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Author/s:
Nesic, K;Wakefield, M;Kondrashova, O;Scott, CL;McNeish, IA

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Wakefield Matthew (Orcid ID: 0000-0001-6624-4698)
McNeish Iain (Orcid ID: 0000-0002-9387-7586)

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Targeting DNA repair: the genome as a potential biomarker

Ksenija Nesic[1,2], Matthew Wakefield[1,3], Olga Kondrashova[1,2], Clare L. Scott[1,2,4,5*], Iain A. McNeish[6*]

1: Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052 Australia

2: Department of Medical Biology, University of Melbourne, Parkville, Victoria 3010, Australia

3: Melbourne Bioinformatics, University of Melbourne, Parkville, Victoria 3010, Australia

4: Royal Women's Hospital, Parkville, Victoria, 3052, Australia

5: Peter MacCallum Cancer Centre, Parkville, Victoria 3010, Australia

6: Imperial College, London, UK

*: Equal contribution

Running title: Predictive genomic biomarkers in cancer

Correspondence

Professor Iain McNeish
Department of Surgery and Cancer
Imperial College
IRBD Building
Hammersmith Hospital
LONDON W12 0NN
UK
i.mcneish@imperial.ac.uk

Professor Clare L. Scott
Walter and Eliza Hall Institute of
Medical Research;
Peter MacCallum Cancer Centre;
Royal Melbourne Hospital,
1G Royal Parade
Parkville Victoria 3052
Australia
scottc@wehi.edu.au

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Abstract

Genomic instability and mutations are fundamental aspects of human malignancies, leading to progressive accumulation of the hallmarks of cancer. For some time, it has been clear that key mutations may be used both as prognostic and predictive biomarkers, the best-known examples being the presence of germline *BRCA1* or *BRCA2* mutations, which are not only associated with improved prognosis in ovarian cancer, but are also predictive of response to poly(ADP-ribose) polymerase (PARP) inhibitors. Although biomarkers as specific and powerful as these are rare in human malignancies, next generation sequencing and improved bioinformatic analyses are revealing mutational signatures, broader patterns of alterations in the cancer genome that have the power to reveal information about underlying driver mutational processes. Thus, the cancer genome can act as a stratification factor in clinical trials and, ultimately, will be used to drive personalised treatment decisions.

In this review, we will use ovarian high grade serous carcinoma (HGSC) as an example of a disease of extreme genomic complexity that is marked by widespread copy number alterations, but which lacks powerful driver oncogene mutations. Understanding of the genomics of HGSC has led to the routine introduction of germline and somatic *BRCA1/2* testing, as well as testing of mutations in other homologous recombination genes, widening the range of patients who may benefit from PARP inhibitors. We will discuss how whole genome-wide analyses, including loss of heterozygosity quantification and whole genome sequencing, may extend this paradigm to allow all patients to benefit from effective targeted therapies.

Keywords

Homologous Recombination; BRCA1; BRCA2; ovarian high grade serous carcinoma; loss of heterozygosity; mutational signatures; next-generation sequencing; copy number signatures;

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Introduction

The integrity of the genome is essential for ensuring survival of cells and organisms. However, genomes are constantly at risk from both intracellular and extracellular agents, such as by-products of cellular metabolism, radiation or chemical exposure as well as spontaneous DNA mutation events. Thus, cells have evolutionarily-conserved mechanisms of DNA repair and maintenance that can cope with these threats to their genetic material, whilst also allowing some mutational alterations to be transferred to their progeny as part of the process of adaptation and evolution [1]. Although this evolution is most obvious at the level of species, it is also visible within the adaptive immune system, which relies on somatic recombination to generate the diverse range of antibodies and immune cell receptors required to combat pathogens and cellular defects [2]. These processes are normally highly regulated, to ensure effective development of the immune system whilst also preventing genomic instability that may lead to hyperactive evolution and malignant transformation. It is, therefore, unsurprising that DNA repair pathway defects can result in not only immune deficiencies, but also the development of cancer [3].

Genomic instability and mutations are now considered key enabling characteristics of cancer, promoting the step-wise accumulation of the hallmarks of malignant cells, including sustained proliferative signalling, resistance to cell death, evasion of growth suppressors, replicative immortality and induced angiogenesis, as well as invasive and metastatic capabilities [4,5]. Genomic instability can also contribute to the ability of some cancers to evade the immune response, and to even promote inflammation that is beneficial to the proliferation and spread of the cancer, effectively enabling hijacking of the immune system [6].

Nonetheless, DNA repair defects that result in tumour-enhancing genomic instability can also act as an Achilles heel of that cancer, and underpin novel targeted therapies that exploit either production of immunogenic neoantigens that prime tumours for treatment with immunotherapy [7], or DNA Double-Strand Break (DSB) repair deficiencies that are enriched in certain cancer types and can be targeted by DNA repair inhibitors [8]. Unfortunately, the same DNA repair defects that can sensitise cancer cells to these therapies also drive diversity and provide possible escape pathways leading to therapeutic resistance.

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This review will examine use of the cancer genome as a predictive biomarker in the context of DNA DSB repair defective cancers, specifically Homologous Recombination Deficient (HRD) ovarian cancer, and DNA repair inhibitor therapies that exploit these defects.

DNA double-strand break repair pathways

DNA DSBs are the most dangerous type of lesion that DNA can sustain, because just a single unrepaired DSB can be sufficient to trigger cell death [9]. This highlights the enormous dependency for cell survival on the capacity of a cell to repair DNA. When DNA DSBs form, there are several competing pathways that can take over their repair. The choice of DNA DSB repair mechanism employed by a cell is complex [10], and depends on multiple factors, including the complexity of damaged DNA DSB ends (one-ended; two-ended; four-ended) [11], the stage of the cell cycle and the specific activity of nucleases such as MRE11 [12]. In the context of cancer, however, the mode of DNA repair also crucially depends upon which DNA repair pathways remain intact in that cancer at that time.

