Homologous recombination deficiency in newly diagnosed FIGO stage III/IV high-grade epithelial ovarian cancer: a multi-national observational study

Robert D. Morgan[1,2], Andrew R. Clamp[1,2], Bethany M. Barnes[2], Kirsten Timms[3], Helene Schlecht[4], Laura Yarram-Smith[5], Yvonne Wallis[6], Mikel Valganon-Petrizan[7], Suzanne MacMahon[7], Rhian White[8], Sian Morgan[8], Sarah McKenna[9], Emma Hudson[10], Laura Tookman[11], Angela George[12,13], Ranjit Manchanda[14,15,16], Sudha Sundar[17,18], Shibani Nicum[19,20], James D. Brenton[21,22], Rebecca S. Kristeleit[23], Susana Banerjee[12,13], Iain A. McNeish[11,24], Jonathan A. Ledermann[19,20], Stephen S. Taylor[2], D. Gareth R. Evans[25,26], Gordon C. Jayson[1,2]

1. *The Christie NHS Foundation Trust, Manchester, United Kingdom*
2. *Division of Cancer Sciences, School of Medical Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom*
3. *Myriad Genetics, Inc., Salt Lake City, Utah, United States*
4. *North West Genomic Laboratory Hub, Manchester University NHS Foundation Trust, Manchester, United Kingdom*
5. *South West Genomic Laboratory Hub, North Bristol NHS Trust, Bristol, United Kingdom*
6. *Central and South Genomic Laboratory Hub, Birmingham Women's and Children's NHS Foundation Trust, Birmingham, United Kingdom*

7. *North Thames Genomic Laboratory Hub, The Royal Marsden Hospital NHS Foundation Trust, Surrey, United Kingdom*
8. *All Wales Genomics Laboratory, Institute of Medical Genetics, University Hospital Wales, Cardiff, United Kingdom*
9. *Belfast Health & Social Care Trust, Belfast, Northern Ireland, United Kingdom*
10. *Velindre University NHS Trust, Cardiff, United Kingdom*
11. *Imperial College Healthcare NHS Trust, London, United Kingdom*
12. *The Royal Marsden NHS Foundation Trust, London, United Kingdom*
13. *The Institute of Cancer Research, London, United Kingdom*
14. *Barts Health NHS Trust, London, United Kingdom*
15. *Department of Health Services Research, The Faculty of Public Health and Policy, London School of Hygiene & Tropical Medicine, London, United Kingdom*
16. *Wolfson Institute of Population Health, Queen Mary's University of London, London, United Kingdom*
17. *Sandwell and West Birmingham Hospitals NHS Trust, Birmingham, United Kingdom*
18. *Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, United Kingdom*
19. *University College London Hospitals NHS Foundation Trust, London, United Kingdom*
20. *UCL Cancer Institute, London, United Kingdom*
21. *Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom*
22. *CRUK Cambridge Institute, University of Cambridge, Cambridge, United Kingdom*
23. *Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom*

24. *Ovarian Cancer Action Research Centre, Department of Surgery and Cancer, Imperial College London, London, United Kingdom*
25. *Manchester University NHS Foundation Trust, Manchester, United Kingdom*
26. *Division of Evolution & Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom*

Corresponding author: Robert Morgan, MD, PhD. Department of Medical Oncology, The Christie Hospital, Wilmslow Road, Manchester M20 4BX, United Kingdom. Tel: +44 (0)161 446 3000. Email: robert.morgan7@nhs.net.

Abstract

Objective

Olaparib plus bevacizumab maintenance therapy improves survival outcomes in women with newly diagnosed, advanced, high-grade ovarian cancers with a deficiency in homologous recombination. We report data from the first year of routine homologous recombination deficiency testing in the National Health Service (NHS) in England, Wales and Northern Ireland between April 2021 and April 2022.

Methods

The Myriad myChoice® companion diagnostic was used to test DNA extracted from formalin-fixed, paraffin-embedded tumour tissue in women with newly diagnosed FIGO (The International Federation of Gynecology and Obstetrics) stage III/IV high-grade epithelial ovarian, fallopian tube or primary peritoneal cancer. Tumours with homologous recombination deficiency were those with a *BRCA1/2* mutation and/or a Genomic Instability Score (GIS) of ≥42. Testing was coordinated by the NHS Genomic Laboratory Hub network.

