Catalytic depolymerisation of suberin rich biomass with precious metal catalysts

DOI:
[10.1039/C8GC00605A](https://doi.org/10.1039/C8GC00605A)

Document Version
Final published version

[Link to publication record in Manchester Research Explorer](#)

Citation for published version (APA):

McCallum, C. S., Strachan, N., Bennett, S. C., Graham Forsythe, W., Garrett, M. D., Hardacre, C., Morgan, K., & Sheldrake, G. N. (2018). Catalytic depolymerisation of suberin rich biomass with precious metal catalysts. *Green Chemistry*, 20(12), 2702-2705. <https://doi.org/10.1039/C8GC00605A>

Published in:
Green Chemistry

Citing this paper

Please note that where the full-text provided on Manchester Research Explorer is the Author Accepted Manuscript or Proof version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version.

General rights

Copyright and moral rights for the publications made accessible in the Research Explorer are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Takedown policy

If you believe that this document breaches copyright please refer to the University of Manchester's Takedown Procedures [<http://man.ac.uk/04Y6Bo>] or contact uml.scholarlycommunications@manchester.ac.uk providing relevant details, so we can investigate your claim.

PARP inhibitors in platinum-sensitive high-grade serous ovarian cancer

Robert D. Morgan^{1,2} · Andrew R. Clamp^{1,2} · D. Gareth R. Evans³ · Richard J. Edmondson^{4,5} · Gordon C. Jayson^{1,2}

Received: 23 November 2017 / Accepted: 25 January 2018
© The Author(s) 2018. This article is an open access publication

Abstract

Purpose Poly(ADP-ribose) polymerase inhibitors (PARPi) have changed the management of high-grade serous ovarian cancer (HGSOC). The rationale for the development of PARPi was based on the concept of synthetic lethality, in which a cell can survive a deficiency of one gene/gene product, but may die if there is a deficiency in a combination of genes/gene products. In women with *BRCA1/2* deficiency within their ovarian cancer tissue, inhibition of PARP imposes an intolerable burden of DNA damage repair deficiency and may induce cell death.

Methods Clinical trials have evaluated PARPi as single-agent therapeutics and as maintenance treatment following platinum-based chemotherapy for HGSOC. Clinical data suggest the most impressive anti-tumour activity occurs in women with platinum-sensitive ovarian cancer and germline or somatic *BRCA1/2* mutations (*g/sBRCAmt*).

Results In the maintenance setting, randomised trials have shown that PARPi compared to placebo reduce the hazard ratio for the development of progressive disease to 0.2–0.27 for patients with a *g/sBRCAmt*; to 0.34–0.38 for patients with putative evidence of DNA damage repair deficiency; and to 0.35–0.45 in an unselected population with HGSOC. Furthermore, phase 1/2 trials have reported single-agent anti-tumour response rates in *gBRCAmt* of approximately 50% in platinum-sensitive and 25% in platinum-resistant disease.

Conclusion Here, we discuss the evidence for the use of PARPi as single-agent therapeutics and maintenance treatment in HGSOC and evaluate the genetic assays used in clinical trials so far. We discuss the emerging role of platinum sensitivity as a broad eligibility criteria for the use of PARPi.

Keywords Ovarian cancer · Platinum sensitivity · PARP inhibitors · BRCA mutation

Introduction

Epithelial ovarian cancer is the fourth commonest cause of female cancer-related death in the west [1]. The commonest histological subtype, high-grade serous carcinoma, accounts for approximately 70% of cases [1]. Epithelial ovarian cancer often presents with advanced disease. Primary treatment is tailored to individual patients but often involves cytoreductive surgery followed by platinum-based chemotherapy. In a minority of patients with advanced stage disease, this approach is associated with long-term survival, but sadly the majority will develop recurrent disease within 12–18 months of their primary treatment.

Upon disease relapse, treatment switches to controlling symptoms and improving survival with sequential lines of platinum- and/or taxane-based chemotherapy, often resulting in diminishing disease-free intervals until drug resistance develops and palliation is required. The 5-year survival for

✉ Gordon C. Jayson
gordon.jayson2@christie.nhs.uk

¹ Department of Medical Oncology, The Christie NHS Foundation Trust, Manchester, UK

² Manchester Cancer Research Centre, The University of Manchester, Wilmslow Road, Manchester M20 4BX, UK

³ Division of Evolution and Genomic Sciences, Manchester Academic Health Science Centre, University of Manchester, Manchester, UK

⁴ Department of Obstetrics and Gynaecology, St Mary's Hospital, Central Manchester NHS Foundation Trust, Manchester, UK

⁵ Division of Cancer Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK

ovarian cancer is approximately 35% [2]. Two drug classes of maintenance therapy have so far been developed [3]. The first group is the VEGF inhibitors, which largely improve progression-free survival (PFS). Improvements in overall survival (OS) have only been reported in occasional trials or trial subgroups [4].

The second class of maintenance therapy agents are the poly(ADP-ribose) polymerase inhibitors (PARPi) [5]. These small molecule inhibitors exploit the susceptibility of cancer cells to defects in DNA damage repair and have shown impressive results in clinical trials as both single-agent therapeutics and maintenance treatments. Herein, we discuss the use of PARPi in high-grade serous ovarian carcinoma and the genetic assays that have been assessed as potential predictive biomarkers.

Homologous recombination repair

Following the initial description of the sensitivity of *BRCA*-deficient cells to PARPi [6, 7], further work tested the hypothesis that impairments in double-strand DNA repair might predict for sensitivity to PARPi. Homologous recombination (HR) repair is a high-fidelity genetic recombination process involved in the repair of DNA double-strand breaks (DSBs) [8]. It occurs during the G2/S phase of the cell cycle where the presence of a sister chromatid/homologous chromosome allows error-free repair of DSBs and preservation of genomic integrity [8]. Germline loss-of-function (LOF) monoallelic mutations in genes involved in HR repair such as *BRCA1*, *BRCA2*, *RAD51C*, *RAD51D* and *BRIP1* are associated with an increased risk of developing familial ovarian cancer [9–15]. For carriers of the high-penetrance cancer-susceptibility genes *BRCA1* and *BRCA2*, the life-time risk of ovarian cancer, and particularly high-grade serous carcinoma, is between 40–70 and 20–50%, respectively [16, 17]. Malignant transformation in *BRCA*-related familial ovarian cancer is thought to occur when the remaining wild-type allele undergoes pathogenic mutation or epigenetic silencing, thereby leaving only the germline *BRCA1/2* mutant allele [18].

A deficiency in *BRCA1* or *BRCA2* within a cell is believed to impair HR repair, termed homologous recombination deficiency (HRD), and place a greater reliance upon alternative DNA repair pathways to repair DSBs, such as non-homologous end joining (NHEJ) and microhomology-mediated end joining (MMEJ) [19]. These alternative pathways are of low fidelity and error-prone, with potential for losses or gains of nucleotide bases within the cell's genome during the repair process [20]. A reliance on NHEJ and/or MMEJ in cells with *BRCA1/2* deficiency increases the probability of mutations occurring within the genome, a process known as genomic instability, and may ultimately lead to malignant transformation [21]. The *BRCA1/2* genes are, therefore, considered

tumour suppressor genes and are likely to require biallelic LOF for tumorigenesis to occur; in keeping with Knudson's double-hit hypothesis [22–24]. In women with a somatic *BRCA1/2* mutation in the tumour tissue only, biallelic LOF occurs through mutations and/or epigenetic silencing in both wild-type alleles and is often clonal [25, 26]. The relationship between possessing a germline monoallelic mutation in other non-*BRCA* HR repair genes and genomic instability/tumorigenesis is less clear.

PARP inhibitors

PARP are a family of cellular enzymes that are involved in a variety of biological functions. The most abundant and well-characterised members are PARP-1 and PARP-2, which have a role in DNA damage detection and repair [27]. Within their catalytic site, nicotinamide adenine dinucleotide is used as a substrate to form polymers of ADP-ribose in a process called poly (ADP-ribosyl)ation or PARYlation [28]. PARP binds to DNA at single-strand breaks (SSBs) and forms PAR on itself and other accessory proteins associated with DNA. These polymers are then able to recruit proteins involved in the base excision repair (BER) pathway; utilised to repair SSBs [29].

