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Inhibited, trapped or adducted: the optimal selective synthetic lethal mix for BRCAness

BRCA1 and BRCA2 proteins have important roles in DNA replication fork stabilisation and a specialised form of DNA repair termed homologous recombination (HR) [1]. They are components of the Fanconi anaemia protein network [2, 3]. A hallmark of deficiency in this network is sensitivity to DNA crosslinks induced by platinum agents and mitomycin C [4, 5]. Historically platinum chemotherapy has only shown modest activity in advanced breast cancer excepting those with chemotherapy naïve disease [6, 7] but recent uncontrolled studies have suggested significant activity for single agent platinum agents in *BRCA1* and *BRCA2* germline mutation carriers [8]. The TNT phase III trial has recently reported positive interaction between the presence of germline mutation but not epigenetic change in these genes and specific treatment effect; with a doubling of response to carboplatin but no effect of mutation on standard of care taxane response in advanced breast cancer [9].

The re-positioning of poly(ADP-ribose) polymerase (PARP) inhibitors, originally developed to enhance the therapeutic effects of DNA damage inducing cancer therapy, as a single agent therapy for BRCA1 or BRCA2 deficient cancers has become a poster child for the therapeutic exploitation of the concept of synthetic lethality [9].

PARPs are a family of enzymes the members of which induce the NAD^+-dependent polymerisation of poly(ADP-ribose) (PAR). PARP1 is thought to be the most relevant family member for therapeutic targeting although current PARP inhibitors target both PARP1 and PARP2.

PARP1 function is required during the repair of lesions in one strand of the DNA template that generate single-strand breaks (SSBs). Upon the generation of an SSB, PARP1 binds to the break and uses NAD^+ to generate PAR polymers upon itself (auto-PARylation) and on chromatin-associated proteins relaxing chromatin and recruiting DNA damage response (DDR) proteins and repair effectors [10–14]. Cumulative auto-PARylation causes the dissociation of PARP1 from DNA. PARP inhibitors block NAD+ binding and PARylation for the duration of the inhibitor's engagement of the NAD+ site. Prolonged occupancy can prevent PARP dissociation from the SSB [15]. This results in both accumulation of unrepaired SSBs and the trapping of PARP1 protein on the chromatin [16]. Repairing the double-strand break that follows arrival at the scene of DNA replication fork, and trapped PARP requires cells to have DDR sensing and signalling proteins, BRCA1 and BRCA2 associated HR repair and DNA replication bypass pathways active for cell survival. PARP1 itself is also directly involved in the repair of 'collapsed forks' and in mechanisms of restart of stalled forks.

Current PARP-targeting agents act both as inhibitors of the catalytic activity of PARP1, effecting the formation of PAR at sites of DNA damage, but can also trap PARP1 onto DNA at sites of the PARP1 DNA interaction. While all PARP inhibitors currently in clinical development significantly inhibit catalytic activity, there is considerable variability between compounds in their PARP trapping effects given equimolar drug exposure [17]. This

is likely explained by variable physico-chemical properties and effects on target binding dynamics [18]. Talazoparib is the most potent of the class at trapping PARP1 with niriparib, olaparib and rucaparib having significant effect but veliparib very little trapping despite catalytic inhibition. It was originally thought that loss of PARP1 and its catalytic function was the main driver of synthetic lethal effect with BRCA1 and BRCA2 mutation. However, preclinical data indicating the need for the presence of PARP1 protein suggest that it is the trapping of PARP as well as its catalytic inhibition that drives therapeutic effect [16]. A number of trials have now reported the maximum tolerated doses and levels of activity of single agent PARP inhibitors with variable trapping effects [15]. Although cross study comparisons must be made with caution, as patient characteristics are variable, two relevant observations emerge: there appears to be a relationship between maximum tolerated dose and trapping potency, and it would seem that single agent response to potent trapping compounds in *BRCA1* and *BRCA2* mutation carriers has been higher [19, 20] than for the less potent trapping agent veliparib [18, 21].

In this issue, Han and colleagues report the BROCADE randomised phase II trial that examines the efficacy of veliparib in combination with temozolomide- or platinum-based chemotherapy in *BRCA1* or *BRCA2* germline mutation associated advanced breast cancer [22]. As well as PARP1's role in the repair of endogenous DNA damage, its activity is also required for the repair of chemotherapy-induced DNA lesions. Synergy with PARP inhibitors is most marked with topoisomerase 1 inhibitors and temozolomide. While synergy with the former relies only on inhibition of the catalytic activity of PARP1, synergy with temozolomide is significantly dependent upon trapping of PARP at DNA SSBs. While PARP inhibitors and platinum agents combine to increase cellular toxicity neither PARP1 catalytic activity nor trapping modulate, the cellular response to platinum DNA adducts and any effects are likely to be due to the independent effects of both agents on DNA [23].

