Advanced Epithelial Ovarian Cancer: An audit of Homologous Recombination Deficiency (HRD) testing and strategies for reducing test failure rate

Vallely K[1], Blake D[2] , Kumar H[2], Slana B[1], Hughes A[3], Rundle S[2], Ang C[2], Ratnavelu N[2], Kucukmetin A[2], Ralte A[1]

[1]South of Tyne and Wear Pathology, Queen Elizabeth Hospital, Gateshead Health NHS Trust, Gateshead, UK
[2]Northern Gynaecological Oncology Centre, Queen Elizabeth Hospital, Gateshead Health NHS Trust, Gateshead, UK
[3]Northern Centre for Cancer Care, Freeman Hospital, Newcastle Hospitals NHS Trust, Newcastle upon Tyne, UK

Background

High grade serous ovarian carcinoma (HGSOC) accounts for 70% of ovarian carcinomas, with the majority of patients presenting with advanced disease (FIGO stage III or IV). Despite high initial responses to chemotherapy most patients recur and median 5-year survival rates are between 15% and 55%[1].

DNA double strand breaks (DSBs) are the most lethal DNA lesions. Homologous recombination repair (HRR) is the high fidelity pathway that processes DSBs (Fig1). Approximately 50% of patients with HGSOC are homologous recombination deficient (HRD) and are unable to repair DSBs by HRR [4]. These tumours exhibit specific clinical behaviours with improved responses to treatments, such as platinum-based chemotherapy and poly (ADP-ribose) polymerase inhibitors (PARPi)[2]

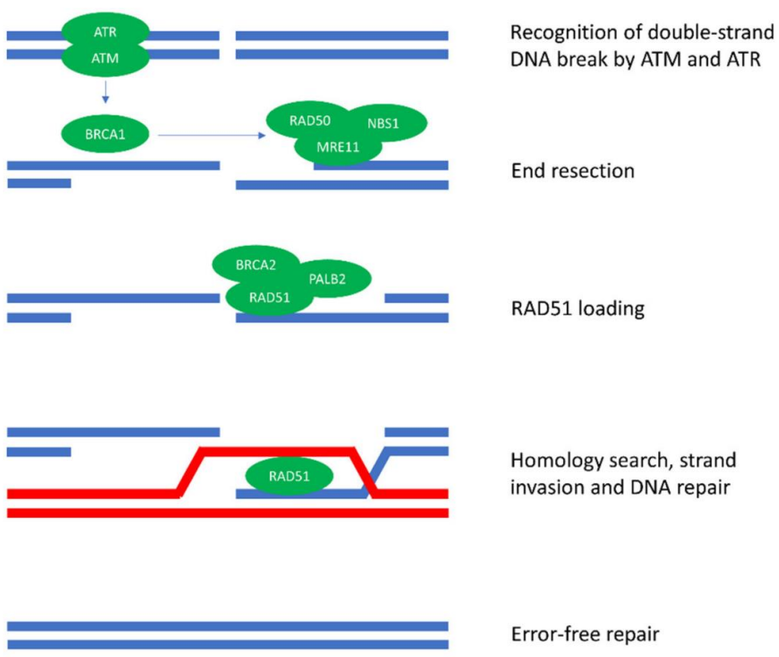

Figure 1. A representation of the process of homologous recombination repair (HRR). HRR occurs during and shortly after DNA replication, during the S and G2 phases of the cell cycle. when sister chromatids are more easily available and starts with the recognition of a DSB by ATM and ATR which activates BRCA1, which plays a key role in the recruitment of a complex of repair proteins, needed for DNA end resection. After end resection, with the help of BRCA2 and PLAB2 , RAD51 is loaded into the single-strand DNA tail enabling it to perform a homology search and 'invade' the sister chromatid. DNA polymerase then extends the sequence using the sister chromatid as a template for the DNA repair, as such this is considered a relatively error free process. 'Of note, besides the proteins depicted here, many other proteins are involved in HRR[3].

HRD tumours rely on alternative pathways for repair, for example error prone non-homologous end joining (NHEJ) . Blocking single strand repair pathways such as base excision repair (BER) with PARPi forces cells to accumulate DSBs resulting in cell death in HRD tumours. This is termed synthetic lethality.

Figure 2. Characteristic biomarkers for genomic instability associated with homologous recombination repair deficiency. Telomeric allelic imbalance is the imbalance in paternal and maternal alleles with or without changes in the overall copy number in the telomeric region. Loss of heterozygosity , which refers to when one of the two alleles that was originally present in the cell is lost and large-scale state transitions which is defined as chromosomal breaks between adjacent regions of at least 10 mb, a situation where a DSB cannot be repaired by HRR leaving a fragment of DNA [3].

Germline and somatic mutations BRCA 1/2 are the most common cause of HRD, however a number of other repair proteins in HRR may have alterations resulting in HRD.[1] For this reason HRD testing by genomic instability is undertaken (Fig2) as this has not only prognostic significance but implications for treatment options in advanced stage disease in the maintenance and recurrent setting.