Small molecule inhibitors of PARP-1/2 were engineered to inhibit the catalytic domain at nanomolar concentrations. By inhibiting PARP, SSBs remain unrepaired and may potentially form lethal DSBs as the ensuing replication fork stalls and/or collapses [19]. More recent evidence also suggests that PARPi trap PARP on DNA and thereby prevent the dissociation required for the BER pathway to proceed [30–32]. There is also evidence to suggest that PARP is involved in NHEJ and that dysregulation of NHEJ may determine PARPi sensitivity [33, 34].

Preclinical work has convincingly demonstrated that cells deficient in *BRCA1/2* were sensitive to PARP inhibition. The proposed mechanism for this effect is synthetic lethality, in which a cell is able to survive with a deficiency in one gene/gene product but may die if a deficiency occurs in a particular combination of two or more genes/gene products [35, 36]. A deficiency in *BRCA1/2* is believed to impair a cell's ability to repair DSBs through HR repair. A lethal second deficiency is brought about by targeted pharmacological inhibition of PARP-1/2 [19].

The use of PARPi is changing how high-grade serous ovarian carcinoma is treated. PARP inhibitors have been evaluated in two distinct clinical settings; as single-agent therapeutics and as maintenance treatment following a response to platinum-based chemotherapy.

Single-agent therapy

PARPi single-agent therapy has been thoroughly investigated in phase 1/2 trials in women with ovarian cancer (see Table 1) [37–53]. These trials predominantly enrolled women with high-grade serous ovarian carcinoma and a

germline *BRCA1/2* mutation (g*BRCAmt*). Most women had been pre-treated with several lines of traditional cytotoxic chemotherapy and in most trials platinum sensitivity was not mandated. A range of doses of individual PARPi were used in the search for recommended phase 2/3 doses. Nevertheless, data from these trials reported encouraging anti-tumour responses across all PARPi in selected groups

Table 1 Objective response rates (ORR) as per RECIST (CR/PR) to PARPi single-agent therapy in ovarian cancer

Clinical trial	PARPi	Dose	<i>BRCA</i> status	ORR, % (95% CI)
Phase 1 trials				
Fong et al. ^a	Olaparib	200 mg BD ^d	g <i>BRCAmt</i>	Plat-Sen 46 Plat-Res 33
Mateo et al. ^a	Olaparib	300 mg BD ^e 400 mg BD ^e	g <i>BRCAmt</i>	Plat-Sen & Plat-Res 38 ^g Plat-Sen & Plat-Res 42 ^g
Kristeleit et al.	Rucaparib	40 mg OD – 840 mg BD	g <i>BRCAmt</i>	Plat-Sen 12 Plat-Res 9
Sandhu et al. ^a	Niraparib	300 mg OD	<i>BRCAmt</i> Sporadic HGSOC	Plat-Sen 50 Plat-Res 33 Plat-Sen 33 Plat-Res 5
De Bono et al. ^a	Talazoparib	0.025–1.1 mg/day OD	g <i>BRCAmt</i>	Plat-Sen 55 Plat-Res 20
Phase 2 trials				
Auden et al.	Olaparib	400 mg BD	g <i>BRCAmt</i>	Plat-Sen 38 Plat-Res 30
Gelmon et al.	Olaparib	400 mg BD	<i>BRCAmt</i> <i>BRCAwt</i> or unknown HGSOC	Plat-Sen 60 Plat-Res 33 Plat-Sen 50 Plat-Res 4
Kaye et al. ^b	Olaparib	400 mg BD	g <i>BRCAmt</i>	Plat-Sen & Plat-Res 31 ^g
Kaufman et al.	Olaparib	400 mg BD	g <i>BRCAmt</i>	Plat-Res only ^h 31 (25–38)
Drew et al.	Rucaparib	Variable oral dosing levels ^f	g <i>BRCAmt</i>	Plat-Sen & Plat-Res 21 ^g
Kristeleit et al.	Rucaparib	600 mg BD	<i>BRCAmt</i>	Plat-Sen 60 (43–74)
ARIEL2 (Part 1) ^b	Rucaparib	600 mg BD	<i>BRCAmt</i> <i>BRCAwt/LOH</i> ^{High} <i>BRCAwt/LOH</i> ^{Low}	Plat-Sen 80 (64–91) Plat-Sen 29 (20–40) Plat-Sen 10 (4–20)
Coleman et al.	Veliparib	400 mg BD	g <i>BRCAmt</i>	Plat-Sen 35 (18–56) ⁱ Plat-Res 20 (9–36) ⁱ
Steffensen et al. ^c	Veliparib	300 mg BD	g <i>BRCAmt</i>	Plat-Sen 56 Plat-Res 45

BRCAmt pathogenic *BRCA1/2* mutation, *BRCAwt* wild type *BRCA1/2*, *BD* twice daily, *CR* complete response, *HGSOC* high-grade serous ovarian cancer, *LOH* loss of heterozygosity, *n* number of patients, *OD* once daily, *PR* partial response, *Plat-Sen* platinum sensitivity, *Plat-Res* platinum resistant

^aPhase 1 dose expansion study

^bRandomised phase 2 trial

^cPhase 1/2 study. The ORR for Phase 2 are included in this table

^dThe majority of patients (*n* = 39/50) received 200 mg twice daily. Dose levels ranged from 40 mg once daily for 2 out of every 3 weeks to 600 mg twice daily

^eORR for the tablet formulation (150 mg tablet), as opposed to capsule formulation, is reported in this table

^fOnly patients receiving oral rucaparib included in this table; oral doses ranged from 92 mg once daily for 7 days q21 days to 600 mg twice daily continuous daily dosing

^gCombined ORR for patients with platinum-sensitive or platinum-resistant disease

^hStudy included women with platinum-resistant disease and those not suitable for further platinum therapy

ⁱ90% confidence interval

of patients. Patients with *gBRCA*mt often had better anti-tumour responses than those with wild-type *BRCA1/2* (*BRCA*wt) and patients with platinum-sensitive disease had better responses than women with platinum-resistant or refractory disease. Data also suggested that women with platinum-resistant disease who carried a *gBRCA*mt achieved better objective response rates (ORRs) than those with *BRCA*wt.

The most impressive anti-tumour response reported was in the phase 2, single arm, multi-centre trial, ARIEL2 (Part 1) in which women with a germline or somatic *BRCA*mt (*g/sBRCA*mt) achieved a RECIST ORR [complete/partial response (CR/PR)] of 80% (95% CI 62–97%) with rucaparib single-agent therapy [53]. The vast majority of women recruited had high-grade serous ovarian cancer and all women had a progression-free interval following the penultimate platinum-based chemotherapy of at least 6 months. The median number of prior lines of chemotherapy was 2 (inter quartile range [IQR] of 1 to 2). In the same trial, women with *BRCA*wt had a more modest, but encouraging, RECIST ORR (CR/PR) of 21% (the objective response by combined RECIST and CA-125 was 35%).

Across all phase 1/2 trials investigating PARPi as a single-agent therapy women with platinum-sensitive ovarian cancer achieved better ORRs compared to those with platinum-resistant/refractory disease (see Table 1). In addition, a number of trials demonstrated that a longer platinum-free interval (PFI) correlated with a higher likelihood of achieving an anti-tumour response [41, 43, 47, 54].

Data from a number of phase 1/2 trials also suggested that in the platinum-resistant setting, eligibility for PARPi single-agent therapy might appropriately include *BRCA1/2* mutational status. Indeed, the ORRs for women with a *gBRCA*mt and platinum-resistant disease were noticeably higher (approximately 20–30%) across all PARPi than that seen in women with *BRCA*wt (see Table 1). In a phase-1 dose expansion trial assessing niraparib, Sandhu et al. reported an ORR of 5% in women with platinum-resistant sporadic high-grade serous ovarian or primary peritoneal cancer compared to 33% in women who had a *gBRCA*mt [51]. In addition, in a non-randomised phase 2 trial assessing olaparib, Gelmon et al. reported an ORR as per RECIST (CR/PR) of 33% in women with a *gBRCA*mt and platinum-resistant high-grade serous or undifferentiated ovarian carcinoma, but a much lower ORR of 4% in those with *BRCA*wt [44]. Interestingly, in the same trial Gelmon et al. also showed that all women with a *gBRCA*mt and platinum-resistant disease achieved a CA-125 treatment response compared to only 40% with *BRCA*wt. These data may suggest that the acquisition of platinum-resistance in women with *BRCA*wt is associated with PARPi single-agent therapy resistance, but that the presence of a *gBRCA*mt maintains PARPi sensitivity. Women with *BRCA*wt and platinum-resistant disease may,

therefore, benefit more from combinational therapy with PARPi and additional targeted treatments, anti-angiogenic inhibitors and/or immune therapy [55–57].