Although a generalisation, it has been challenging to combine PARP inhibitors with chemotherapy that causes lesions in DNA for anything other than brief periods of concomitant exposure. Veliparib has provided the greatest quantity of study data in this regard perhaps reflecting a great ability to find tolerable combinations with chemotherapy.

In the BROCADE, phase II study patients were treated with veliparib for the first 7 days of the treatment cycle combined with either paclitaxel and carboplatin on day 1 or temozolomide from days 1 to 5. The dose of veliparib was substantially lower in the temozolomide arm (40 mg versus 120 mg BID) reflecting the recommended phase II doses of the combinations of these agents and the presence of mechanistic synergy between veliparib and temozolomide described above.

The combination of paclitaxel, veliparib and carboplatin has been tested in early sporadic breast cancer in the I-SPY 2 programme. This regimen used a lower 50 mg BID dose of veliparib given continuously concomitant with chemotherapy and was found to be both tolerable and to cause high levels of pathological complete response in comparison to paclitaxel alone in the 'triple negative' breast cancer subgroup [24]. An important question arose as to whether the increase in activity could have been achieved by carboplatin alone or was dependent upon the additional effect of PARP inhibition by veliparib. The BrightTNess study, recently reported at ASCO [25], attempted to address this question using the same regimen in an early 'triple negative' breast cancer population analogous to I-SPY2. The BROCADE study reported here addresses a similar question: whether veliparib adds to the efficacy of a carboplatin paclitaxel combination in the context of *BRCA1* or *BRCA2* germline mutation associated advanced breast cancer. The study also tests the additional hypothesis that the mechanistically synergistic combination of temozolomide and veliparib would show greater activity than the combination of carboplatin DNA adducts and paclitaxel.

So, what have we learnt from BROCADE? Firstly, when we look at the comparator arm we see confirmation of very high activity for a carboplatin-containing regimen in a population approximately evenly split between *BRCA1* and *BRCA2* mutation carriers. This is evidenced by an objective response rate of 61% with durable progression-free survival (PFS; median 12 months). The TNT trial has reported an objective response rate of 68% for single agent carboplatin in the same population in contrast to more modest activity for the licensed dose of docetaxel [9] suggesting the BROCADE response rate is not a consequence of unusually high paclitaxel response in BRCA mutation carriers but rather that this is an effect of DNA platinum adducts on an HR repair deficient tumour. Secondly, veliparib has a modest but statistically significant effect on activity increasing objective response rates from 61% to 78% but insufficient to significantly improve PFS over that of the carboplatin and paclitaxel regimen alone. The authors highlight the short duration of veliparib exposure in each cycle and protocol prohibition of the continuation of single agent veliparib in the face of withdrawal of the chemotherapy combination due to toxicity. This is not the case in the follow-on phase III trial BROCADE3. Thirdly, this modest increase in activity does not appear to come at the price of any significant increase in normal tissue toxicity. Finally, we see that the mechanistically synergistic combination of temozolomide and veliparib has significantly less activity than paclitaxel and carboplatin. The latter suggesting that veliparib, despite catalytic inhibition of repair at temozolamide-induced SSB sites sufficient to create cytotoxicity and define a recommended maximum phase II dose, has less selective synthetic lethal effect in BRCA1 or BRCA2 deficient breast cancer cells than that of a carboplatin regimen. This supports the notion that to exploit an HR deficiency to maximum effect, it is the trapping of PARP at DNA breaks or the creation of adducts on DNA caused by platinum salts that generate the toxic lesions that are required. That said, veliparib despite lack of synergy with platinum and use of a brief low-dose schedule, does somewhat add to the effect of platinum while appearing more tolerable than the combinations of potent PARP1 trapping inhibitors with platinum reported to date [26]. We have also to infer that veliparib given intermittently, even when enhanced by temozolomide-induced SSBs, appears to have substantially less efficacy than single agent PARP inhibitors with potent PARP1 trapping activity used in a similar metastatic treatment contexts [27].

Although the context, design and veliparib dosing differ in the BrightTNess TNBC neo-adjuvant trial [24], this study supports the BROCADE result in several ways. BrightTNess, like I-SPY2 showed an increase in activity of paclitaxel chemotherapy, assessed by the primary objective of effect on pathological complete response, with the addition of veliparib and carboplatin.

Importantly the design and result support the conclusion that this effect was driven by carboplatin with little additional effect of veliparib, even in the small *BRCA1* and *BRCA2* mutation carrier sub-population recruited.

