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INVITED COMMENTARY

Biomarkers of PARP inhibitor sensitivity

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The PARP inhibitors represent one of the most exciting recent developments in cancer therapy. Substantial efficacy has been shown with PARP inhibitors in the treatment of hereditary *BRCA1/2* related Breast and Ovarian cancer as single agents [1–3] and in combination with temozolomide [4]. Similarly, encouraging activity has been shown in sporadic ovarian cancer with a PARP inhibitor as a single agent [5], and in sporadic triple negative breast cancer in combination with gemcitabine/carboplatin chemotherapy [6]. Yet the picture is not universally positive. Negative studies have been reported in heavily pre-treated sporadic triple negative cancer, with PARP inhibitor as a single agent [5] and no evidence of activity in combination with temozolomide [4]. To understand the reasons some studies have succeeded, and others failed, the development of biomarkers that will predict the sensitivity, or resistance, to PARP inhibitors is required.

There are two conceptually independent ways in which PARP inhibitors are thought to act as anti-cancer agents. First, PARP inhibitors work as single agents targeting homologous recombination (HR) deficient cancers through synthetic lethality. Second, PARP inhibitors also act as chemotherapy or radiotherapy sensitizers in the absence of single agent activity. For example, PARP inhibitors substantially increase the potency of temozolomide in vitro. Whether this translates to an increased therapeutic window in cancers with normal DNA repair in unclear; substantial bone marrow toxicity has been demonstrated with PARP inhibitor and chemotherapy combinations. In reality many PARP inhibitors are being developed assuming a combination of these two strategies, on the assumption that a combination of a PARP inhibitor with chemotherapy may target HR deficient cancers more effectively than PARP inhibitor alone.

Single gene biomarkers for PARP inhibitor sensitivity

In sporadic cancers there are multiple mechanisms through which HR may be lost and PARP inhibitors, at least in vitro, target HR defective cancers independent of the underlying molecular defect [7]. There is in vitro evidence, of varying strength, to support as potentially biomarkers of PARP inhibitor sensitivity, *BRCA1/2* sporadic mutation [8], *BRCA1* promoter methylation [9, 10], *BRCA1* suppression in the absence of methylation [10], *PTEN* deficient cancers [11], *ATM* mutation [12, 13], *MRE11* dominant negative mutations in mismatch repair deficient cancers [14] and *FANCF* promoter methylation [15]. This list can likely be simplified in that each tumour type differs in the potential mechanism of HR deficiency, and therefore each tumour type may ultimately be defined by a panel of biomarkers. However, there is a pressing need to identify unifying biomarkers of HR deficiency that detect the underlying defect in HR that all these diverse mechanisms share. Such a marker of 'BRCAness' [16] may predict the benefit from PARP inhibitors in multiple cancer types, regardless of the underlying molecular mechanism.

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Set against this background Gonçalves et al. report an assessment of *PARP1* mRNA levels in sporadic breast cancer. Reanalysing data from their own previously published whole genome gene expression arrays they show that *PARP1* mRNA expression is highly heterogenous, and related to proliferative rate of the tumour [37]. This association with proliferation has been suggested previously [17], and is a pattern characteristic of multiple DNA repair genes where expression is higher in S phase with active DNA repair required to facilitate DNA replication. This is particularly true in cancer cells as oncogene driven replication is abnormal and prone to the generation of DNA damage [18]. Gonçalves et al. also show that *PARP1* levels are associated with genomic copy number, yet the functional significance of this is unclear. They go on to 'meta-analyse' multiple data sets, and combining such data sets is technically challenging and fraught with pitfalls. A particular difficulty is that and the authors attribute tumour subtype using predictors that are not robust [19], and therefore for this reason alone this data should be treated with caution.

The ultimate question posed by Gonçalves et al. is whether PARP1 levels, assessed either at the mRNA or protein level, predict benefit from PARP inhibitors. Others have previously suggested that high PARP1 levels may predict for the benefit of cytotoxic DNA damaging chemotherapy [20, 21], providing some support for PARP1 levels as a potential biomarker, although this may possibly reflect the association of PARP1 levels with proliferation. So what evidence is there that high PARP1 levels would predict benefit from a PARP inhibitor? It is first important to recall that there is no evidence that PARP1 acts as an oncogene or promotes tumourigenesis, and that biomarkers to predict benefit of DNA repair inhibitors are unlikely to follow the same paradigms as biomarkers of therapies that target oncogenic drivers such as trastuzumab. In vitro sensitivity to PARP inhibitors as single agents is limited to cell lines with HR deficiency. This suggests that PARP1 levels are only likely to be a biomarker for PARP inhibitor sensitivity if HR deficient cancers have higher PARP1 levels, or to put it another way if HR deficiency results in compensatory increase in PARP1 levels and that results in an increase in PARP1 function. Yet at present there is little evidence to support this being the case. PARP1 protein levels do not differ between isogenic pairs of HR deficient and proficient cancers [22], and moreover there is in vitro evidence that PARP1 levels do not correlate with PARP1 activity [23]. In contrast, PARP activity is substantially increased in HR deficient cancers; In vitro PARP1 is hyperactive in HR deficient cell lines [22]. So rather than total PARP1 levels, a functional assay such as assessment of PAR [poly(ADP)ribose] polymer levels [24] may potentially assay HR function and possibly be a biomarker of BRCAness.