Homologous Recombination (HR) and classical non-homologous end joining (C-NHEJ) are the two main DNA DSB repair pathways available in mammalian cells (Figure 1). The contributions of other DNA DSB pathways, such as alternative End-Joining (alt-EJ) and Single-Strand Annealing (SSA), long believed to play back-up roles, are now also being recognised as significant [10]. HR DNA repair is considered to be of high fidelity, as it relies on the presence of a sister chromatid to act as a template for repair and results in largely error-free repair. This use of a sister chromatid means that HR can only occur during S and G2 phases of the cell cycle following DNA replication. Furthermore, the crucial HR genes *BRCA2* and *RAD51* are only expressed during S/G2, again ensuring that HR cannot occur in G0/G1 [13]. By contrast, C-NHEJ can occur throughout the cell cycle, and dominates in G0/G1 and early G2. It is not homology-directed, and is a more error-prone DNA repair pathway than HR, causing chromosomal rearrangements and mutations when not regulated [10,14,15]. Therefore, HRD, defined as a loss of HR DNA repair capacity, is associated with genomic instability leading to increased genome-wide losses of heterozygosity (LOH) [16,17], sometimes called ‘genomic scarring’ [18]. Loss of HR DNA repair is frequently observed in human malignancies, including ovarian high grade serous carcinoma (HGSC),

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triple negative breast cancer (TBNC) and prostate cancer [19-21] and will be discussed below. The consequences of C-NHEJ pathway loss are more complex, and lie beyond the scope of this review. Here, we will focus on HRD, given its prevalence in human malignancies and the promising targeted therapeutic opportunities now available.

BRCA1/2 defects in High Grade Epithelial Ovarian Carcinoma

BRCA1 and *BRCA2* were first identified as breast cancer susceptibility genes in 1994 and 1995 respectively [22-24], and play fundamental roles in HR [25,26]. BRCA1 has multiple roles, but importantly it assists with recruitment of other DNA repair proteins, including BRCA2, to sites of DNA DSB. BRCA2 is then able to catalyse the formation of RAD51 filaments, thereby initiating strand invasion of the sister chromatid and homology-directed repair [27,28]. For a more detailed review the HR DNA repair pathway, please refer to [29].

The clinical importance of *BRCA1/2* mutations has had the greatest impact in the management of ovarian cancer so far. Although cervical cancer is the commonest gynaecological malignancy worldwide, and endometrial cancer the commonest gynaecological malignancy in Western countries, ovarian cancer remains a significant clinical problem, with little improvement in overall survival in the past twenty years [30]. Long thought to be a single disease, ovarian cancer is now recognised to be at least five separate diseases (high grade serous - HGSC, low grade serous, endometrioid, clear cell and mucinous carcinomas), driven by different genomic aberrations and with different cells of origin, and linked only by their broad anatomical location [30]. HGSC is the commonest subtype – approximately 70% ovarian cancer cases and 80% ovarian cancer deaths are due to HGSC – and typically presents at an advanced stage where treatment is rarely curative [31]. HGSC, endometrial and cervical cancers have all been the subjects of analysis by The Cancer Genome Atlas (TCGA) Network [21,32,33], which demonstrated significant differences between these three cancer types, although there is some clear overlap between HGSC and copy-number high endometrial cancer, as both have near-universal *TP53* mutation and extensive copy number alterations [33].

It has been estimated that approximately half of all HGSC contain defects in HR at the time of diagnosis [21,34], the commonest causes of which are either germline *BRCA* mutation

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(approximately 8-12% for *BRCA1*, 5-7% for *BRCA2*) or somatic *BRCA* mutation (approximately 3% for both *BRCA1* and *BRCA2*) [21,35,36]. A further 5-20% HGSC are HRD due to epigenetic silencing of *BRCA1* via promoter hypermethylation, which is considered mutually exclusive of *BRCA1/2* mutations [21,37]. Conversely *BRCA2* promoter hypermethylation is rare in HGSC, and, unlike *BRCA1* promoter hypermethylation, does not correlate with reduced mRNA expression [38]. In a recent study of nearly 10,000 *BRCA* carriers, the cumulative risk of developing ovarian cancer (OC) prior to age 80 was estimated to be 44% (95% CI, 36%-53%) for *BRCA1* and 17% (95% CI, 11%-25%) for *BRCA2* [39], highlighting the importance of these genes in the development of OC.

Therapeutic exploitation of HR DNA repair defects in Ovarian Cancer

The exciting finding that inhibition of poly(ADP-ribose) polymerase 1 (PARP1) is cytotoxic to *BRCA1/2* null cells *in vitro* [40,41] led to the subsequent development of PARP inhibitors (PARPi) as a new class of targeted anti-cancer drug for HRD cancers, initially only for tumours lacking BRCA1/2 function. This specific sensitivity of *BRCA1/2* mutated cells to PARP inhibition is an excellent manifestation of the concept of 'synthetic lethality'. This term was first used in the 1940s in relation to *Drosophila* [42] and describes the phenomenon whereby deletion or inactivation of two genes is lethal when deletion of either one alone is not (Figure 2). Further examples of this phenomenon in relation to DNA damage repair are reviewed in [8].

Over the past decade, multiple clinical studies have shown that tumours harbouring *BRCA1/2* mutations are sensitive to PARPi and platinum-based chemotherapy, and are associated with improved overall survival, especially *BRCA2* mutant cases [43]. As a result, *BRCA1/2* mutation testing has become routine in clinical management of OC patients as well as a key stratification factor in clinical trials. Despite the high frequency of *BRCA1* methylation in HGSOC, its impact on platinum or PARPi responses has not been established, with mixed reports on its effects on overall survival in clinical studies [18,21,44].