Results

The myChoice® assay was performed on 2,829 tumours. Of these, 2,474 (87%) and 2,178 (77%) successfully underwent *BRCA1/2* and GIS testing, respectively. All complete and partial assay failures occurred due to low tumour cellularity and/or low tumour DNA yield. Three-hundred-and-eighty-five tumours (16%) contained a *BRCA1/2* mutation and 814 (37%) had a GIS ≥42. Tumours with a GIS ≥42 were more likely to be *BRCA1/2* wild-type (n=510) than *BRCA*1/2 mutant (n=304). The distribution

of GIS was bimodal, with *BRCA1/2* mutant tumours having a higher mean score than *BRCA1/2* wild-type tumours (61 versus 33, respectively, chi-squared test P<0.0001).

Conclusion

This is the largest real-world evaluation of homologous recombination deficiency testing in newly diagnosed FIGO stage III/IV high-grade epithelial ovarian, fallopian tube or primary peritoneal cancer. It is important to select tumour tissue with adequate tumour content and quality to reduce the risk of assay failures. The rapid uptake of testing across England, Wales and Northern Ireland demonstrates the power of centralised NHS funding, centre specialisation and the NHS Genomic Laboratory Hub network.

Key messages

What is already known on this topic?

Olaparib plus bevacizumab maintenance therapy improves survival outcomes in women with newly diagnosed, advanced, high-grade ovarian cancers with a deficiency in homologous recombination. There is a scarcity of real world evidence describing the prevalence of homologous recombination deficiency.

What this study adds?

This is the largest real world evaluation of homologous recombination deficient tumour testing in newly diagnosed FIGO (The International Federation of Gynecology and Obstetrics) stage III/IV high-grade epithelial ovarian, fallopian tube or primary peritoneal cancer. We report the prevalence of homologous recombination deficient tumours in England, Wales and Northern Ireland as 37% using Myriad's myChoice® companion diagnostic. The complete and partial failure rate of the assay was 13% and 23%, respectively. Tests failed due to low tumour cellularity and/or low tumour DNA yield.

How might this study affect research, practice or policy?

This study highlights the importance of testing tumour DNA for a deficiency in homologous recombination to optimise outcomes for women with newly diagnosed FIGO stage III/IV high-grade epithelial ovarian, fallopian tube or primary peritoneal cancer. Tumour tissue must be carefully selected prior to testing to reduce the chance of assay failure. The rapid uptake of homologous recombination deficient tumour

testing demonstrates the power of centralised NHS funding, centre specialisation and the NHS Genomic Laboratory Hub network.

Introduction

Ovarian cancer is the most common cause of gynaecological cancer-related death in Europe (1). Epithelial ovarian cancer accounts for approximately 85 to 90% of all ovarian cancers. The majority of women diagnosed with high-grade epithelial ovarian cancer present with advanced disease (The International Federation of Gynaecology and Obstetrics [FIGO] stage III and IV), meaning despite good response to first-line multi-modality therapy, at least 80% develop relapsed disease, at which point cure is unlikely (2). Consequently, the five-year overall survival for advanced ovarian cancer is approximately 35% (3).

Maintenance therapy aims to extend relapse-free survival in patients at high risk of recurrence, without impacting on quality of life (4). Randomised, phase III trials have demonstrated that maintenance therapy with a poly(ADP-ribose) polymerase-1/2 inhibitor (PARPi) improves progression-free survival in women with newly diagnosed FIGO stage III/IV or platinum-sensitive, relapsed high-grade serous and/or endometrioid ovarian cancer (5, 6, 7, 8, 9, 10, 11, 12, 13, 14). These small molecule inhibitors of PARP-1/2 are synthetically lethal to cells deficient in homologous recombination, a high-fidelity DNA double-strand break repair pathway that maintains genomic stability (15, 16). The best-studied causes of homologous recombination deficiency are loss-of-function mutations in *BRCA1* and *BRCA2*, which occur in 20 to 25% of high-grade serous ovarian cancers (17).

The myChoice® companion diagnostic (Myriad Genetics, Inc.) is a next-generation sequencing assay used to detect a deficiency in homologous recombination in genomic DNA derived from formalin-fixed, paraffin-embedded tumour tissue (18). The assay reports homologous recombination deficient tumours

as those that harbour a *BRCA1/2* mutation and/or have a Genomic Instability Score (GIS) of ≥42. The phase III trial, PAOLA-1, showed an improved hazard ratio (HR) for disease progression or death in women with newly diagnosed, advanced, high-grade ovarian cancer who were randomised to maintenance olaparib plus bevacizumab versus placebo plus bevacizumab, following a response to first-line platinum-taxane chemotherapy (HR 0.59; 95% confidence interval [CI] 0.49-0.72) (11, 19). The greatest reduction in HR was reported in women with tumours positive for homologous recombination deficiency (HR 0.33; 95%CI 0.25-0.45). By contrast, those women with homologous recombination proficient tumours gained no benefit from the addition of olaparib to bevacizumab (HR 1.00, 95%CI 0.75-1.35). More recently, data presented from PAOLA-1 also showed that olaparib plus bevacizumab improved the overall survival of women with homologous recombination deficient tumours (HR 0.62, 95%CI 0.45-0.85), but not in women with homologous recombination proficient tumour (HR 1.19, 95%CI 0.88-1.63) (20).