As single agents, PARPi brought about modest and manageable toxicities. Specific grade 3–4 adverse events (AEs) included most commonly anaemia but also fatigue, nausea and vomiting. These AEs are now recognised class effects of which the majority are grade 1–2. Rucaparib caused a noticeable comparable increase in grade 3–4 ALT/AST (> 10%) although these increases often occurred within the first few weeks after starting treatment and were not associated with any symptoms, infrequently led to treatment discontinuation (< 1%), and, reversed following dose omission [58]. The recommended phase 2/3 dose of niraparib (300 mg once daily) did cause notable myelosuppression [51]. Rare ($\leq 1\%$) but important class-specific AEs also included myelodysplastic syndrome/acute myeloid leukaemia (MDS/AML) and pneumonitis. The incidence of MDS/AML was similar across all PARPi and did not depend upon duration of treatment [37, 45, 51, 54].

A number of trials are ongoing to assess PARPi as single-agent therapy in the platinum-sensitive and platinum-resistant setting (see Table 2).

Maintenance therapy

More recently, the utilisation of PARPi has focused on maintenance treatment following a response to platinum-based chemotherapy. Several phase 2/3 trials have assessed PARPi as maintenance treatment in women with relapsed high-grade ovarian cancer [59–63] (see Table 3). In each trial, PARPi were commenced following a treatment response [RECIST (CR/PR) $\pm$ CA-125 response] to platinum therapy. These trials primary outcome was progression-free survival (PFS); either investigator assessed or blinded independent central review (BICR; see Table 3). In addition, secondary efficacy outcomes included time to first subsequent therapy (TFST) and time to second subsequent therapy (TSST) as there was concern that platinum-resistance may be induced by PARPi and so these exploratory endpoints allow analysis of this (see Table 4).

Randomised phase 2 maintenance therapy trial

Study 19, a randomised, placebo-controlled, phase 2 trial, first assessed PARPi as maintenance treatment in ovarian cancer (see Table 3) [60]. All enrolled women had recurrent, platinum sensitive (defined as a progression-free interval of 6 months or more from the penultimate platinum chemotherapy) high-grade serous ovarian carcinoma and had achieved a CR/PR to their latest platinum-based chemotherapy. Patients were randomised to receive either

Table 2 Ongoing phase 2/3 trials assessing PARPi as single-agent therapy and maintenance monotherapy

Single-agent therapy	
SOLO-3 (NCT02282020)	Olaparib vs. chemotherapy (gBRCAmt, platinum-sensitive)
OCTOVA (NCT03117933)	Olaparib ± cediranib vs. chemotherapy (g/s BRCAmt, platinum-resistance)
QUADRA (NCT02354586)	Niraparib after 3 or 4 lines of chemotherapy (single arm, phase 2)
ARIEL2 Part 2 (NCT01891344)	Rucaparib after ≥ 3 lines of chemotherapy (single arm, phase 2)
ARIEL4 (NCT02855944)	Rucaparib vs. chemotherapy (g/s BRCAmt, platinum-sensitive or resistance)
Maintenance monotherapy treatment	
SOLO-1 (NCT01844986)	Olaparib vs. placebo (g/sBRCAmt, following response to first-line platinum therapy)
OReO (NCT03106987)	Olaparib vs. placebo (re-challenge maintenance olaparib monotherapy after response to repeat platinum therapy)
PRIMA (NCT02655016)	Niraparib vs. placebo (following response to first-line platinum-based therapy)
GOG3005 (NCT02470585)	Veliparib vs. placebo (following concurrent first-line carboplatin, paclitaxel & veliparib)

g germline, g/s germline or somatic

Table 3 Progression-free survival (PFS) in phase 2/3 trials assessing PARPi as maintenance treatment

Clinical Trial	Subgroups	Study arms	PFS/months (95% CI)	HR (95% CI)	P value
Study 19 ^a	HGSOC	Olaparib vs. Placebo	8.4 vs. 4.8	0.35 (0.25–0.49)	< 0.001
	g/sBRCAmt	Olaparib vs. Placebo	11.2 (8.3–NC) vs. 4.3 (3.0–5.4)	0.18 (0.10–0.31)	< 0.0001
SOLO2 ^a	gBRCAmt	Olaparib vs. Placebo	19.1 (16.3–25.7) vs. 5.5 (5.2–5.8)	0.30 (0.22–0.41)	< 0.0001
NOVA ^b	gBRCAmt	Niraparib vs. Placebo	21.0 vs. 5.5	0.27 (0.17–0.41)	< 0.001
	Non-gBRCAmt / HRD-carcinoma	Niraparib vs. Placebo	12.9 vs. 3.8	0.38 (0.24–0.59)	< 0.001
ARIEL3 ^a	Non-gBRCAmt	Niraparib vs. Placebo	9.3 vs. 3.9	0.45 (0.34–0.61)	< 0.001
	g/sBRCAmt	Rucaparib vs. Placebo	16.6 (13.4–22.9) vs. 5.4 (3.4–6.7)	0.23 (0.16–0.34)	< 0.0001
	HRD carcinoma	Rucaparib vs. Placebo	13.6 (10.9–16.2) vs. 5.4 (5.1–5.6)	0.32 (0.24–0.42)	< 0.0001
	ITT population	Rucaparib vs. Placebo	10.8 (8.3–11.4) vs. 5.4 (5.3–5.5)	0.36 (0.30–0.45)	< 0.0001

CI confidence interval, g/sBRCAmt germline or somatic, HGSOC high-grade serous ovarian carcinoma, HRD Homologous Recombination Deficiency, ITT intention to treat, NC not calculable

^aInvestigator assessed PFS

^bBlinded independent central review PFS

olaparib 400 mg twice daily (50 mg capsules) or placebo. The median number of previous chemotherapy regimens in the experimental arm was 3 (range 0–11). Investigators reported that olaparib led to a significantly longer PFS than placebo (8.4 vs. 4.8 months, HR 0.35, 95% CI 0.25–0.49, $P < 0.001$) [60]. A subsequent pre-planned retrospective analysis showed that median PFS was even longer in patients with g/sBRCAmt (11.2 vs. 4.3 months, HR 0.18, 95% CI 0.10–0.31, $P < 0.0001$) (see Table 3) [61]. Although the study showed a significant improvement in overall survival (OS) in the primary analysis (HR 0.73, 95% CI 0.55–0.96, $P = 0.025$) particularly in women with g/sBRCAmt (HR 0.62, 95% CI 0.41–0.94, $P = 0.025$), these results are considered descriptive as the trial was not originally powered to detect a difference in OS [64, 65].

Phase 3 maintenance treatment trials

Three randomised, placebo-controlled, double-blinded phase 3 trials have investigated PARPi as maintenance treatment in women with ovarian cancer (see Table 3) [59, 62, 63]. All patients recruited had recurrent, platinum sensitive (defined as a progression-free interval of 6 months or more from the penultimate platinum chemotherapy) high-grade serous or endometrioid ovarian cancer and had achieved a CR/PR to their last platinum-based chemotherapy. Approximately 60% of patients had received two previous lines of chemotherapy before study enrolment and 60% had a PFI of at least 12 months. All participants were enrolled within 8 weeks of their last cycle of platinum.

