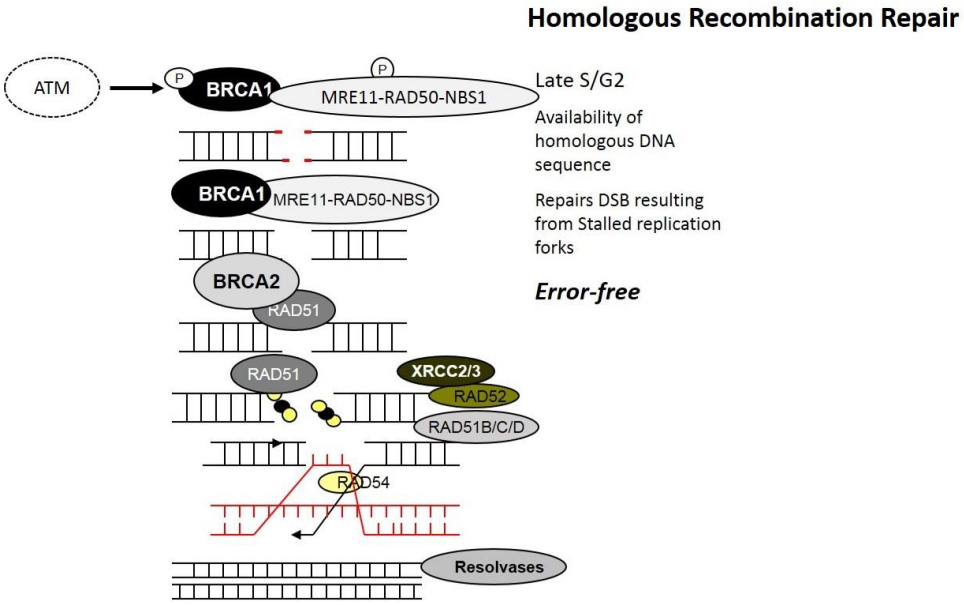
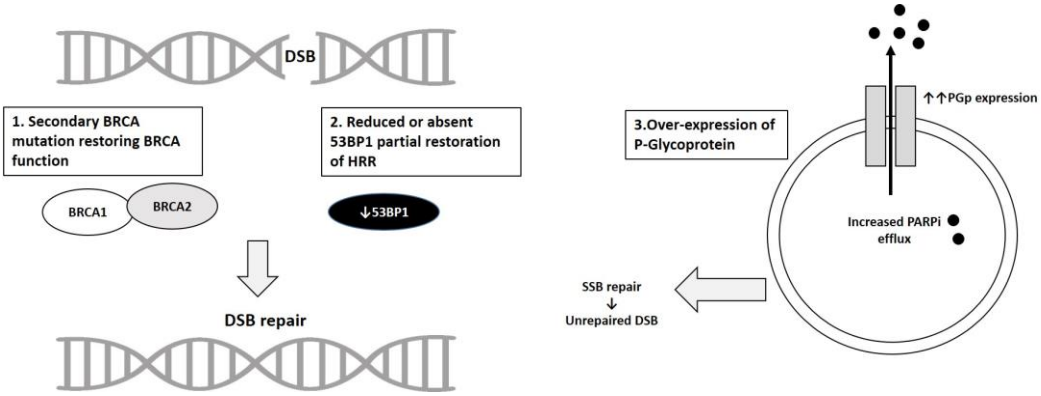


Figure 3: The three known mechanisms of acquired resistance to PARP inhibitors. 53BP1 (p53 binding protein, also called TP53BP1) is a chromatin-associated factor that promotes immunoglobulin class switching and DNA double-strand-break (DSB) repair by non-homologous end joining.
PGp = P-glycoprotein



Conflict of Interest: JAL was the Chief Investigator for AstraZeneca study 19 and is the Co-Chief Investigator for the Clovis Oncology ARIEL3 trial. He has participated in Advisory Boards for both companies but has not received any personal remuneration. He has participated in AstraZeneca sponsored symposia and received travel costs to attend but no personal remuneration.
RSK is an investigator on PARPi trials with olaparib, rucaparib, niraparib and advisory board member to Clovis
YD is an investigator on several PARPi trials with olaparib and rucaparib. Advisory board member to Clovis and Astrazeneca for which I have received honoraria

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