The results from PAOLA-1 led to European licensing of maintenance olaparib plus bevacizumab for women with newly diagnosed FIGO stage III/IV high-grade ovarian, fallopian tube or primary peritoneal cancers that responded to platinum-based chemotherapy and were homologous recombination deficient. Consequently, access to Myriad's myChoice® companion diagnostic became available in the United Kingdom (UK) from April 2021 onwards. We report data from the first year of routine tumour testing for homologous recombination deficiency in the National Health Service (NHS) in England, Wales and Northern Ireland between April 2021 and April 2022.

Methods

Eligibility criteria

Eligibility criteria for myChoice® testing included women with newly diagnosed FIGO stage III/IV high-grade epithelial ovarian, fallopian tube or primary peritoneal cancer. Tumour testing was requested during first-line treatment. No patient who underwent tumour testing as part of a clinical trial was included.

Tumour testing

Tumour testing was co-ordinated by the NHS Genomic Laboratory Hub network. The hubs co-ordinating testing for England were North West, South West, Central and South, and North Thames. All Wales Genomics Laboratory co-ordinated testing for Wales. The North West Genomic Laboratory Hub co-ordinated testing for cancer centres in Northern Ireland.

Myriad's myChoice® companion diagnostic became available in England, Wales and Northern Ireland from April 2021, October 2021 and September 2021, respectively. The UK includes Scotland, although myChoice® testing did not become available in Scotland until after April 2022, therefore no cases from Scottish cancer centres were included in this study. Local medical teams obtained informed consent from the patient prior to tumour testing. All cancer centres were asked to provide 10 x slide mounted formalin-fixed, paraffin-embedded tumour sections at a thickness of 5 μm.

Myriad's myChoice® companion diagnostic

The myChoice® test was performed by Myriad Genetics, Inc. (Salt Lake City, Utah) (18). The GIS was calculated as a composite score (range 0 to 100) based on three bioinformatic algorithms that assessed genome-wide putative biomarkers of homologous recombination deficiency including Loss of Heterozygosity, Telomeric Allelic Imbalance and Large-scale State Transitions. The Loss of Heterozygosity score was defined by the number of loss of heterozygosity regions longer than 15 megabases but shorter than the whole chromosome. The Telomeric Allelic Imbalance score was defined by the number of regions with allelic imbalance that extend to one of the sub telomeres, did not cross the centromere, and were longer than 11 megabases. The Large-scale State Transitions score was defined by the number of chromosomal breaks between two adjacent regions of at least 10 megabases, after filtering out regions less than 3 megabases and adjusting for ploidy. To quantify Loss of Heterozygosity, Telomeric Allelic Imbalance and Large-scale State Transitions the myChoice® test interrogated >27,000 genome-wide single nucleotide polymorphisms.

Tumours with a GIS of ≥42 were reported as 'GIS-positive', while those with a GIS of <42 were reported as 'GIS-negative'. Tumours with a *BRCA1*/*2* mutation and/or a GIS of ≥42 were reported as homologous recombination deficient, while those with *BRCA1/2* wild-type and a GIS of <42 were reported as homologous recombination proficient. Only tumour *BRCA1/2* pathogenic or likely pathogenic variants were reported (21).

Statistical analysis

Categorical data were reported as number (percentage). Continuous data were reported as median (range and interquartile range) and mean (standard deviation). The chi-squared test was used to determine if there were statistically significant differences between categorical variables, with a p-value of <0.05 defined as significant. The t-test was used to determine if there were statistically significant differences between the mean averages of two groups, with a p-value of <0.05 defined as significant.

In accordance with the journal's guidelines, we will provide our data for independent analysis by a selected team by the Editorial Team for the purposes of additional data analysis or for the reproducibility of this study in other centres if such is requested.

Results

The myChoice® assay was performed on 2,829 tumours. The tumour content was ≥30% in 83% (n=2,362) of formalin-fixed, paraffin-embedded tissue sections tested. Of the 2,829 tumours tested, 2,474 (87%) and 2,178 (77%) were successfully tested for *BRCA1/2* and GIS, respectively. Testing failed due to low tumour cellularity and/or low tumour DNA yield. In the UK, early testing (April to July 2021) showed a very high rate of quantity insufficient cancellations as higher DNA inputs were required for version 1 (legacy version) of the myChoice® test. The myChoice® test version 2 (improved version, due to higher yield DNA extraction and lower DNA input minimum) was implemented from August 2021 onwards and the rate of sample failures dramatically dropped (21.7% between April to July 2021, down to 5.3% between August 2021 to April 2022).