Table 4 Median time to first subsequent therapy (TFST) and time to second subsequent therapy (TSST) reported in phase 2/3 trials assessing PARPi as maintenance treatment

Clinical trial	Subgroups	Study arms	TFST/months (95% CI)	TSST/months (95% CI)
Study 19	HGSOC	Olaparib vs. Placebo	13.4 (11.3–15.7) vs. 6.7 (5.7–8.2) HR 0.39 (0.29–0.51) <i>P</i> < 0.0001	19.1 (16.5–22.0) vs. 14.8 (14.0–17.2) HR 0.52 (0.39–0.68) <i>P</i> < 0.0001
	<i>g/sBRCA</i> mt	Olaparib vs. Placebo	15.6 (12.3–28.2) vs. 6.2 (5.3–9.2) HR 0.32 (0.22–0.48) <i>P</i> < 0.0001	22.0 (17.7–34.9) vs. 15.3 (14.0–18.7) HR 0.41 (0.28–0.62) <i>P</i> < 0.0001
SOLO2	<i>gBRCA</i> mt	Olaparib vs. Placebo	27.9 (22.6–NC) vs. 7.1 (6.3–8.3) HR 0.28 (0.21–0.38) <i>P</i> < 0.0001	NR (NC) vs. 18.2 (15.0–20.5) HR 0.37 (0.26–0.53) <i>P</i> < 0.0001
NOVA	<i>gBRCA</i> mt	Niraparib vs. Placebo	21 (17.5–NR) vs. 8.4 (6.6–10.6) HR 0.31 (0.21–0.48) <i>P</i> < 0.001	Not reported
	Non- <i>gBRCA</i> mt	Niraparib vs. Placebo	11.8 (9.7–13.1) vs. 7.2 (5.7–8.5) HR 0.55 (0.41–0.72) <i>P</i> < 0.001	Not reported

CI confidence interval, *HR* Hazard Ratio, *NR* not reached, *NC* not calculable

In SOLO2/ENGOT-Ov21 (see Table 3: olaparib vs. placebo), only women with a *g/sBRCA*mt were eligible to participate, although no women with a somatic *BRCA* mutation were enrolled [63]. The dose and formulation of olaparib assessed in SOLO2 (300 mg twice daily in 100 or 150 mg tablets) differed from Study 19 (400 mg twice daily in 50 mg capsules) following phase 1/2 data showing improved bio-availability with 300 mg twice daily in a tablet formulation compared to 400 mg twice daily in a capsule formulation [49]. In ENGOT-OV16/NOVA [62] (see Table 3: niraparib vs. placebo), eligible patients had less than 2 cm of residual disease and normalisation of CA-125 following the last cycle of platinum therapy. In ARIEL3 [59] (rucaparib vs. placebo) no restrictions were made regarding the volume of residual disease following the last cycle of platinum chemotherapy and women with any persistent lesion greater than 2 cm diameter were defined as having bulky disease (rucaparib arm 19%; placebo 15%).

All three trials reported a significant improvement in the primary outcome of median PFS in women receiving PARPi compared to placebo (see Table 3). NOVA reported the longest PFS in the experimental arm (21.0 months; BICR PFS).

In addition, in SOLO2 and ARIEL3 additional anti-tumour responses were reported beyond those achieved with the latest platinum therapy, confirming the cytotoxic effect of PARPi demonstrated in single-agent therapeutic trials (see Table 5) [58, 59].

The most encouraging results from NOVA and ARIEL3 were that cohorts of women with or without a *g/sBRCA*mt achieved a significantly improved PFS compared with patients who received placebo. Niraparib and rucaparib improved the median PFS by 5.4 months in the non-germline *BRCA1/2* mutation group (NOVA) and the intention-to-treat (ITT) population (ARIEL3) demonstrating the efficacy of the drugs in a genetically unselected population (see Table 3).

The data from these trials, in combination with the primary analysis from Study 19, demonstrate that PARPi are likely to benefit women with recurrent, platinum-sensitive high-grade serous ovarian cancer with or without a *g/sBRCA*mt. As all participants in these trials had platinum-sensitive disease this may be an appropriate and broader predictive biomarker to screen eligibility for PARPi.

There were some initial concerns that PARPi maintenance therapy may lead to platinum-resistance [66–70].

Table 5 Objective response rates (ORR) as per RECIST criteria (CR/PR) for PARPi as maintenance treatment

Clinical Trial	PARP inhibitor	Subgroups	ORR, % (<i>n</i>)
Study 19	Olaparib 400 mg BD vs. placebo	HGSOC	12 (7/57) vs. 4 (2/48)
SOLO2	Olaparib 300 mg BD vs. placebo	<i>gBRCA</i> mt	41 (30/73) vs. 17 (6/35)
ARIEL3	Rucaparib 600 mg BD vs. placebo	<i>g/sBRCA</i> mt	38 (15/40) vs. 9 (2/23)
		HRD carcinoma	27 (23/85) vs. 7 (3/41)
		ITT population	18 (26/141) vs. 8 (5/66)

BD twice daily, *g* germline, *g/s* germline or somatic, *HRD* Homologous Recombination Deficiency, *ITT* intention-to-treat

In those phase 3 trials with mature data, the median TFST and TSST were significantly longer in women treated with PARPi compared to placebo (see Table 4) [59, 61, 63, 71]. The TFST in women with a *gBRCA*mt was consistently in excess of 12 months across trials. Furthermore, the significant improvement in TSST across trials provides a surrogate marker of improved OS before full maturation of data. Thus, overall, there are no significant concerns that PARPi treatment induces platinum-resistance in a clinical setting.

As maintenance therapy, PARPi class-based AEs of fatigue, nausea, vomiting and anaemia were again evident. There was noticeably higher grade 3–4 neutropenia, thrombocytopenia and hypertension in patients receiving niraparib and grade 3–4 increases in ALT/AST in patients receiving rucaparib (see Table 6). The incidence of MDS/AML was around 1% across all PARPi. Safety data were not reported for individual cohorts of women and so it is unclear if the incidence and severity of off-target AEs was worse in women with a *gBRCA*mt vs. *sBRCA*mt or *BRCA* wt. Adverse events appeared to be manageable with dose reductions/interruptions and did not significantly affect quality of life [72–75].

Ongoing trials are also assessing the use of PARPi maintenance treatment as part of first-line chemotherapy as well as re-challenge PARPi maintenance treatment (see Table 2) [76–78].

Genetic biomarkers

The clinical development of PARPi was predicted on the need for functional PARP1/2 in the absence of *BRCA1/2* function or other causes of homologous recombination deficiency (HRD). In keeping with these observations, genetic assays were investigated in NOVA and ARIEL3 to predict response to PARPi. Up to 50% of women with high-grade serous ovarian carcinoma may harbour a mutation in, or epigenetic silencing of, genes involved in HR repair [14, 79–84] and preclinical data suggested cells deficient in *BRCA1/2* and non-*BRCA* HR repair genes are sensitive to PARPi [6, 7, 10, 85, 86]. Other surrogate markers of HRD proposed as predictive biomarkers for PARPi include genetic mutations within the cancer genome suggestive of a greater reliance on alternative error-prone DNA damage repair mechanisms

Table 6 Key treatment-related adverse events in phase 3 trials assessing PARPi as maintenance treatment

	SOLO2 Olaparib 300 mg BD		NOVA Niraparib 300 mg OD		ARIEL3 Rucaparib 600 mg BD	
	G1-2 (%)	G3-4 (%)	Any grade (%)	G3-4 (%)	G1-2 (%)	G3-4 (%)
<i>Haematological adverse events</i>						
Anaemia	24	19	50	25	19	19
Neutropenia	14	5	30	20	11	7
Thrombocytopenia	13	1	61	34	13	5
<i>Gastrointestinal adverse events</i>						
Nausea	73	3	74	3	72	4
Vomiting	35	3	34	2	33	4
Diarrhoea	32	1	19	<1	31	1
Abdominal pain	22	3	23	1	27	2
Constipation	21	0	40	<1	35	2
Dyspepsia	11	0	11	0	14	<1
<i>Other key adverse events</i>						
Fatigue	62	4	59	8	63	7
Dysgeusia	27	0	10	0	39	0
Headache	25	1	25	<1	18	<1
Decreased appetite	22	0	25	<1	23	1
Dyspnea	11	1	19	1	13	0
Insomnia	6	0	24	<1	14	0
Hypertension	3	0	19	8	NR	NR
Increase ALT/AST	5	3	10	4	23	10
Increase blood creatinine	11	1	NR ^a	NR ^a	15	<1

ALT alanine aminotransaminase, AST aspartate aminotransaminase, BD twice daily, NR not reported, OD once daily

^aFDA label for Niraparib states increase in blood creatinine identified in $\geq 1\%$ to $<10\%$ participants but grades not reported

such as NHEJ and/or MMEJ [19, 87]. These putative biomarkers include genome-wide loss of heterozygosity (LOH), telomeric allelic imbalance (TAI) and large-scale state transitions (LST). Loss of heterozygosity is the irreversible loss of one parental allele [88]. Telomeric allele imbalances are regions of allelic imbalance that extend to one of the subtelomeres but do not cross the centromere [89]. Large-scale state transitions are chromosomal breaks between two adjacent regions of at least 10 Mb after filtering out regions less than 3 Mb [90]. Both *BRCA1/2* deficiency [89, 91] and sensitivity to platinum therapy [90] have been associated with greater LOH and increased regions of TAI and LST.