Olaparib (Lynparza; AstraZeneca) was the first PARPi to become approved both by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), and since December 2014 has been used to treat relapsed OC with both germline and somatic

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BRCA1/2 mutation. Olaparib was also approved by Australia's Therapeutic Goods Administration (TGA) in January 2016, and, since February 2017, has been reimbursed by the Pharmaceutical Benefits Scheme (PBS), with the approval restricted tightly to germline *BRCA1/2*-mutated HGSC in the maintenance setting. Additional PARPi, including niraparib (Zejula; Tesaro) and rucaparib (Rubraca; Clovis Oncology), have also been approved in the U.S.A. for the treatment of recurrent OC in the maintenance setting after successful treatment with platinum-based chemotherapy, and as single agent treatment for those with proven *BRCA1/2*-mutated OC respectively. Though not yet approved by the EMA, both Tesaro and Clovis Oncology have applied for licences to sell within Europe.

Homologous Recombination defects beyond *BRCA1/2* in High Grade Epithelial Ovarian Carcinoma

Although loss of BRCA1 or BRCA2 function through mutation or epigenetic alteration is the commonest cause of defective HR, the efficacy of PARPi therapy has also been demonstrated in cells with loss of other HR proteins, a trait sometimes described as "*BRCA*ness" [45,46]. As stated above, it has been estimated that approximately half of HGSC contain defects in HR DNA repair at the time of diagnosis [21,34], of which germline or somatic *BRCA1/2* mutations accounts for about 20%. In the Cancer Genome Atlas Research Network study, the remaining 30% were attributed to other alterations including mutations in Fanconi Anaemia genes (*FANCC, FANCD2, FANCA, FANCE, FANCI, FANCL* and *FANCM*), other genes associated with DNA damage repair and genome stability (*ATM*, *ATR*, *CHEK2*, *NBN, PALB2*, *RAD50* and *RAD51)*, *BRCA1* promoter hypermethylation, *EMSY* amplification, and loss of *PTEN* [21].

Many DNA repair genes have been associated with PARPi synthetic lethality when deficient *in vitro* or *in vivo* (Table 1). However, more consistent evidence of HR involvement and PARPi sensitivity is available *ATM* [47,48], *RAD51C* [18,48,49], *RAD51D* [49], *NBN* [18] and *PALB2* [50]. On the other hand, there appears to be conflicting evidence for the involvement of *PTEN* loss [51,52], and *EMSY* amplification [53]. Interestingly, many HGSC will display *BRCA* mutant-like phenotype, despite the absence of other known HR gene mutations [17,18,54].

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Limitations of Homologous Recombination DNA Repair Gene Testing

In keeping with the preclinical data, a key finding in early phase trials of PARPi was that some patients lacking a germline or somatic mutation in *BRCA1/2* derived marked clinical benefit [55]. This implied that extending mutation testing beyond *BRCA1/2* to include a wider panel of HR genes might have utility and could potentially identify a greater number of patients who might benefit from PARP inhibitor therapy. However, germline/somatic mutations in other core HR genes are detected in fewer than 10% of relapsed HGSC, and these panels are also unable to detect promoter methylation, which requires additional tests such as pyrosequencing or methylation-specific polymerase chain reaction. Thus, simply sequencing a wide panel of HR genes may still fail to detect all patients who could derive benefit from PARPi therapy [17,18,54]. Furthermore, despite extensive characterization and diagnostic testing of the *BRCA1* and *BRCA2* genes for pathogenic (Class 4-5) variants, there are still many Variants of Uncertain Significance (VUS; Class 2-3,) within these genes that remain to be clarified [56]. These variants pose a significant problem in the clinic, as their effects on disease risk and treatment response are undetermined, and their identification often necessitates both functional experimental analysis [57] and complex discussions with patients and their families. As next generation sequencing costs continue to decline, and somatic and germline testing become more prevalent, an increasing number of VUS are being detected, as other less well-characterized genes are being screened [58].

One very specific use for HR gene sequencing is to identify secondary or reversion mutations that correct the primary HR gene defects, which is a known mechanism of HR repair rescue and PARPi/platinum resistance. These secondary and reversion mutations are the best studied mechanism of HR repair rescue and PARPi/platinum resistance. Secondary mutations have been described in *BRCA1/2* and *RAD51C/D* in recurrent HGSC and prostate cancer patients, and appear to be enriched in acquired platinum-resistant patients rather than primary resistance [49,59,60]. However, not all PARPi and platinum resistance can be explained by secondary and reversion mutations. Cruz et al. (2016, abstract P4-07-05), recently reported the presence of RAD51 foci, a marker of functional HR DNA repair, in *BRCA1* and *BRCA2* mutant breast and ovarian cancer PDX models, despite no secondary mutations being detected. Indeed, multiple alternative mechanisms of HR restoration have been reported [61-63], which will not be detected with HR gene sequencing. Two genes of

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specific note are *53BP1* and *REV7.* In the context of DNA DSB, 53BP1 can promote NHEJ and suppress HR, acting in opposition to BRCA1 by preventing DSB end-resection [64]. Crucially, loss of 53BP1 function permits restoration of HR repair in cells lacking BRCA1 [61,65] and induces resistance to PARP inhibition [62]. More recently, REV7 (also known as MAD2L2) has been shown to work downstream of 53BP1 to co-ordinate DSB pathway choice, whilst loss of REV7 restores HR in *BRCA1*-deficient cells leading to PARP inhibitor resistance [63]. In addition, refolding of BRCA1 into an HR-competent conformation can restore HR function and thus induce PARP inhibitor resistance [66]. The specific contribution of these mechanisms to clinical drug resistance remains to be determined, however. Nonetheless, these novel mechanisms highlight that, although HR gene sequencing panels can detect some resistance events, other correcting mutations or deletions may be missed, along with alternative mechanisms of restored HR.