Tumour BRCA1/2 mutations

Of the 2,474 tumours successfully tested, 385 (16%) *BRCA*1/2 mutations were detected (**Supplementary Table 1**). These included 220 (9%) *BRCA1* and 165 (7%) *BRCA2* mutations. There were 308 (80%) distinct tumour *BRCA1/2* mutations (178 *BRCA1* and 130 *BRCA2*). There were no mutational hotspots in *BRCA1* or *BRCA2*, with mutations detected across the length of each gene (**Figure 1**).

The majority of tumour *BRCA1/2* mutations were small deletions (172/385) or single nucleotide variants (143/385) leading to premature protein terminations (201/385 frameshift-deletions and 103/385 nonsense mutations) (**Table 1**). Small deletions, duplications and insertions ranged from 1 to 116 base pairs in length. Of the 385 tumour *BRCA1/2* mutations, 360 (94%) were ≤40 base pairs in length and would have been detected using local tumour *BRCA1/2* next-generation sequencing assays used in the UK (22, 23). Twenty-one (5%) pathogenic large genomic rearrangements were detected. All pathogenic large genomic rearrangements were large deletions (21/21), with no pathogenic large duplications (0/21) detected. One whole gene deletion was detected, in *BRCA2*.

We were unable to confirm which tumour *BRCA1/2* mutations were germline or somatic. No genetic assay has been validated to distinguish between germline and somatic *BRCA1/2* mutations from tumour DNA alone. However, multiple European *BRCA1/2* founder mutations have been described, thereby allowing us to predict those tumour *BRCA1/2* mutations that were most likely to be germline. Of the 385 tumour *BRCA1/2* mutations, 79 (21%) were European *BRCA1/2* founder mutations, including 51 *BRCA1* and 28 *BRCA2*. There were 34 individual *BRCA1/2* founder mutations, of

which 16 (47%) were detected in 2 or more tumours. By contrast, of the remaining 306 tumour *BRCA1/2* mutations, there were 274 individual mutations, of which only 25 (25/274; 9%) were detected in 2 or more tumours (chi-squared test P<0.0001). The commonest European *BRCA1/2* founder mutations were *BRCA2*:c.6275_6276delTT (n=9), *BRCA1*:c.68_69delAG (n=6), *BRCA1*:c.5266dupC (n=6) and *BRCA2*:c.5946delT (n=6;). *BRCA2*:c.6275_6276delTT is a founder mutation from the UK and the other three *BRCA1/2* mutations are Ashkenazi Jewish founder mutations (24, 25).

Genomic Instability Score

Of the 2,178 tumours successfully tested, 814 (37%) had a GIS of ≥42. Of these, 304 (37%) had a *BRCA1/2* mutation, while 510 (63%) were *BRCA1/2* wild-type.

The GIS had a bimodal distribution (**Figure 2**). Tumours with a *BRCA1/2* mutation had higher GIS than those with *BRCA1/2* wild-type. The mean GIS for tumours with a *BRCA1/2* mutation was 61 (median 62; range 3-90; interquartile range 54-70; standard deviation 13) compared to 33 for *BRCA1/2* wild-type tumours (median 28; range 0-100; interquartile range 18-45; standard deviation 20; t-test P<0.0001) (**Figure 3**).

Of the 337 tumours with a *BRCA1/2* mutation that were successfully tested for GIS, 33 (10%) were GIS-negative. Although these tumours did not meet the GIS threshold for homologous recombination deficiency, they were classified as homologous recombination deficient due to the presence of a tumour *BRCA1/2* mutation.

Prevalence of tumours positive for homologous recombination deficiency

By including the number of tumours successfully tested for *BRCA1/2* mutation or a GIS, the prevalence of homologous recombination deficiency was 36% (895/2,474) and 37% (814/2,178), respectively.