References:
1. WHO Classification of Tumours Editorial Board. Female genital tumours. Lyon (France): International Agency for Research on Cancer; 2020. (WHO classification of tumours series, 5th ed.; vol. 4).
2. da Cunha Colombo Bonadio RR, Fogace RN, Miranda VC, Diz MDPE. Homologous recombination deficiency in ovarian cancer: a review of its epidemiology and management. *Clinics (Sao Paulo)*. 2018;73(suppl 1):e450s. Published 2018 Aug 20. doi:10.6061/clinics/2018/e450s
3. van Wilpe, S.; Tolmeijer, S.H.; Koornstra, R.H.T.; de Vries, I.J.M.; Gerritsen, W.R.; Ligtenberg, M.; Mehra, N. Homologous Recombination Repair Deficiency and Implications for Tumor Immunogenicity. Cancers 2021, 13, 2249. https://doi.org/10.3390/ cancers13092249
4. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma [published correction appears in Nature. 2012 Oct 11;490(7419):298]. Nature. 2011;474(7353):609-615. Published 2011 Jun 29. doi:10.1038/nature1016

Aims

HRD somatic tumour testing has replaced BRCA1/2 somatic mutation testing as the first line method to characterise HGOCs as it is recognised that BRCA alone does not account for all HRD cancers. Samples from our Centre are tested by Myriad Oncology (USA) using Myriad myChoice^{R} /CDX myChoice^{R} CDx Plus assay to determine a Genomic Instability Score (GIS) and BRCA mutation status. A high score (>42) is suggestive of HRD. We aimed to compare sample adequacy rates and the identified mutations of HRD somatic tumour testing versus somatic BRCA1/2.

Method

SOTW, QEH laboratory system was interrogated for results of somatic tBRCA and HRD testing sent for high grade, high stage (Stage III and above), non mucinous tubo-ovarian cancers between 6th June 2019 and 31st December 2021. Specimen reports were reviewed for type, molecular testing method and test result.

Results and Conclusions

A total of 206 HGOCs molecular test reports were received during that time period, 111 for BRCA1/2 and 95 for HRD. HRD testing increased detection of a pathogenic BRCA mutation from 15.3% to 17.9% with an additional 20% found to have high GIS suggesting HRD (Fig 3.). tBRCA1/BRCA2 mutation analysis was performed by Manchester Genomic Medicine from June 2019 to March 2021. From April 2021 onwards, the combined tBRCA/HRD test was performed by Myriad Oncology USA (funded by AstraZeneca) routed via Manchester Genomics.

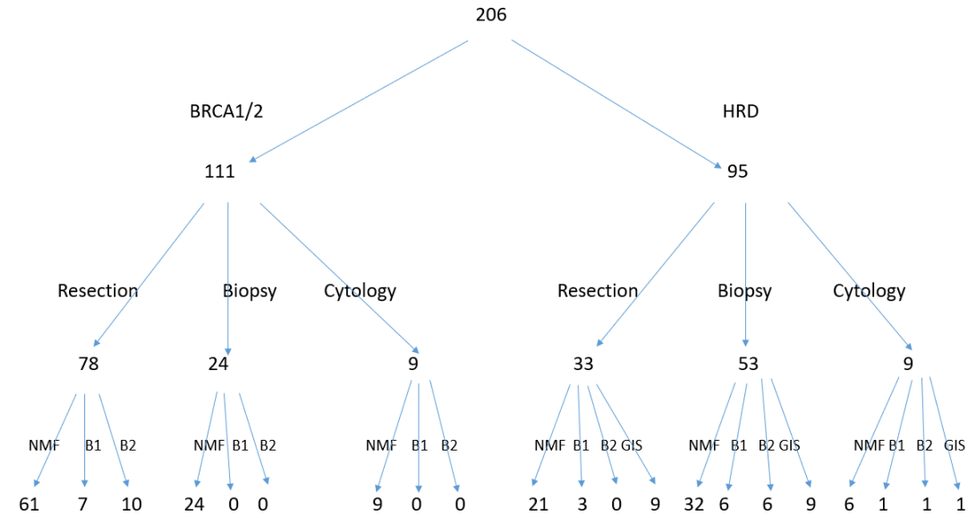

Figure 3. Diagram of results by molecular test, specimen type and result received. 206 samples sent for molecular testing, 111 for BRCA and 95 for HRD. (NMF- no mutation found, B1- BRCA1 mutation found, B2 –BRCA2 mutation found, GIS- high genomic instability score)

There was increase in biopsy specimens sent for HRD testing compared to BRCA1/2 alone, but failed analysis rates increased from 3.6% to 15.7% (Table 1). The only unsuccessfully analysed sample was taken following neoadjuvant chemotherapy. Success rates of HRD analysis have improved by selecting optimal samples and ensuring quality control in the assessment of tumour cellularity in small biopsies. We have observed that pooling of multiple cores into one block increased our success rate compared to dividing or separating the cores in separate blocks. Failed HRD analysis rates reduced from 15.7% to 5.8% in second half of 2021 and early 2022 (additional data since submission of this study) due to combination of improved tissue pathways and move to myChoice Plus assay. Superficial first face sections are used for initial tumour diagnosis followed by a limited panel of confirmatory immunostains (WT1, p53, PAX8, ER) performed on serial sections.

As our experience with HRD testing increased, we have received support from cancer satellite units. Tumour biopsy samples are often directly transported from the Cancer Units to Pathology in Gateshead, either as a wet specimen or as a paraffin block for primary reporting, in patients with a strong clinical suspicion of metastatic tubo-ovarian cancer. The advantage of this is the expeditious process while preserving the tissue for genomic studies.

Additional data obtained after submission of this abstract also showed an improved HRD+ (inc tBRCA) rates from 46.5% to 50% in line with TCGA (The Cancer Genomic Atlas) molecular characterisation of ovarian cancer.

We would like to thank our colleagues in pathology, radiology, and gynaecology in Northumbria Healthcare Trust, University of North Durham, North Cumbria and Gateshead as well as South Tyneside and Sunderland for their continued support. This has helped ensure a more successful HRD analysis.
All authors confirm that this is work is not affiliated with any commercial sponsor or external organisation that could affect independence or objectivity.