Given the potential shortcomings of relying solely on HR gene sequencing, novel strategies to use genomic alterations as prognostic and predictive biomarkers have been developed in parallel.

Genome-wide biomarkers of Homologous Recombination Deficiency

The mutations and copy number alterations within a cancer genome act as an archaeological record of all the unrepaired insults that have accumulated during the evolution of that tumour. It has been known for some time that specific carcinogenic processes in certain tumours, such as UV light in skin cancer or tobacco exposure in lung cancer, give rise to specific patterns of base-pair mutations. Although the majority of mutations observed in cancer are not driver mutations, these passenger mutations are frequent and can be informative of the underlying mutational process [67]. However, it is only recently that the application of unsupervised methods to large quantities of next generation sequencing data have permitted the identification of distinct mutational landscapes (or 'mutational signatures') in malignancies that lack obvious aetiologies. This can include cancers that may be defective in HR through a variety of mechanisms. In addition, methods to analyse and categorise the complex copy number and structural variant alterations are now available, some of which may have immediate clinical utility.

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Single Nucleotide Variant signatures

Using nearly 5 million somatic substitutions and small insertions/deletion (indels) identified in Whole Genome Sequencing (WGS) and Whole Exome Sequencing (WES) data from over 7000 primary tumours, Alexandrov, et al. studied the six classes of SNV (C>A, C>G, C>T, T>A, T>C, T>G), as well as the nucleotides immediately 5' and 3' to each mutated base, thus analysing 96 possible classes of nucleotide change. They identified 21 distinct single nucleotide variant (SNV) signatures across thirty different cancer types [68]. Key points were that all signatures contained multiple different classes of nucleotide change, and that multiple different signatures could be identified in any given tumour type. Nonetheless, it was clear that SNV signature 3, which was characterised by a large number of indels (up to 50bp) rather than specific nucleotide changes, was strongly associated with the presence of *BRCA1/2* mutations. In addition, there were breast, ovary and pancreatic cancers whose genomes contained a significant contribution from SNV signature 3 but which lacked mutation in either of these genes. This suggested that SNV signature 3 might represent a genomic hallmark of defective HR that could potentially be applied to tumours both with and without known *BRCA1/2* mutations. However, it has yet to be demonstrated that the presence of this signature *per se* is either prognostic or can predict response to therapy in patients, although in oesophageal cancer, mutational signatures that defined three broad disease subgroups had some predictive ability in cell line models [69]. Oesophageal cancer is a good example, along with HGSC and TNBC, of cancers that are dominated by copy number changes [70], and which are generally difficult to classify through mutational signatures as they have relatively low mutational burden, low rates of driver oncogenic mutations and highly complex genomic profiles [71]. Alternative strategies are required to analyse their genomes more comprehensively.

Loss of Heterozygosity (LOH) Assays

One genomic consequence of loss of HR is loss or duplication of chromosomal regions, also known as genomic LOH [16,17]. LOH was first investigated following reports of increased copy number alterations in HRD cancers [72,73], and this feature of HRD cancers is now being explored as a potential predictive biomarker.

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Recently, the phase 2 trial of rucaparib (ARIEL2 – Assessment of Rucaparib In Ovarian Cancer Trial – phase 2) demonstrated the utility of an LOH signature as a surrogate marker of HRD to identify *BRCA* wild-type (WT) HGSC patients who may benefit from PARPi therapy [18]. Next generation sequencing (NGS) was used to measure the percentage of genome-wide LOH in tumours, using approximately 3500 SNPs. A cut-off of 14% LOH was initially used to define HRD. In the first part of ARIEL2, patients with HGSC relapsing in the platinum-sensitive time frame (ie >6 months following last platinum chemotherapy) were categorised into three groups - *BRCA* mutant, *BRCA* WT LOH high and *BRCA* WT LOH low. All patients received single agent rucaparib (600 mg bd until disease progression), with a primary endpoint of progression-free survival (PFS). The trial met its primary endpoint as PFS was significantly longer in the *BRCA* mutant group (HR 0·27, 95% CI 0·16–0·44, p<0·0001) and *BRCA* WT LOH high group (HR 0·62, 0·42–0·90, p=0·011) compared to the *BRCA* WT LOH low subgroup. Response rates were highest in the *BRCA* mutant group (80%, 95% CI 64–91), compared to 29% (95% CI 20–40) *BRCA* WT LOH high group and 10% (95% CI 4–20) in the *BRCA* WT LOH low group. Interestingly, in those who did respond, median response duration was similar in the *BRCA* mutant (9·2 months, 95% CI 6·4–12·9) and *BRCA* WT LOH high groups (10·8 months, 5·7–not reached), and both were higher (p=0.013 and 0.022 respectively) than in the LOH low subgroup (5·6 months, 4·6–8·5).

In the follow-on randomised phase 3 ARIEL3 study, women responding to platinum chemotherapy in the relapsed setting were randomised to receive rucaparib (600 mg bd or placebo) until disease progression, with stratification again into the three subgroups described in ARIEL2. However, a refined 16% LOH cut-off was utilised [74]. Outcomes were best in the *BRCA* mutant group: median PFS for those treated with rucaparib was 16·6 months (95% CI 13·4-22·9), compared to 5·4 months (95% CI 3·4-6·7) in those treated with placebo (HR=0.23, p<0.0001). Positive results were also seen in both the HRD population (*BRCA* mutant and *BRCA* WT LOH high) and the overall intention-to-treat population. However, interestingly, even in *BRCA* WT LOH low group, rucaparib treatment induced a significant improvement in PFS compared to placebo: 6·7 (95% CI 5·4-9·1) versus 5·4 (95% CI 4·1-5·7) months respectively (HR=0·58 (95% CI 0·40–0·85); p=0·0049).