Discussion

Summary of Main Results

This observational study reports the largest real world evaluation of routine tumour testing for homologous recombination deficiency in women diagnosed with ovarian cancer (26). Over 12 months of testing, 895 women with newly diagnosed FIGO stage III/IV high-grade epithelial ovarian, fallopian tube or primary peritoneal cancer in England, Wales and Northern Ireland were found to have a homologous recombination deficient tumour. In the UK, around 7,500 women are diagnosed with ovarian cancer each year. Of these, approximately 5,000 will be diagnosed with FIGO stage III/IV high-grade epithelial ovarian, fallopian tube or primary peritoneal cancer. The number of cases tested for homologous recombination deficiency in this study represents almost half of these women. Moreover, it is notable that tumour testing was not available in Wales and Northern Ireland until Autumn 2021, meaning fewer than 12 months of eligible women were included from these countries. No cases from Scotland were included in this study either. The rapid uptake of homologous recombination deficiency testing across the NHS demonstrates a concerted effort amongst multi-disciplinary teams to identify women most likely to respond to first-line

maintenance PARPi. The substantially higher number of GIS-positive tumours with *BRCA1/2* wild-type compared to *BRCA1/2* mutations demonstrates the value of using mutational scar assays to identify potentially PARPi sensitive tumours, above and beyond standard germline and somatic *BRCA1/2* testing (18, 27, 28).

Results in the Context of Published Literature

The prevalence of homologous recombination deficient tumours in this study was lower than anticipated. It has been suggested that approximately 50% of high-grade serous ovarian cancers harbour a genetic or epigenetic mutation that brings about homologous recombination deficiency (17). The relative lower prevalence in this study may have occurred because of a number of reasons. Firstly, eligibility criteria specified FIGO stage III/IV high-grade epithelial ovarian, fallopian tube or primary peritoneal cancer. As a result, non-high-grade serous carcinomas such as endometrioid (grade 2 or grade 3) and clear cell will have been tested. These subtypes account for approximately 10 to 15% of high-grade ovarian cancers and are rarely deficient in homologous recombination (26, 29, 30, 31, 32, 33). Secondly, eligibility criteria did not mandate a response to first-line platinum-based chemotherapy prior to tumour testing. Thus, tumours that did not respond to first-line platinum would have been tested, and these are highly unlikely to be homologous recombination deficient (18). Thirdly, fewer germline *BRCA1/2* mutations may have been included in this study. Women with newly diagnosed FIGO stage III/IV high-grade ovarian cancer who are known germline *BRCA1/2* heterozygotes can access maintenance olaparib plus bevacizumab without requiring tumour testing (11). Fourthly, observational data suggests that tumour samples with higher chemotherapy response scores (2/3 versus 1) following

neoadjuvant chemotherapy are more likely to be deficient in homologous recombination, but also more likely to fail testing (34). Therefore, homologous recombination proficient tumours are often disproportionately reported in cases treated with neoadjuvant chemotherapy plus delayed primary surgery. Fifthly, this study shows a relatively higher rate of complete and partial assay failure compared to clinical trials, meaning a significant number of patients with a tumour *BRCA1/2* mutation may have been missed (10, 11). Finally, our real world data is likely to include more elderly patients compared to clinical trials. Observational data from the BriTROC-1 study has demonstrated that the presence of homologous recombination deficient-related single nucleotide variant-signature 3 inversely correlates with age (35).

Strengths and Weaknesses

There are three main limitations with this study. Firstly, no clinical data have been provided. This information was not mandated on the test request form. Thus, we are unable to determine the distribution of homologous recombination deficiency across demographic groups. Secondly, no follow-up data have been provided. Therefore, we cannot determine whether testing influenced clinical decision making. The UK testing scheme did not mandate treatment with first-line maintenance olaparib plus bevacizumab for *BRCA1/2* mutant or GIS-positive tumours. In fact, because the optimal first-line maintenance therapy for FIGO stage III/IV high-grade epithelial ovarian, fallopian tube or primary peritoneal cancer has not been precisely defined, several alternative options are available including olaparib, niraparib or bevacizumab (8, 10, 36). Thirdly, the germline and somatic status of each tumour *BRCA1/2* mutation

is unknown. No tumour DNA sequencing assay is able to distinguish between germline and somatic *BRCA1/2* variants. Thus, we are unable to report whether GIS was affected by germline or somatic status. Interestingly, 10% of tumours with a *BRCA1/2* mutation had a GIS of <42. The reason for this unusual genotype is unclear but may suggest certain *BRCA1/2* mutations having a passenger role in carcinogenesis. Those patients found to have a *BRCA1/2* mutant/GIS-negative tumour should be more closely observed for poorer response to PARPi therapy.

Implications for Practice and Future Research

We report data from the largest observational study evaluating homologous recombination deficiency in newly diagnosed FIGO stage III/IV high-grade epithelial ovarian, fallopian tube or primary peritoneal cancer. These data show the value of tumour testing to identify women most likely to respond to first-line maintenance PARPi. These data demonstrate the importance of homologous recombination deficiency testing to optimise outcomes for eligible women. The relatively high failure rate of testing, resulting from formalin-fixed, paraffin-embedded tissue with low tumour cellularity and/or low tumour DNA yield, also highlights the need for local multi-disciplinary teams to carefully select tumour tissue to be tested. Finally, the rapid uptake of homologous recombination deficiency testing in England, Wales and Northern Ireland demonstrates the power of centralised NHS funding, centre specialisation and the NHS Genomic Laboratory Hub network.