Biomarkers of BRCAness

Where are we with other potential markers of BRCAness? Potential signatures of BRCAness have been reported based on the differences in gene expression between hereditary *BRCA1/2* related cancers and sporadic cancers [25, 26]. These gene expression signatures may enrich for a group of 'hereditary-like' sporadic cancers, and these cancers are more responsive to DNA damaging chemotherapy [25, 26]. At present these studies are interesting proofs of principle, but validation of these signatures is insufficient for use in the clinic. Perhaps the most promising potential biomarker of BRCAness would be if the genomic 'scar' of HR deficiency could be identified. In a way analogous to microsatellite instability in mismatch repair deficient cancers, there is likely to be a genomic mutational pattern that predicts for underlying HR deficiency. In part this may be a characteristic degree of gross genomic instability, but there is also some indication that certain complex mutations may be a marker of HR deficiency. For example, *BRCA1* mutant breast cancers frequently have characteristic mutations in *TP53* that are infrequent in sporadic cancer [27]. The presence of these complex mutations in sporadic triple negative breast cancers may predict benefit to neoadjuvant cisplatin [28], providing some support for their use as a biomarkers of HR deficiency, although further validation study is required.

PARP inhibitors in combination or as single agents?

The PARP1 inhibitors have been proposed to target HR deficient cells through inhibiting single strand break repair, perhaps trapping PARP1 on the break inhibiting further repair [29]. Ultimately this may cause the collapse of replication forks leading to a DNA double strand breaks that are only effectively repaired by HR [8]. Yet recent data has added layers of complexity to the story. PARP1 itself may be required for the repair of stalled replications forks [30], but not for replication forks that have collapsed to generate a double strand break. Whether the involvement of PARP1 in stalled fork repair is significant to our understanding of the potency of PARP inhibitors is unclear, but this data does emphasise that the repair of stalled replication forks is complex, and that PARP inhibition may force repair down a particular pathway.

A major issue in biomarker development for PARP inhibitors is to try and dissect the issue of whether PARP inhibitors should be developed as single agents or in combination with chemotherapy. At present it is generally assumed that same biomarkers, of homologous recombination deficiency, will predict for the benefit of PARP inhibitors as single agents, as well as PARP inhibitors in

combination with chemotherapy. However, it is probable that different molecular defects in HR may predict sensitivity to PARP inhibitors alone, whereas other defects for benefit to PARP inhibitors in combination with chemotherapy. For example, cancers with a 'hard' defect in HR, such as *BRCA1/2* mutations, may be responsive to PARP inhibitors as single agents, whereas potentially cancers with a 'soft' or mild defect in HR may not show sensitivity to single agent PARP inhibition, but could show substantial benefit when the cell is stressed by the combination of PARP inhibition and more complex damage created by chemotherapy.

Biomarkers for resistance to therapy

Mechanisms of resistance to PARP inhibitor therapy are beginning to emerge. In hereditary breast cancers reversion mutation of *BRCA1/2* gene, either directly to wild type [31] or by intragenic deletion [32], restoring normal BRCA1/2 protein function is highly likely to be a major mechanism of resistance, with the mutation selected by prior platinum therapy [33]. Further potential resistance mechanisms identified include 53BP1 loss as a mechanism of resistance in BRCA1 deficiency [34, 35], that partly rescues the deficiency in HR seen BRCA1 deficient cells. Overexpression of RAD51 has also been suggested to partially rescue HR deficiency [36], but it is uncertain if this is clinically relevant. It is also important to recall that many of the PARP inhibitors in current clinical development are P-glycoprotein substrates.

Finally, the issue of tumour heterogeneity, and the influence of prior therapy, on a biomarker is a likely to be a major confounding factor in predicting response. PARP inhibitors share potential resistance mechanisms with conventional chemotherapy, and this emphasises the importance of not relying fully on biomarkers present in the primary tissue, or germline, when treating the patients in the metastatic setting. Therefore, to maximise clinical potential it will be important to assess PARP inhibitors and companion biomarkers either in previously untreated cancers, such as the neoadjuvant setting, or with metastatic disease biopsies immediately prior to treatment.

Conflict of interest Professor Ashworth may benefit financially from the development of PARP inhibitors through patents held jointly with AstraZeneca through the Institute of Cancer Research 'rewards to inventors' scheme.

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