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The randomised, placebo-controlled, phase 3 trial of the PARPi niraparib [75] also grouped patients based on germline *BRCA* status using BRACAnalysis CDx (Myriad Genetics), and further stratified the germline *BRCA* WT group based on their HRD status using the myChoice HRD test (Myriad Genetics) – this test combines quantification of LOH, telomeric allelic imbalance and large-scale state transitions [76,77]. Again, PARPi treatment induced a highly significant improvement in PFS in the germline *BRCA* mutant group (21·0 vs 5·5 months, HR 0·27; 95% CI, 0·17-0·41), but, like ARIEL3, results in the *BRCA* WT HRD negative cohort indicated a significant improvement in PFS (6·9 vs 3·8 months, HR 0·58; 95% CI 0·36-0·92) for those treated with niraparib compared to placebo.

Thus, the two LOH-based biomarkers, whilst potentially useful in predicting outcome to PARPi therapy as a single agent treatment, lacked specificity, and were not able to override the power of platinum response as a surrogate marker for maintenance PARPi benefit in the relapsed setting. There are additional limitations to the use of LOH assays as a biomarker, in particular the fact that genomic scarring does not disappear if the HR pathway is restored, highlighting it as an historical marker of defective DNA repair, rather than a reflection of the current DNA repair capacity of a tumour. For example, in ARIEL2 part 1, LOH analyses were undertaken in matched tumour samples from 117 patients at the time of diagnosis and at study entry. Although there was strong correlation (rho=0·86, p<0·0001) between archival and study-entry tumour samples, of the 50 cases classified as LOH low at the time of diagnosis, 17 (34%) were LOH high at relapse, whilst no cases went from LOH high at diagnosis to LOH low a low at relapse [18]. For this reason, LOH assays may be most useful in first-line trials of untreated OC that have had no prior exposure to any chemotherapy and where acquired resistance has not yet developed. However, in relapsed cases, combining LOH assays with other methods to measure HR capacity may provide better predictive power.

Complex genomic algorithms including Structural Variant (SV) and Copy Number (CN) signatures

Further analyses of the genomes of ovarian, breast and other cancer types are revealing signatures within the complex structural and copy number variants that have the potential to act as tools for stratification within clinical trials and to direct therapy.

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Analysis of genomic rearrangements identified in WGS data from 560 breast cancer samples, based upon 32 separate features (including clustered vs non-clustered change; deletions vs inversions vs tandem duplications; size of rearrangement; presence of interchromosomal translocations) revealed six rearrangement signatures [78]. Interestingly, three of these rearrangements signatures associated with defective HR; signature 3 was associated with loss of BRCA1 (but not BRCA2) function, signature 5 was associated with loss of either BRCA1 or BRCA2 function, whilst signature 1, with large tandem duplications, did not associate with *BRCA1/2* mutations nor with mutations in other DNA DSB repair/HR genes (*ATM*, *ATR*, *PALB2*, *RAD51C*, *RAD50*, *TP53*, *CHEK2* and *BRIP1*).

Leading on from this work, Serena Nik-Zainal's group then utilised deep WGS data from 22 breast cancers from women with known germline *BRCA1* or *BRCA2* mutations, in which loss of heterozygosity (LOH) of the wild-type allele was also observed, to develop a genome-wide assay of defective HR, which they called 'HRDetect' [79]. The HRDetect score is based upon the weighted quantification of six genomic features, including (in descending order of weighting) the presence of microhomology-mediated deletions, SNV signature 3, rearrangement signatures 3 and 5, widespread LOH and SNV signature 8. When HRDetect was applied prospectively to a cohort of 560 breast cancers, it was able to detect *BRCA1/2* mutations (receiver operating characteristic (ROC) of 0.98) and was able to outperform LOH-based assays. Of the 560 breast cancers analysed, 124 were identified as having a high HRDetect score (>0.70), of which 47 had no detectable mutation or epigenetic alteration in *BRCA1/2*. In a separate ovarian cancer cohort of 73 cases, 46 (63%) were predicted to have defective HR, of which 16 had no mutations in either *BRCA1 or BRCA2*. Together, these results reinforce the message that the cancer genome may be used as a biomarker and, specifically, that a significant proportion of cancers may have an HRD phenotype without abnormalities in either *BRCA1* or *BRCA2*. However, again like SNV signatures, HRDetect has yet to be evaluated prospectively in clinical trials.

A specific pattern of structural abnormality identified in HGSC is the tandem duplicator phenotype (Figure 3). This was first described in 2012 in both breast and ovarian cancers as large (>100 Kb) duplicated fragments, and was originally thought to be mutually exclusive

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with *BRCA1/2* mutations [80,81]. However, further work revealed two distributions of tandem duplications [82]; the second group contained smaller (c.10 Kb) fragments with microhomology breakpoints, and was associated with loss of *BRCA1*, but not *BRCA2*, function [78,83]. Across multiple TCGA analyses, the tandem duplicator phenotype was identified in TBNC, HGSC and serous-like endometrial cancers, and was strongly associated with platinum sensitivity [83]. Very recently, the underlying mechanism of the smaller tandem duplications associated with *BRCA1* loss has been elucidated – the duplications arise at stalled replication forks as a result of an aberrant replication restart-bypass mechanism, which terminates with end-joining [84]. Moreover, a similar pattern of duplications appears common across *BRCA1*-mutated cancers and is also observed upon loss of BARD1 and CtIP function [84], suggesting that identification of the tandem duplicator phenotype might be a mechanism of identifying cancers with defective DNA damage repair function.