Conclusions

The real world prevalence of homologous recombination deficient tumours in women with newly diagnosed, FIGO stage III/IV high-grade epithelial ovarian, fallopian tube or primary peritoneal cancer in England, Wales and Northern Ireland was 37%. Most of tumours positive for homologous recombination deficiency were *BRCA1/2* wild-type

Funding

The Myriad myChoice® companion diagnostic was funded by AstraZeneca PLC (Address: 1 Francis Crick Avenue, Cambridge Biomedical Campus, Cambridge CB2 0AA, United Kingdom; Tel: +44 (0)20 3749 5000).

Acknowledgement

The data reported in this manuscript has been presented at the European Society of Medical Oncology 2022 Congress (*Annals of Oncology* 2022;33(suppl_7):S235-S282).

Conflicts of interest

RDM, BMB, SN, HS, LY-S, YW, MV-P, RW, SM, SMc, EH, AG, SS, SN, SST and DGRE declare no conflicts of interest.

KT is an employee and stockholder of Myriad Genetics.

ARC declares research funding from AstraZeneca.

SMac declares travel funding and speaker fees from AstraZeneca, Merck and Eli Lily.

LT declares honoraria from AstraZeneca, Clovis Oncology, GSK and Tesaro.

RM declares honoraria from AstraZeneca, Merck Sharp & Dohme and GSK and research funding from Barts & The London Charity, Rosetrees Trust, GSK and Yorkshire Cancer Research.

JB declares honoraria from AstraZeneca and GSK and consulting and advisory roles in Clovis Oncology and GSK.

SB declares institutional grants from AstraZeneca and GSK, personal fees for speaker, consulting/advisory roles from Amgen, AstraZeneca, Clovis Oncology, Epsilogen, GSK, Immunogen, Mersana, Merck Sharp & Dohme, Merck Serono, Novartis, OncXerna, Pfizer, Roche, Shattuck Labs & Takeda.

RSK declares honoraria from AstraZeneca, Clovis Oncology, GSK and Incyte and travel support from AstraZeneca, GSK and Sierra Oncology and trial grants from Merck Sharp & Dohme and consulting fees from Basilea Pharmaceutica and Shattuck Pharma.

IMcN declares honoraria from AstraZeneca, Clovis Oncology, Epsila Bio, GSK, Roche and Scancell and institutional funding from AstraZeneca.

JAL declares honoraria from AstraZeneca, Clovis Oncology, GSK, Eisai, Neopharm, Artios Pharma, Merck/Merck Sharp & Dohme, Mersana, Regeneron, VBL Therapeutics, Nuvation and Bristol Myers Squibb.

GCJ declares research funding from AstraZeneca.

References

1. Lheureux S, Gourley C, Vergote I, Oza AM. Epithelial ovarian cancer. Lancet. 2019;393(10177):1240-53.

2. du Bois A, Reuss A, Pujade-Lauraine E, Harter P, Ray-Coquard I, Pfisterer J. Role of surgical outcome as prognostic factor in advanced epithelial ovarian cancer: a combined exploratory analysis of 3 prospectively randomized phase 3 multicenter trials: by the Arbeitsgemeinschaft Gynaekologische Onkologie Studiengruppe Ovarialkarzinom (AGO-OVAR) and the Groupe d'Investigateurs Nationaux Pour les Etudes des Cancers de l'Ovaire (GINECO). Cancer. 2009;115(6):1234-44.

3. Colombo N, Sessa C, du Bois A, Ledermann J, McCluggage WG, McNeish I, et al. ESMO-ESGO consensus conference recommendations on ovarian cancer: pathology and molecular biology, early and advanced stages, borderline tumours and recurrent diseasedagger. Ann Oncol. 2019;30(5):672-705.

4. Markman M. Maintenance chemotherapy in the management of epithelial ovarian cancer. Cancer Metastasis Rev. 2015;34(1):11-7.

5. Mirza MR, Monk BJ, Herrstedt J, Oza AM, Mahner S, Redondo A, et al. Niraparib Maintenance Therapy in Platinum-Sensitive, Recurrent Ovarian Cancer. N Engl J Med. 2016;375(22):2154-64.

6. Coleman RL, Oza AM, Lorusso D, Aghajanian C, Oaknin A, Dean A, et al. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2017;390(10106):1949-61.