Next generation sequencing (NGS) assays can detect mutations in non-*BRCA* HR repair genes as well as the presence of LOH, TAI and/or LST in DNA extracted from tumour tissue. Two of these assays were prospectively evaluated in NOVA and ARIEL3, including Myriad myChoice® HRD test [62] (Myriad Genetics, Inc., UT, US) and Foundation Medicine T5 NGS assay [59] (Foundation Medicine, Inc., MA, US) respectively. The comparative results of these two technologies are presented below. These NGS-based assays may determine the presence/absence of mutations in genes involved in HR repair but do not provide information on function [92, 93].

Myriad myChoice® HRD test

Myriad's myChoice® HRD test was assessed in NOVA as a predictive biomarker for niraparib maintenance treatment [62]. In women without a *gBRCAmt* (non-*gBRCAmt*), DNA was extracted from archival ovarian tumour tissue and sequenced for somatic mutations in *BRCA1/2* and 54,091 genome-wide single nucleotide polymorphisms (SNPs) to quantify LOH, TAI and LST [62]. In this assay, genomic LOH was specifically defined as the number of LOH regions greater than 15 Mb within the tumour genome, but less than a whole chromosome [91]. The assay calculates an algorithmic score (range 0 to 100) based on the accumulation of LOH, TAI and LST and produces a HRD "status". All patients with a *sBRCAmt* and/or an algorithmic score of 42 or above were classified as HRD-positive, suggestive of HR deficiency [94, 95]. Patients with non-*gBRCAmt* and HRD-positive were enrolled into an independent cohort for the primary outcome analysis of BICR PFS (see Table 3). In addition, investigators assessed the primary outcome in all women with non-*gBRCAmt* including those with *sBRCAmt*, HRD-positive and HRD-negative tumour tissue (see Table 3).

Foundation Medicine T5 NGS assay

The Foundation Medicine T5 NGS assay was assessed in ARIEL3 as a predictive biomarker for rucaparib maintenance treatment [59]. This assay was used to detect

mutations in 30 HR repair genes and to quantify genome-wide LOH (as a percentage) by sequencing over 3500 evenly distributed SNPs in DNA extracted from archival ovarian tumour tissue [96]. The assay was unable to differentiate between *g/sBRCAmt* so blood could also be tested for germline mutations using the BRACAnalysis CDx test (Myriad Genetics, Inc., UT, US). Any patient found to have a *g/sBRCAmt* or genomic LOH of 16% or more were grouped together in a predefined cohort called "HRD carcinoma" for assessment of the primary outcome of investigator-assessed PFS (see Table 3). The cut off of 16% for LOH was determined following a retrospective analysis of ARIEL2 (Part 1) in which a clinically significant benefit in PFS was achieved in patients treated with rucaparib single-agent therapy who had at least 16% genomic LOH [53, 97]. The trial investigators in ARIEL3 also assessed the primary outcome in the ITT population, including all patients with either a *g/sBRCAmt* or *BRCA* wild-type/LOH^{High} or *BRCA* wild-type/LOH^{Low} (see Table 3).

Neither the Myriad myChoice® HRD test nor Foundation Medicine T5 NGS assay could fully predict which patients with relapsed, platinum-sensitive high-grade serous ovarian cancer were likely to benefit from niraparib or rucaparib maintenance therapy, respectively (see Table 3). The results from these trials raise important questions about the utility of genetic assays as predictive biomarkers in this selected group of patients. Both trials showed a tiered treatment effect, with the greatest benefit in PFS achieved in women with a *gBRCAmt*, however they also demonstrated that most women with platinum-sensitive high-grade serous ovarian carcinoma benefit from maintenance PARPi. This suggests that a conceptually simpler and much less expensive predictive biomarker for PARPi therapy could be platinum sensitivity.

The clinical relevance of possessing a germline or somatic mutation in a non-*BRCA* gene involved in HR repair as a predictive biomarker for PARPi also remains unclear. Data from both ARIEL2 (Part 1) and ARIEL3 reported around 10% of patients with high-grade serous ovarian cancer had a germline or somatic mutation in a non-*BRCA* HR repair gene [53, 59]. This represents a potential sizeable target population. Nevertheless, between these trials only patients with germline/somatic *RAD51C* or *RAD51D* mutations consistently achieved treatment responses to rucaparib [53, 98]. Indeed, the presence of a mutation in *RAD51C* or *RAD51D* correlated with an ORR (as per RECIST [CR/PR] and/or CA-125) to single-agent therapy with rucaparib in ARIEL2 (Part 1) and also led to encouraging median PFS [rucaparib: 16.4 months (range 5.4–30.4 months) versus placebo: 5.4 months (range 3.9–5.5 months)] with rucaparib maintenance treatment [53, 98]. Interestingly, all *RAD51C/D* mutations recorded in ARIEL2 (Part 1) and ARIEL3 correlated with the putative marker of genomic scarring "LOH^{High}",

and, in ARIEL3 were reported as homozygous, suggesting biallelic LOF may bring about genomic instability and predispose to PARPi sensitivity. As a notable comparison approximately 80–90% of *gBRCA*mt carriers with ovarian cancer are reported to have LOH of the wild-type allele; in keeping with biallelic LOF and consistent with Knudson's double hit hypothesis for tumour suppressor genes [23, 80, 82]. Therefore, if NGS-based assays are to be used on tumour tissue to determine eligibility for PARP inhibitors, it may be appropriate to consider screening for biallelic LOF (i.e. homozygosity) in *RAD51C* and *RAD51D* as well as *BRCA1* and *BRCA2*.

Circulating tumour DNA as genetic biomarkers

Despite women with platinum-sensitive high-grade serous ovarian cancer achieving a significant PFS benefit to PARPi, the majority eventually develop progressive disease. A number of mechanisms of resistance to PARPi have been reported including intragenic reversions in the germline mutant alleles, loss of 53BP1, reduction in PAR expression and up-regulation of P-glycoprotein efflux pumps (reviewed in Lord et al. [99]). In clinical practice, the most frequently reported resistance mechanism is secondary reversion mutations/intragenic deletions in germline *BRCA1* [100, 101] or *BRCA2* [67–69, 100] mutations following platinum-based therapy or in germline *BRCA1* [102], *BRCA2* [66], *RAD51C* and *RAD51D* [102] mutations following PARPi therapy. The incidence of *BRCA1/2* reversion mutations in women with platinum-resistant ovarian cancer has been reported as high as 46% (95% CI 29–65%) [100]. In women with *sBRCA1/2* mutations, copy number alternation and up-regulation of the remaining wild-type allele has also been suggested as potential resistance mechanisms to PARPi [103]. Multiple reversions in germline mutations have been described in the same patient at different sites of metastatic disease as well as single biopsy sites [83, 102]. Reversion mutations have also been detected in circulating tumour DNA (ctDNA) in women who develop resistance to platinum [104–106] and in tumour tissue/ctDNA from patients with prostate and pancreatic cancer after progression following PARPi therapy [107–109]. Indeed, the use of temporal sampling of ctDNA may circumvent the challenges of determining differences in tumour heterogeneity and tumour evolution when analysing tumour tissue biopsies alone [110–112].