WGS data have been used to identify other patterns in ovarian cancer with potential clinical utility. Sohrab Shah and David Huntsman's group undertook WGS on a large ovarian cancer cohort, identifying twenty genomic features from SNV, indels, copy number alterations (CNA) and structural variant (SV) analyses. When applied to a mixed cohort of ovarian cancer subtypes (including HGSC, endometrioid, clear cell carcinoma and granulosa cell tumours), they identified seven distinct groups [85]. Two of these groups were comprised almost exclusively of HGSC cases – an HRD cohort and a cohort with a high proportion of fold-back inversions (FBI), which are duplicated regions of the genome where the two copies face opposing directions from the break-point (distinguishing them from tandem duplications – Figure 3) and which may arise as result of breakage-fusion-bridge cycles [86]. Again, the HRD cohort contained a significant number of cases that lacked *BRCA1/2* mutations. However, importantly, it was also possible to demonstrate that tumours with high rates of FBI had worse prognosis than low FBI, and that FBI was associated with known poor prognostic features including *CCNE1* amplification. Thus, this assay of genome structure identified features that could act as a prognostic biomarker (high vs low FBI) as well as a potential predictive biomarker (HRD vs non-HRD) for treatment with a PARP inhibitor. Clearly, again, this biomarker will need to be evaluated prospectively within up-coming clinical trial cohorts.

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One disadvantage of rearrangement signatures, the HRDetect assay and the analysis of FBI events is the current requirement for deep coverage WGS. It was noticeable that use of either WES data or lower coverage WGS resulted in a significant reduction in sensitivity of the HRDetect assay [79]. Although the cost of deep WGS is falling rapidly, the requirement for relatively large quantities of DNA from fresh-frozen tumour samples as well as the length and complexity of the bioinformatic analyses suggests that deep WGS is likely to remain a research tool rather than a prospective clinical stratifier for some time yet.

Recently, we have used low-cost shallow WGS (sWGS) to identify copy number signatures in the genomes of HGSC [87]. We used biopsy samples from the BriTROC-1 study (the first study of the UK Translational Research in Ovarian Cancer Consortium – detailed in [88]) of relapsed HGSC, and combined relative copy number data from sWGS (mean 0·1x) and *TP53* mutant allele frequency from deep tagged-amplicon sequencing [89] to generate absolute copy number profiles. Using non-negative matrix factorisation protocols, we were able to identify distinct copy number (CN) signatures in the initial cohort of 117 samples from the BriTROC-1 study that were then validated on samples from both PCAWG [60] and TCGA [21]. We also undertook deep WGS on a subset of the BriTROC-1 cases to allow comparison of the CN signatures with other genomic features. We believe that there are three critical features of the CN signatures. Firstly, they are prognostic; secondly, the signatures appear to correlate with other known features of HGSC genomes (for example one CN signature is highly enriched in cases with *BRCA1/2* mutation); and finally, they can be derived using only 100 ng DNA isolated from fixed tumour material [90] and thus have the potential to be assessed in small biopsy cores. However, again, these will still need to be prospectively evaluated to assess their ultimate utility.

Conclusion

There has been enormous progress in our ability to interrogate the genomes of cancer in recent years and with it the ability to stratify patients at the time of diagnosis. Currently, patients undergo germline *BRCA1/2* mutation testing and, in some cases, NGS analysis of a wider gene panel. Our improved understanding of the mutagenic processes in cancer and their effects on the cancer genome provide powerful and cost-effective tools that have great potential for clinical utility, and so we propose a pathway (Figure 4) where genomic

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biomarkers are incorporated routinely. As genomic scarring reflects the historic rather than current mutational state, the role of genome wide biomarkers should be predominantly in the analysis of newly diagnosed cancers, to determine first-line treatment approaches. With rapid and low-cost methods, ongoing monitoring of changes in genomic scarring may allow monitoring for reversion of HRD. Challenges remain in the application of genomic signatures of HRD, with prospective studies required to optimally calibrate thresholds that correspond to each test and ultimately their clinical utility.

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Author contributions statement

KN, MW, OK, CLS and IAMcN wrote the manuscript
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Figure Legends

Figure 1. Repair of DNA double strand breaks – homologous recombination and classical non-homologous end-joining
Homologous recombination DNA repair (left) is activated upon detection and binding of DSBs by the MRE11-RAD50-NBS1 (MRN) complex. MRN activates the serine threonine protein kinase ataxia telangiectasia mutated (ATM), which initiates sequential phosphorylation and recruitment of several HR proteins, including BRCA1, to sites of DNA damage. Phosphorylated CtIP is also recruited and activates the exonuclease function of MRE11. MRE11 resects each strand of the DNA to generate short, single-strand DNA (ssDNA) 3'-overhangs, which are further resected to several thousand base-pairs by exonucleases ExoI, DNA2 and BLM helicase. These long ssDNA regions are rapidly coated with replication protein A (RPA) to maintain their single-stranded form and to protect them from exonucleases. RPA is required for the loading of RAD51 onto ssDNA, where partner and localizer of BRCA2 (PALB2) creates a bridge between the C-terminus of BRCA1 and N-terminus of BRCA2. The BRCA2/PALB2/BRCA1 complex then binds phosphorylated RAD51 and targets it to the ssDNA, displacing RPA. RAD51 family proteins RAD52 and RAD54, complexed with ssDNA, form nucleofilaments that facilitate homology searching and invasion of homologous duplex DNA sequences of the sister chromatid. Resected ends are thus annealed to complementary DNA strand sequences, and the missing DNA sequence is synthesized and ligated using the un-broken homologous strand as a template.
Classical non-homologous end-joining (right) is a faster, but more error-prone repair pathway. Upon sensing a DNA DSB, 53BP1 recruits RIF1 to the site of DNA damage. BRCA1 and phosphorylated CtIP prevent the recruitment of RIF1 and subsequent displacement of 53BP1, while 53BP1 and REV7 (also known as MAD2L2) prevent extensive end resection in the absence of BRCA1. REV7 is recruited downstream of 53BP1 and promotes C-NHEJ repair, through pathways that remain unclear. KU70 and KU80 subsequently form a heterodimer that binds to the ends of the DNA strands and recruits serine/threonine kinase DNA-PKcs. DNA-PKcs can then phosphorylate itself and other proteins, signaling the presence of a DSB and activating other C-NHEJ repair proteins. The 5′–3′ nuclease Artemis performs nucleolytic processing of incompatible (complex) DSB ends, which can result in DNA deletions and loss of genetic information. Ligation is carried out by the XRCC4-DNA ligase IV complex and XLF. All of these proteins are critical for C-NHEJ [10,29,63,64,91].