7. Pujade-Lauraine E, Ledermann JA, Selle F, Gebski V, Penson RT, Oza AM, et al. 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. 2017;18(9):1274-84.

8. Moore K, Colombo N, Scambia G, Kim BG, Oaknin A, Friedlander M, et al. Maintenance Olaparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. N Engl J Med. 2018;379(26):2495-505.

9. Coleman RL, Fleming GF, Brady MF, Swisher EM, Steffensen KD, Friedlander M, et al. Veliparib with First-Line Chemotherapy and as Maintenance Therapy in Ovarian Cancer. N Engl J Med. 2019;385(25):2403-15.

10. Gonzalez-Martin A, Pothuri B, Vergote I, DePont Christensen R, Graybill W, Mirza MR, et al. Niraparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. N Engl J Med. 2019;381(25):2391-402.

11. Ray-Coquard I, Pautier P, Pignata S, Perol D, Gonzalez-Martin A, Berger R, et al. Olaparib plus Bevacizumab as First-Line Maintenance in Ovarian Cancer. N Engl J Med. 2019;381(25):2416-28.

12. Wu XH, Zhu JQ, Yin RT, Yang JX, Liu JH, Wang J, et al. Niraparib maintenance therapy in patients with platinum-sensitive recurrent ovarian cancer using an individualized starting dose (NORA): a randomized, double-blind, placebo-controlled phase III trial. Ann Oncol. 2021;32(4):512-21.

13. Li N, Zhang Y, Wang J, Zhu J, Wang L, Wu X, et al. Fuzuloparib Maintenance Therapy in Patients With Platinum-Sensitive, Recurrent Ovarian Carcinoma (FZOCUS-2): A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Phase III Trial. J Clin Oncol. 2022:40(22):2436-2446.

14. Monk BJ, Parkinson C, Lim MC, O'Malley DM, Oaknin A, Wilson MK, et al. A Randomized, Phase III Trial to Evaluate Rucaparib Monotherapy as Maintenance Treatment in Patients With Newly Diagnosed Ovarian Cancer (ATHENA-MONO/GOG-3020/ENGOT-ov45). J Clin Oncol. 2022:40(34):3952-3964.

15. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature. 2005;434(7035):913-7.

16. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature. 2005;434(7035):917-21.

17. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474(7353):609-15.

18. Telli ML, Timms KM, Reid J, Hennessy B, Mills GB, Jensen KC, et al. Homologous Recombination Deficiency (HRD) Score Predicts Response to Platinum-Containing Neoadjuvant Chemotherapy in Patients with Triple-Negative Breast Cancer. Clin Cancer Res. 2016;22(15):3764-73.

19. Gonzalez-Martin A, Desauw C, Heitz F, Cropet C, Gargiulo P, Berger R, et al. Maintenance olaparib plus bevacizumab in patients with newly diagnosed advanced high-grade ovarian cancer: Main analysis of second progression-free survival in the phase III PAOLA-1/ENGOT-ov25 trial. Eur J Cancer. 2022;174:221-31.

20. Ray-Coquard IL, Leary A, Pignata S, Cropet C, Gonzalez Martin AJ, Bogner G, et al. Final overall survival (OS) results from the phase III PAOLA- 1/ENGOT-ov25 trial evaluating maintenance olaparib (ola) plus bevacizumab (bev) in patients (pts) with newly diagnosed advanced ovarian cancer (AOC). Ann Oncol. 2022;33:S1396.

21. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-24.

22. Ellison G, Huang S, Carr H, Wallace A, Ahdesmaki M, Bhaskar S, et al. A reliable method for the detection of BRCA1 and BRCA2 mutations in fixed tumour tissue utilising multiplex PCR-based targeted next generation sequencing. BMC Clin Pathol. 2015;15:5.

23. Frugtniet B, Morgan S, Murray A, Palmer-Smith S, White R, Jones R, et al. The detection of germline and somatic BRCA1/2 genetic variants through parallel testing of patients with high-grade serous ovarian cancer: a national retrospective audit. BJOG. 2022;129(3):433-42.

24. Metcalfe KA, Poll A, Royer R, Llacuachaqui M, Tulman A, Sun P, et al. Screening for founder mutations in BRCA1 and BRCA2 in unselected Jewish women. J Clin Oncol. 2010;28(3):387-91.

25. Scottish/Northern Irish BBC. BRCA1 and BRCA2 mutations in Scotland and Northern Ireland. Br J Cancer. 2003;88(8):1256-62.

26. Denkert C, Romey M, Swedlund B, Hattesohl A, Teply-Szymanski J, Kommoss S, et al. Homologous Recombination Deficiency as an Ovarian Cancer Biomarker in a Real-World Cohort: Validation of Decentralized Genomic Profiling. J Mol Diagn. 2022;24(12):1254-63.