As yet, no prospective trial data are available regarding the incidence of reversion mutations in women with ovarian cancer who develop resistance to PARPi or their treatment responses to subsequent systemic anti-cancer therapy [70, 106]. Prospective trials are, therefore, required that utilise repeat tumour sampling and ctDNA to assess tumour evolution during therapy in women with *g/sBRCA*mt and *BRCA* wt ovarian cancer. In this respect, serial molecular analyses

of tumour evolution should parallel traditional methods of determining progressive disease such as clinical, biochemical and radiological assessment, especially in those women with mutations in genes that may initially confer sensitivity to PARPi therapy and then potentially revert/mutant to bring about resistance.

Conclusion

PARPi have anti-tumour activity as single agent therapy and offer effective maintenance treatment in recurrent, platinum-sensitive high-grade serous ovarian carcinoma, with the greatest benefits being demonstrated in women with *g/sBRCA*mt. Similar efficacy outcomes were demonstrated across a range of PARPi without any one drug showing superior efficacy. A number of class-based side effects have emerged, with early suggestions of particular drug-related side effects. Next generation sequencing-based assays that detect putative markers of HRD in tumour tissue or ctDNA, beyond, *BRCA1/2* mutational status, may have potential as predictive biomarkers in certain clinical settings but further research is required.

Funding Dr. Robert D. Morgan is supported by a research grant from AstraZeneca and The Christie Hospital NHS Foundation Trust.

Compliance with ethical standards

Conflict of interest Dr. Robert D. Morgan is supported by a research grant from AstraZeneca and The Christie NHS Foundation Trust. Prof. Gordon C. Jayson and Dr. Andrew R. Clamp have received research grants from AstraZeneca. Prof. D. Gareth R. Evans has received travel grants from AstraZeneca and Amgen. Prof. Richard J Edmondson declares that he has no conflict of interest.

Human and animal statement This article does not contain any studies with human participants or animals performed by any of the authors.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

1. Jayson GC, Kohn EC, Kitchener HC, Ledermann JA (2014) Ovarian cancer Lancet 384:1376–1388
2. Ledermann JA (2017) Front-line therapy of advanced ovarian cancer: new approaches. Ann Oncol 28:viii46–viii50
3. Pujade-Lauraine E (2017) New treatments in ovarian cancer. Ann Oncol 28:viii57–viii60
4. Jayson GC, Kerbel R, Ellis LM, Harris AL (2016) Antiangiogenic therapy in oncology: current status and future directions. Lancet 388:518–529

5. Ledermann JA (2016) PARP inhibitors in ovarian cancer. *Ann Oncol* 27(Suppl 1):i40–i44
6. Bryant HE, Schultz N, Thomas HD et al (2005) Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* 434:913–917
7. Farmer H, McCabe N, Lord CJ et al (2005) Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* 434:917–921
8. Heyer WD, Ehmsen KT, Liu J (2010) Regulation of homologous recombination in eukaryotes. *Annu Rev Genet* 44:113–139
9. Lilyquist J, LaDuca H, Polley E et al (2017) Frequency of mutations in a large series of clinically ascertained ovarian cancer cases tested on multi-gene panels compared to reference controls. *Gynecol Oncol* 147:375–380
10. Loveday C, Turnbull C, Ramsay E et al (2011) Germline mutations in RAD51D confer susceptibility to ovarian cancer. *Nat Genet* 43:879–882
11. Meindl A, Hellebrand H, Wiek C et al (2010) Germline mutations in breast and ovarian cancer pedigrees establish RAD51C as a human cancer susceptibility gene. *Nat Genet* 42:410–414
12. Norquist BM, Harrell MI, Brady MF et al (2016) Inherited mutations in women with ovarian carcinoma. *JAMA Oncol* 2:482–490
13. Rafnar T, Gudbjartsson DF, Sulem P et al (2011) Mutations in BRIP1 confer high risk of ovarian cancer. *Nat Genet* 43:1104–1107
14. Walsh T, Casadei S, Lee MK et al (2011) Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. *Proc Natl Acad Sci USA* 108:18032–18037
15. Peltari LM, Heikkinen T, Thompson D et al (2011) RAD51C is a susceptibility gene for ovarian cancer. *Hum Mol Genet* 20:3278–3288
16. Antoniou A, Pharoah PD, Narod S et al (2003) Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. *Am J Hum Genet* 72:1117–1130
17. Nielsen FC, van Overeem Hansen T, Sorensen CS (2016) Hereditary breast and ovarian cancer: new genes in confined pathways. *Nat Rev Cancer* 16:599–612
18. Bowtell DD (2010) The genesis and evolution of high-grade serous ovarian cancer. *Nat Rev Cancer* 10:803–808
19. Helleday T (2011) The underlying mechanism for the PARP and BRCA synthetic lethality: clearing up the misunderstandings. *Mol Oncol* 5:387–393
20. Tutt A, Bertwistle D, Valentine J et al (2001) Mutation in BRCA2 stimulates error-prone homology-directed repair of DNA double-strand breaks occurring between repeated sequences. *EMBO J* 20:4704–4716
21. Venkitaraman AR (2014) Cancer suppression by the chromosome custodians, BRCA1 and BRCA2. *Science* 343:1470–1475
22. Venkitaraman AR (2009) Linking the cellular functions of BRCA genes to cancer pathogenesis and treatment. *Annu Rev Pathol* 4:461–487
23. Maxwell KN, Wubbenhorst B, Wenz BM et al (2017) BRCA locus-specific loss of heterozygosity in germline BRCA1 and BRCA2 carriers. *Nat Commun* 8:319
24. Riaz N, Blecua P, Lim RS et al (2017) Pan-cancer analysis of bi-allelic alterations in homologous recombination DNA repair genes. *Nat Commun* 8:857
25. Dougherty BA, Lai Z, Hodgson DR et al (2017) Biological and clinical evidence for somatic mutations in BRCA1 and BRCA2 as predictive markers for olaparib response in high-grade serous ovarian cancers in the maintenance setting. *Oncotarget* 8:43653–43661
26. Hennessy BT, Timms KM, Carey MS et al (2010) Somatic mutations in BRCA1 and BRCA2 could expand the number of patients that benefit from poly (ADP ribose) polymerase inhibitors in ovarian cancer. *J Clin Oncol* 28:3570–3576
27. Gibson BA, Kraus WL (2012) New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs. *Nat Rev Mol Cell Biol* 13:411–424
28. Rouleau M, Patel A, Hendzel MJ et al (2010) PARP inhibition: PARP1 and beyond. *Nat Rev Cancer* 10:293–301
29. Malanga M, Althaus FR (2005) The role of poly(ADP-ribose) in the DNA damage signaling network. *Biochem Cell Biol* 83:354–364
30. Murai J, Huang SY, Das BB et al (2012) Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors. *Cancer Res* 72:5588–5599
31. Murai J, Huang SY, Renaud A et al (2014) Stereospecific PARP trapping by BMN 673 and comparison with olaparib and rucaparib. *Mol Cancer Ther* 13:433–443
32. Hopkins TA, Shi Y, Rodriguez LE et al (2015) Mechanistic dissection of PARP1 trapping and the impact on in vivo tolerability and efficacy of PARP inhibitors. *Mol Cancer Res* 13:1465–1477
33. McCormick A, Donoghue P, Dixon M et al (2017) Ovarian cancers harbor defects in nonhomologous end joining resulting in resistance to rucaparib. *Clin Cancer Res* 23:2050–2060
34. Patel AG, Sarkaria JN, Kaufmann SH (2011) Nonhomologous end joining drives poly(ADP-ribose) polymerase (PARP) inhibitor lethality in homologous recombination-deficient cells. *Proc Natl Acad Sci USA* 108:3406–3411
35. Ashworth A (2008) A synthetic lethal therapeutic approach: poly(ADP-ribose) polymerase inhibitors for the treatment of cancers deficient in DNA double-strand break repair. *J Clin Oncol* 26:3785–3790
36. Kaelin WG Jr. (2005) The concept of synthetic lethality in the context of anticancer therapy. *Nat Rev Cancer* 5:689–698
37. Audeh MW, Carmichael J, Penson RT et al (2010) Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial. *Lancet* 376:245–251
38. Coleman RL, Sill MW, Bell-McGuinn K et al (2015) A phase II evaluation of the potent, highly selective PARP inhibitor veliparib in the treatment of persistent or recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer in patients who carry a germline BRCA1 or BRCA2 mutation - An NRG Oncology/Gynecologic Oncology Group study. *Gynecol Oncol* 137:386–391
39. de Bono J, Ramanathan RK, Mina L et al (2017) Phase I, dose-escalation, two-part trial of the PARP inhibitor talazoparib in patients with advanced germline BRCA1/2 mutations and selected sporadic cancers. *Cancer Discov* 7:620–629
40. Domchek SM, Aghajanian C, Shapira-Frommer R et al (2016) Efficacy and safety of olaparib monotherapy in germline BRCA1/2 mutation carriers with advanced ovarian cancer and three or more lines of prior therapy. *Gynecol Oncol* 140:199–203
41. Drew Y, Ledermann J, Hall G et al (2016) Phase 2 multicentre trial investigating intermittent and continuous dosing schedules of the poly(ADP-ribose) polymerase inhibitor rucaparib in germline BRCA mutation carriers with advanced ovarian and breast cancer. *Br J Cancer* 114:723–730
42. Fong PC, Boss DS, Yap TA et al (2009) Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N Engl J Med* 361:123–134
43. Fong PC, Yap TA, Boss DS et al (2010) Poly(ADP-ribose) polymerase inhibition: frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval. *J Clin Oncol* 28:2512–2519
44. Gelmon KA, Tischkowitz M, Mackay H et al (2011) Olaparib in patients with recurrent high-grade serous or poorly differentiated ovarian carcinoma or triple-negative breast cancer: a phase