Figure 2. Synthetic lethality.
The phenomenon of synthetic lethality describes the situation where loss of two genes or pathways (in this case A and B) is lethal when loss of either alone is non-lethal.

Figure 3. Tandem duplications and foldback inversions.
The figure shows highly simplified representations of complex genomic rearrangements that are observed in many malignancies. In both cases, there is duplication of a genomic region. In the tandem duplicator phenotype (left), the duplicated regions lie in the same orientation; one cause of this is a restart-bypass mechanism around stalled replication forks in cells lacking BRCA1 function. In foldback inversions (right), a DNA double strand break in G0/G1 is replicated in S phase, creating two duplicated end sequences that are fused together in opposite orientation, possibly as a result of breakage fusion bridge cycles.

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Figure 4. The potential of genomic biomarkers in clinical practice.
Historically, patients diagnosed with HGSC were referred for *BRCA1/2* testing and genetic counselling only if they had a family history of breast or ovarian cancer meaning that *BRCA* mutations were missed in sporadic HGSC cases. Currently, all patients with HGSC should be offered germline *BRCA1/2* testing regardless of family history. However, first line treatment of HGSC is still determined by clinical factors and response to platinum chemotherapy. We suggest that extensive molecular characterization of the cancer should also be performed at the time of diagnosis, with analysis of HR gene mutations and methylation state, as well as broader genomic biomarkers. Low cost tests could be used for this, such as sWGS, CN profiling or LOH analysis. These would inform therapy, especially as maintenance following platinum. Following relapse, tumours could be screened for mechanisms of drug (e.g. PARPi) resistance in a research setting, using more in-depth molecular testing and genomic signature assessment. The results of these tests could then inform subsequent clinical decisions regarding treatment.

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Table 1. Genes alterations reported to cause PARPi sensitivity *in vitro* or *in vivo*.

Gene altered	Evidence from models	Publications
ATM	*in vitro* and *in vivo**	[92]; [93] [48]
ATR	*in vitro* only	[48], [94] [48]
BRCA1	*in vitro* and *in vivo**	[41] [35]; [36] [18]
BRCA2	*in vitro* and *in vivo**	[40] [35]; [36] [18]
CCDC6	*in vitro* only	[95]
CDK12	*in vitro*	[96] [97]
CDK5	*in vitro* and *in vivo**	[95]; [93]
CHK1	*in vitro* only	[48]
CHK2	*in vitro* only	[48]
CTIP	*in vitro* only	[98]
DDB1	*in vitro* only	[94]
DSS1	*in vitro* only	[48]
FANCA	*in vitro* and *in vivo**	[48]; [93]
FANCC	*in vitro* only	[48]
FANCD2	*in vitro* only	[48]
FANCF	*in vitro* and *in vivo**	[48]; [93]
LIG1	*in vitro* only	[94]
MRE11	*in vitro* only	[98]
NBN	*in vivo**	[18]
NBS1	*in vitro* only	[48]
PALB2	*in vitro* and *in vivo**	[98]; [50]; [99] [100]
PCNA	*in vitro* only	[94]
PLK3	*in vitro* only	[95]
PNKP	*in vitro* only	[95]
PTEN	*in vitro* and *in vivo**	[51] [93]
RAD51B	*in vitro* only	[48]
RAD51C	*in vitro* and *in vivo**	[48]; [18]
RAD51D	*in vitro* only	[48]; [101]; [18]

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RAD54	*in vitro* only	[48]
RPA1	*in vitro* only	[48]
STK22C	*in vitro* only	[95]
STK36	*in vitro* only	[95]
USP1	*in vitro* only	[95]
USP11	*in vitro* only	[95]
XAB2	*in vitro* only	[94]
XRCC1	*in vitro* only	[94]

*reported in clinical trials.

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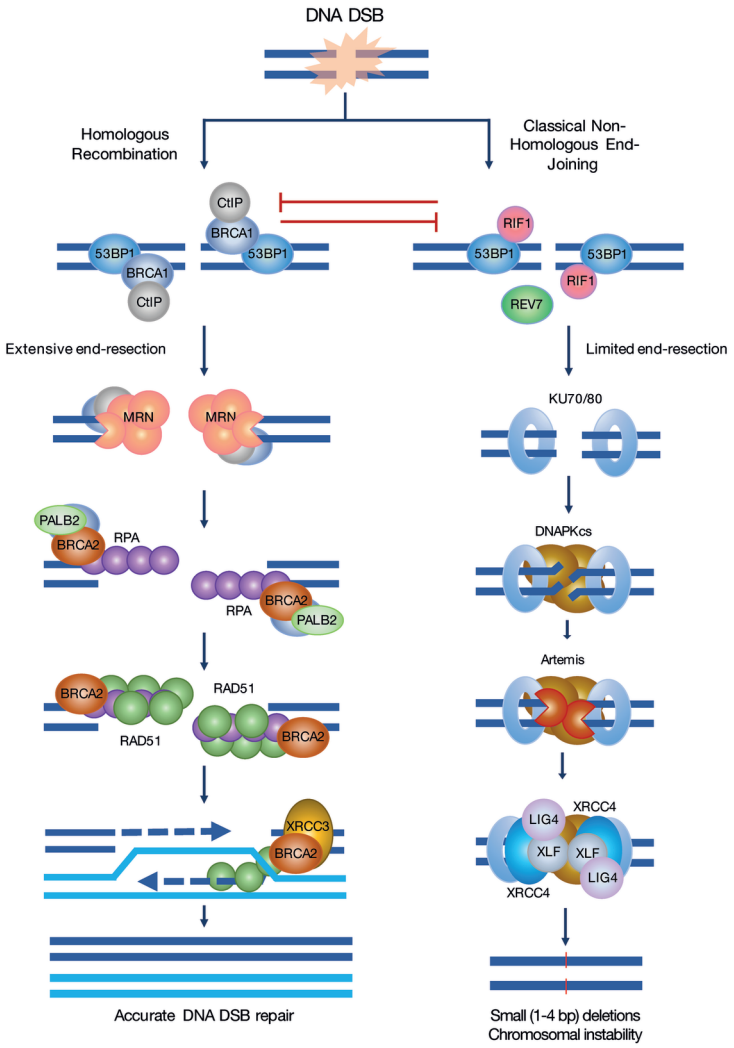