27. Frampton GM, Fichtenholtz A, Otto GA, Wang K, Downing SR, He J, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol. 2013;31(11):1023-31.

28. Miller RE, Leary A, Scott CL, Serra V, Lord CJ, Bowtell D, et al. ESMO recommendations on predictive biomarker testing for homologous recombination deficiency and PARP inhibitor benefit in ovarian cancer. Ann Oncol. 2020;31(12):1606-22.

29. Chao A, Lai CH, Wang TH, Jung SM, Lee YS, Chang WY, et al. Genomic scar signatures associated with homologous recombination deficiency predict adverse clinical outcomes in patients with ovarian clear cell carcinoma. J Mol Med (Berl). 2018;96(6):527-36.

30. Hollis RL, Thomson JP, Stanley B, Churchman M, Meynert AM, Rye T, et al. Molecular stratification of endometrioid ovarian carcinoma predicts clinical outcome. Nat Commun. 2020;11(1):4995.

31. How JA, Timms KM, Jazaeri AA, Lu KH, Yates MS. Characterization of homologous recombination deficiency in uncommon epithelial ovarian cancer subtypes. 2020;159:90.

32. Iida Y, Okamoto A, Hollis RL, Gourley C, Herrington CS. Clear cell carcinoma of the ovary: a clinical and molecular perspective. Int J Gynecol Cancer. 2021;31(4):605-16.

33. Zalaznick M, Clegg B, Cogan E, Perry M, Trost J, Mancini-DiNardo D, et al. Rates of homologous recombination deficiency across different subtypes of ovarian cancer and in pre- and post-neoadjuvant chemotherapy tumor samples. Gynecologic Oncology. 2022;166:S86-S7.

34. Zalaznick M, Clegg W, Cogan ES, Perry M, Trost J, Mancini-DiNardo D, et al., editors. Rates of homologous recombination deficiency across different subtypes of

ovarian cancer and in pre- and post-neoadjuvant chemotherapy tumour samples. Gynecol Oncol. 2022;166(Supp_1):S86-S87.

35. Macintyre G, Goranova TE, De Silva D, Ennis D, Piskorz AM, Eldridge M, et al. Copy number signatures and mutational processes in ovarian carcinoma. Nat Genet. 2018;50(9):1262-70.

36. Perren TJ, Swart AM, Pfisterer J, Ledermann JA, Pujade-Lauraine E, Kristensen G, et al. A phase 3 trial of bevacizumab in ovarian cancer. N Engl J Med. 2011;365(26):2484-96.

	BRCA1 **(N=220)**	***BRCA2*** **(N=165)**	**Total (N=385)**
Nucleotide level			
Small deletions	82 (37)	90 (55)	172 (45)
Single nucleotide variants	95 (43)	48 (29)	143 (37)
Small duplications	19 (9)	19 (12)	38 (10)
Large genomic rearrangements	18 (8)	3 (2)	21 (5)
Small insertion-deletions	6 (3)	3 (2)	9 (2)
Small insertions	0	2 (1)	2 (1)
Protein level			
Frameshift-deletions	96 (44)	105 (64)	201 (52)
Nonsense	59 (27)	44 (27)	103 (27)
Splice	22 (10)	8 (5)	30 (8)
Large genomic rearrangements	18 (8)	3 (2)	21 (5)
Missense	16 (7)	4 (2)	20 (5)
Intron	9 (4)	1 (1)	10 (3)

Table 1. Types of tumour *BRCA1/2* pathogenic and likely pathogenic variants. Data is presented as number (percentage).

Figure Legends

Figure 1. Lollipop diagram showing the loci of each pathogenic or likely pathogenic variant in *BRCA1* and *BRCA2*. Key: (**A**) *BRCA1* and (**B**) *BRCA2*; splice site, intronic variants and large genomic rearrangements are not included; the number of circles on each lollipop stick indicates the number tumours containing that variant; the exons of BRCA1 and BRCA2 proteins are numbered; reference sequences are LRG_292(BRCA1) and LRG_293(BRCA2).

Figure 2. Bar graph showing the distribution of Genomic Instability Scores in tumours with a *BRCA1/2* mutation or wild-type. Two-thousand-one-hundred-and-seventy-eight tumours were successfully tested for Genomic Instability Score.

Figure 3. Dot plot diagram showing the Genomic Instability Score of tumours with a *BRCA1/2* mutation or wild-type. Key: each dot represents the Genomic Instability Score (GIS) for a single tumour; the dotted line at GIS = 42 represents the threshold at which a tumour is classified as deficient in homologous recombination.