- 2, multicentre, open-label, non-randomised study. *Lancet Oncol* 12:852–861
45. Kaufman B, Shapira-Frommer R, Schmutzler RK et al (2015) Olaparib monotherapy in patients with advanced cancer and a germline BRCA1/2 mutation. *J Clin Oncol* 33:244–250
46. Kaye SB, Lubinski J, Matulonis U et al (2012) Phase II, open-label, randomized, multicenter study comparing the efficacy and safety of olaparib, a poly (ADP-ribose) polymerase inhibitor, and pegylated liposomal doxorubicin in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer. *J Clin Oncol* 30:372–379
47. Kristeleit R, Shapira-Frommer R, Oaknin A et al (2016) Clinical activity of the poly(ADP-ribose) polymerase (PARP) inhibitor rucaparib in patients (pts) with high-grade ovarian carcinoma (HGO) and a BRCA mutation (BRCAmut): analysis of pooled data from study 10 (parts 1, 2a, and 3) and ARIEL2 (parts 1 and 2). *Ann Oncol* 27(suppl_6):856O
48. Kristeleit R, Shapiro GI, Burris HA et al (2017) A phase I–II study of the oral PARP inhibitor rucaparib in patients with germline BRCA1/2-mutated ovarian carcinoma or other solid tumors. *Clin Cancer Res* 23:4095–4106
49. Mateo J, Moreno V, Gupta A et al (2016) An adaptive study to determine the optimal dose of the tablet formulation of the PARP inhibitor olaparib. *Target Oncol* 11:401–415
50. Matulonis UA, Penson RT, Domchek SM et al (2016) Olaparib monotherapy in patients with advanced relapsed ovarian cancer and a germline BRCA1/2 mutation: a multistudy analysis of response rates and safety. *Ann Oncol* 27:1013–1019
51. Sandhu SK, Schelman WR, Wilding G et al (2013) The poly(ADP-ribose) polymerase inhibitor niraparib (MK4827) in BRCA mutation carriers and patients with sporadic cancer: a phase 1 dose-escalation trial. *Lancet Oncol* 14:882–892
52. Steffensen KD, Adimi P, Jakobsen A (2017) Veliparib monotherapy to patients with BRCA germ line mutation and platinum-resistant or partially platinum-sensitive relapse of epithelial ovarian cancer: a phase I/II study. *Int J Gynecol Cancer* 27:1842–1849
53. Swisher EM, Lin KK, Oza AM et al (2017) Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial. *Lancet Oncol* 18:75–87
54. Oza AM, Tinker AV, Oaknin A et al (2017) Antitumor activity and safety of the PARP inhibitor rucaparib in patients with high-grade ovarian carcinoma and a germline or somatic BRCA1 or BRCA2 mutation: Integrated analysis of data from Study 10 and ARIEL2. *Gynecol Oncol*
55. Lee JM, Cimino-Mathews A, Peer CJ et al (2017) Safety and clinical activity of the programmed death-ligand 1 inhibitor durvalumab in combination with poly (ADP-Ribose) polymerase inhibitor olaparib or vascular endothelial growth factor receptor 1–3 inhibitor cediranib in women's cancers: a dose-escalation, phase I study. *J Clin Oncol* 35:2193–2202
56. Liu JF, Barry WT, Birrer M et al (2014) Combination cediranib and olaparib versus olaparib alone for women with recurrent platinum-sensitive ovarian cancer: a randomised phase 2 study. *Lancet Oncol* 15:1207–1214
57. Matulonis UA, Wulf GM, Barry WT et al (2017) Phase I dose escalation study of the PI3kinase pathway inhibitor BKM120 and the oral poly (ADP ribose) polymerase (PARP) inhibitor olaparib for the treatment of high-grade serous ovarian and breast cancer. *Ann Oncol* 28:512–518
58. Oza A, Combe P, Ledermann J et al (2017) Evaluation of tumour responses and olaparib efficacy in platinum-sensitive relapsed ovarian cancer patients (PSROC) with or without measurable disease in the SOLO2 trial (ENGOT Ov-21). *Ann Oncol* 28:P344 (abstract 965P)
59. Coleman RL, Oza AM, Lorusso D et al. (2017) Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 390:1949–196
60. Ledermann J, Harter P, Gourley C et al (2012) Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. *N Engl J Med* 366:1382–1392
61. Ledermann J, Harter P, Gourley C et al (2014) Olaparib maintenance therapy in patients with platinum-sensitive relapsed ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. *Lancet Oncol* 15:852–861
62. Mirza MR, Matulonis UA (2017) Niraparib in recurrent ovarian cancer. *N Engl J Med* 376:801–802
63. Pujade-Lauraine E, Ledermann JA, Selle F et al. (2017) Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Oncol* 18:1274–1284
64. Ledermann JA, Harter P, Gourley C et al (2016) Overall survival in patients with platinum-sensitive recurrent serous ovarian cancer receiving olaparib maintenance monotherapy: an updated analysis from a randomised, placebo-controlled, double-blind, phase 2 trial. *Lancet Oncol* 17:1579–1589
65. Matulonis UA, Harter P, Gourley C et al (2016) Olaparib maintenance therapy in patients with platinum-sensitive, relapsed serous ovarian cancer and a BRCA mutation: Overall survival adjusted for postprogression poly(adenosine diphosphate ribose) polymerase inhibitor therapy. *Cancer* 122:1844–1852
66. Barber LJ, Sandhu S, Chen L et al (2013) Secondary mutations in BRCA2 associated with clinical resistance to a PARP inhibitor. *J Pathol* 229:422–429
67. Edwards SL, Brough R, Lord CJ et al (2008) Resistance to therapy caused by intragenic deletion in BRCA2. *Nature* 451:1111–1115
68. Sakai W, Swisher EM, Jacquemont C et al (2009) Functional restoration of BRCA2 protein by secondary BRCA2 mutations in BRCA2-mutated ovarian carcinoma. *Cancer Res* 69:6381–6386
69. Sakai W, Swisher EM, Karlan BY et al (2008) Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. *Nature* 451:1116–1120
70. Ang JE, Gourley C, Powell CB et al (2013) Efficacy of chemotherapy in BRCA1/2 mutation carrier ovarian cancer in the setting of PARP inhibitor resistance: a multi-institutional study. *Clin Cancer Res* 19:5485–5493
71. Matulonis U, Herrstedt J, Tinker A et al (2017) Long-term benefits of niraparib treatment of recurrent ovarian cancer (OC). *J Clin Oncol* 35(suppl_15):5534
72. Fabbro M, Moore KN, Dorum A et al (2017) Safety and efficacy of niraparib in elderly patients with recurrent ovarian cancer. *Ann Oncol* 28:P332 (abstract 934PD).
73. Friedlander M, GebSKI V, Gibbs E et al. (2017) Health-related quality of life (HRQOL) and patient-centred outcomes with maintenance olaparib compared with placebo following chemotherapy in patients with germline (g) BRCA-mutated (m) platinum-sensitive relapsed serous ovarian cancer (PSR SOC): SOLO2 phase 3 trial. *J Clin Oncol* 35(suppl_15):5507
74. Ledermann J, Lortholary A, Penson RT et al (2017) Adverse events (AEs) with maintenance olaparib tablets in patients (pts) with BRCA-mutated (BRCAm) platinum-sensitive relapsed serous ovarian cancer (PSR SOC): Phase III SOLO2 trial. *J Clin Oncol* 35(suppl_15):5518
75. Oza AM, Matulonis UA, Malander S et al (2017) Quality of life in patients with recurrent ovarian cancer (OC) treated with niraparib: results from ENGOT-OV16/NOVA trial. *Ann Oncol* 28(suppl_5):v330–v330