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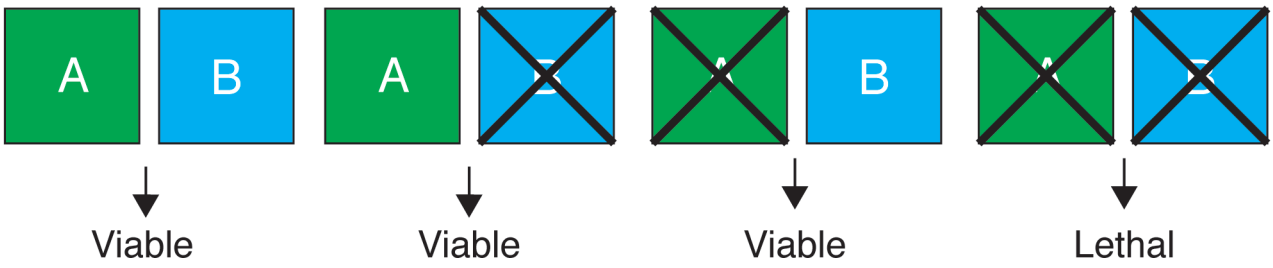

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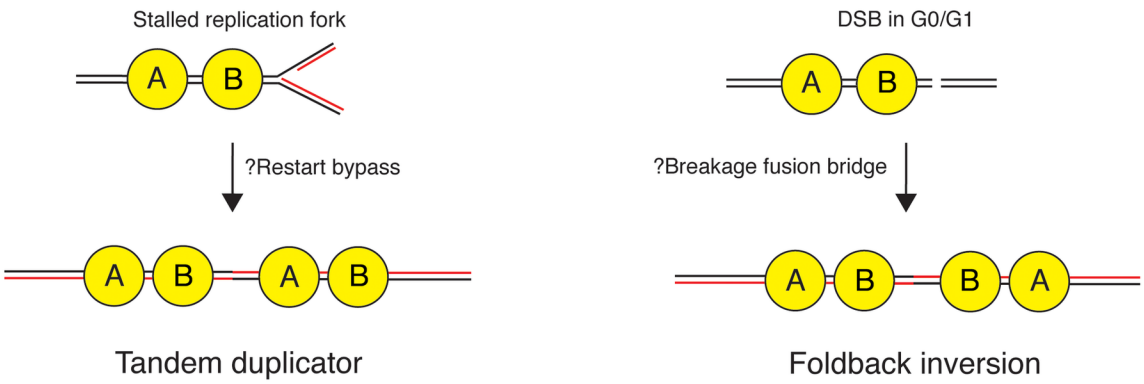

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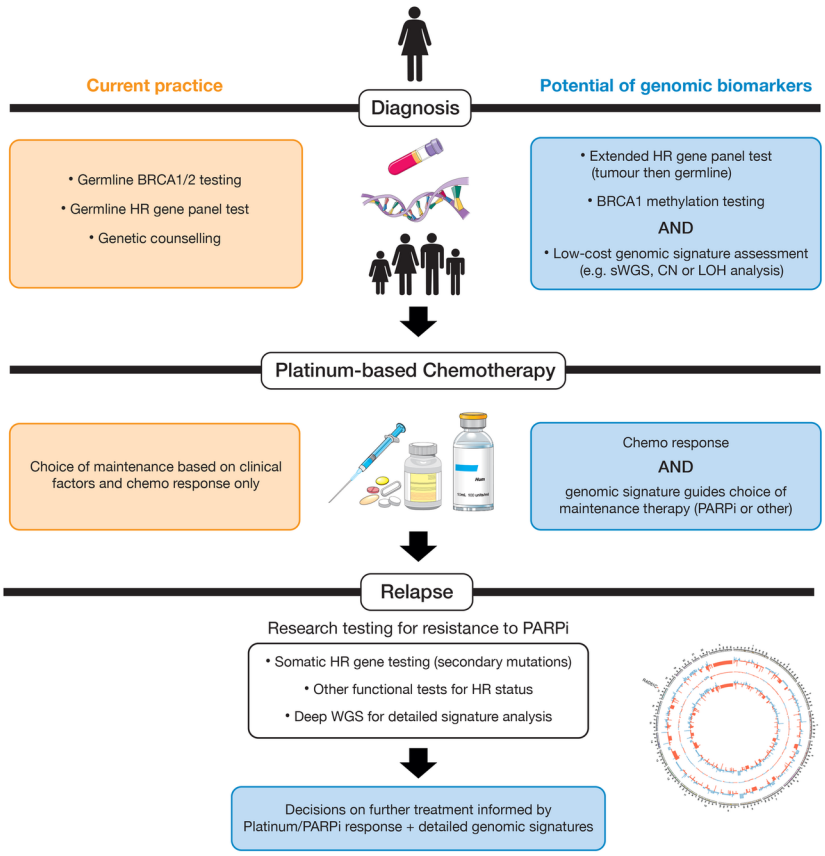

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