76. Clamp A, Jayson G (2015) PARP inhibitors in BRCA mutation-associated ovarian cancer. *Lancet Oncol* 16:10–12
77. Oza AM, Cibula D, Benzaquen AO et al (2015) Olaparib combined with chemotherapy for recurrent platinum-sensitive ovarian cancer: a randomised phase 2 trial. *Lancet Oncol* 16:87–97
78. Gonzalez-Martin A, Backes FJ, Baumann KH et al (2016) A randomized, double-blind phase III trial niraparib maintenance treatment in patients with HRD + advanced ovarian cancer after response to front-line platinum-based chemotherapy. *J Clin Oncol* 34(suppl_15):TPS5606
79. Alsop K, Fereday S, Meldrum C et al (2012) BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: a report from the Australian Ovarian Cancer Study Group. *J Clin Oncol* 30:2654–2663
80. Cancer Genome Atlas Research N. Integrated genomic analyses of ovarian carcinoma. *Nature* 2011; 474: 609–615
81. Cunningham JM, Cicek MS, Larson NB et al (2014) Clinical characteristics of ovarian cancer classified by BRCA1, BRCA2, and RAD51C status. *Sci Rep* 4:4026
82. Kanchi KL, Johnson KJ, Lu C et al (2014) Integrated analysis of germline and somatic variants in ovarian cancer. *Nat Commun* 5:3156
83. Patch AM, Christie EL, Etemadmoghadam D et al (2015) Whole-genome characterization of chemoresistant ovarian cancer. *Nature* 521:489–494
84. Pennington KP, Walsh T, Harrell MI et al (2014) Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas. *Clin Cancer Res* 20:764–775
85. Gottipati P, Vischioni B, Schultz N et al (2010) Poly(ADP-ribose) polymerase is hyperactivated in homologous recombination-defective cells. *Cancer Res* 70:5389–5398
86. McCabe N, Turner NC, Lord CJ et al (2006) Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. *Cancer Res* 66:8109–8115
87. Watkins JA, Irshad S, Grigoriadis A, Tutt AN (2014) Genomic scars as biomarkers of homologous recombination deficiency and drug response in breast and ovarian cancers. *Breast Cancer Res* 16:211
88. Ryland GL, Doyle MA, Goode D et al (2015) Loss of heterozygosity: what is it good for? *BMC Med Genomics* 8:45
89. Birkbak NJ, Wang ZC, Kim JY et al (2012) Telomeric allelic imbalance indicates defective DNA repair and sensitivity to DNA-damaging agents. *Cancer Discov* 2:366–375
90. Popova T, Manie E, Rieunier G et al (2012) Ploidy and large-scale genomic instability consistently identify basal-like breast carcinomas with BRCA1/2 inactivation. *Cancer Res* 72:5454–5462
91. Abkevich V, Timms KM, Hennessy BT et al (2012) Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer. *Br J Cancer* 107:1776–1782
92. Mukhopadhyay A, Elattar A, Cerbinskaite A et al (2010) 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
93. Mukhopadhyay A, Plummer ER, Elattar A et al (2012) Clinicopathological features of homologous recombination-deficient epithelial ovarian cancers: sensitivity to PARP inhibitors, platinum, and survival. *Cancer Res* 72:5675–5682
94. Telli ML, Timms KM, Reid J et al (2016) Homologous recombination deficiency (HRD) score predicts response to platinum-containing neoadjuvant chemotherapy in patients with triple-negative breast cancer. *Clin Cancer Res* 22:3764–3773
95. Wilcoxon KM, Becker M, Neff C et al (2015) Use of homologous recombination deficiency (HRD) score to enrich for niraparib sensitive high grade ovarian tumors. *J Clin Oncol* 33(suppl_15):5532
96. Frampton GM, Fichtenholtz A, Otto GA et al (2013) Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol* 31:1023–1031
97. Coleman RL, Swisher EM, Oza AM et al (2016) Refinement of prespecified cutoff for genomic loss of heterozygosity (LOH) in ARIEL2 part 1: A phase II study of rucaparib in patients (pts) with high grade ovarian carcinoma (HGOC). *J Clin Oncol* 34(suppl_15):5540
98. O'Malley DM, Coleman RL, Oza A et al (2017) Results from the phase 3 study ARIEL3: mutations in non-BRCA homologous recombination repair genes confer sensitivity to maintenance treatment with the PARP inhibitor rucaparib in patients with recurrent platinum-sensitive high-grade ovarian carcinoma. In: AACR-NCI-EORTC molecular targets and cancer therapeutics conference
99. Lord CJ, Ashworth A (2013) Mechanisms of resistance to therapies targeting BRCA-mutant cancers. *Nat Med* 19:1381–1388
100. Norquist B, Wurz KA, Pennil CC et al (2011) Secondary somatic mutations restoring BRCA1/2 predict chemotherapy resistance in hereditary ovarian carcinomas. *J Clin Oncol* 29:3008–3015
101. Swisher EM, Sakai W, Karlan BY et al (2008) Secondary BRCA1 mutations in BRCA1-mutated ovarian carcinomas with platinum resistance. *Cancer Res* 68:2581–2586
102. Kondrashova O, Nguyen M, Shield-Artin K et al. (2017) 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
103. Lheureux S, Bruce JP, Burnier JV et al (2017) Somatic BRCA1/2 recovery as a resistance mechanism after exceptional response to poly (ADP-ribose) polymerase inhibition. *J Clin Oncol* 35:1240–1249
104. Christie EL, Fereday S, Doig K et al (2017) Reversion of BRCA1/2 germline mutations detected in circulating tumor DNA from patients with high-grade serous ovarian cancer. *J Clin Oncol* 35:1274–1280
105. Mayor P, Gay LM, Lele S, Elvin JA (2017) BRCA1 reversion mutation acquired after treatment identified by liquid biopsy. *Gynecol Oncol Rep* 21:57–60
106. Weigelt B, Comino-Mendez I, de Bruijn I et al. (2017) Diverse BRCA1 and BRCA2 reversion mutations in circulating cell-free DNA of therapy-resistant breast or ovarian cancer. *Clin Cancer Res* 23:6708–6720
107. Goodall J, Mateo J, Yuan W et al (2017) Circulating cell-free DNA to guide prostate cancer treatment with PARP inhibition. *Cancer Discov* 7:1006–1017
108. Quigley D, Alumkal JJ, Wyatt AW et al (2017) Analysis of circulating cell-free DNA identifies multiclonal heterogeneity of BRCA2 reversion mutations associated with resistance to PARP inhibitors. *Cancer Discov* 7:999–1005
109. Pishvaian MJ, Biankin AV, Bailey P et al (2017) BRCA2 secondary mutation-mediated resistance to platinum and PARP inhibitor-based therapy in pancreatic cancer. *Br J Cancer* 116:1021–1026
110. Wan JC, Massie C, Garcia-Corbacho J et al (2017) Liquid biopsies come of age: towards implementation of circulating tumour DNA. *Nat Rev Cancer* 17:223–238
111. Parkinson CA, Gale D, Piskorz AM et al (2016) Exploratory analysis of TP53 mutations in circulating tumour DNA as biomarkers of treatment response for patients with relapsed high-grade serous ovarian carcinoma: a retrospective study. *PLoS Med* 13:e1002198
112. Murtaza M, Dawson SJ, Tsui DW et al (2013) Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. *Nature* 497:108–112