What can we take home from this study? BROCADE provides further evidence, supporting the report of the TNT trial that platinum containing regimens are highly active and have important effects on survival end points in BRCA mutation carriers, in a first or second line relapse setting. A combination of carboplatin and paclitaxel with veliparib, an oral PARP inhibitor with PARP binding characteristics that causes catalytic inhibitory effect but low PARP trapping, is highly tolerable and shows a signal of additive efficacy insufficient to significantly change PFS. The authors posit that this signal may be converted to a clinically meaningful effect on a PFS end point through longer exposure to higher dose single agent veliparib after cessation of chemotherapy. We must wait for BROCADE3 (NCT02163694) to report to know if this is indeed the case. Meanwhile the potent PARP inhibitor olaparib has been shown to be superior as a single agent targeted therapy compared with physicians choice of non-platinum post-taxane standard of care chemotherapies in the phase III OlympiaD study in the same population with marketing approval being sought in this indication [27]. In OlympiaD objective response with a non-chemotherapy oral regimen was similar to that shown with carboplatin in TNT and to three-weekly paclitaxel and carboplatin in BROCADE. The PFS achieved using the two paclitaxel carboplatin-based regimens used in BROCADE compare favourably with those in the OlympiaD study. Cross-study comparisons should again be interpreted with caution as the OlympiaD trial accrued a more heavily pre-treated population approximately 30% of whom had received prior platinum-based therapy.

Beyond the primary hypotheses around effect of the two mechanistically distinct PARP inhibitors they test, BROCADE and OlympiaD leave a number of unanswered questions around the use of platinums and PARP inhibitors in advanced *BRCA1* and *BRCA2* mutation associated breast cancer. The TNT trial, conducted in advanced TNBC or *BRCA1*/*BRCA2* mutation associated breast cancer prospectively tested the interaction between genetic or epigenetic BRCAness and platinum versus taxane treatment effect in first-line therapy in TNBC. The final result has been presented [28] and awaits publication but the abstract presentation suggests *BRCA1* and *BRCA2* genetic testing should be widely adopted as a patient selection biomarker with platinum being the standard of care chemotherapy in the setting of mutation. If olaparib is approved which should we use first, a platinum regimen or a potent PARP inhibitor capable of trapping PARP1? What remains unknown is how the efficacy of single agent potent trapping PARP inhibitors compares with a platinum regimen and in which sequence they should best be used. OlympiaD indicates activity in a population 30% of whom had received but not progressed on a prior platinum regimen. The non-randomised ABRAZO study recently reported the activity of talazoparib in platinum pre-treated disease and a positive relationship between time since platinum exposure and level of activity [19]. Mechanisms of resistance to PARP inhibitors are increasingly understood and seem to only partially overlap with those of platinum resistance [15]. The activity of platinums after PARP inhibitor failure has not been significantly studied. As yet we do not have the answer to which agent to use in what sequence, but it will become increasingly important to understand the distinct and overlapping biology of PARP inhibitor and platinum resistance in this population of patients we now more frequently identify in our oncology clinics.

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Novel treatment options for refractory Germ Cell Tumours: back to the bench!

Multi-disciplinary management of germ-cell tumours (GCT) is highly effective and associated with excellent outcomes. However, treatment of refractory GCT remains a therapeutic challenge with very limited treatment options [1]. In patients who are refractory to or recur after standard dose first-line therapy and subsequent therapies including high-dose chemotherapy, only a few systemic treatments have demonstrated activity and the prognosis of refractory patients is extremely poor with cure rates of <5% [2]. Hence, the exploration of novel treatment options for these patients remains one of the remaining priorities in management of GCT.

Results for novel, targeted agents including vascular endothelial growth factor-tyrosine kinase inhibitors (TKI), epidermal growth factor receptor-TKIs, mammalian target of rapamycin (mTOR) inhibitors, and c-Kit inhibitors have been generally disappointing [3, 4]. The vast majority of these studies were based on a limited preclinical rationale, a limited understanding of critical molecular mechanisms and were not biomarker driven. Without strong underlying rationale, it is not surprising to find that responses to targeted agents are exceedingly rare and long-term survival virtually non-existent.

Cancer immunotherapy with checkpoint inhibitors is the new hope in oncology and is expected to significantly improve outcomes with promising results having been reported in a variety of tumour types. However, despite all the euphoria about immunotherapy, only a small number of patients benefit from immunotherapy long-term due to significant inter-tumour immune-response heterogeneity.

In this issue of *Annals of Oncology*, Adra et al. [5] report the results of the first prospective phase II study of a programmed cell death-1 receptor (PD-1) inhibitor in refractory GCT. Of note, this study included an unselected, not biomarker selected group of refractory patients. On archival tissue, only two patients were found to have programmed death ligand-1 (PD-L1)-positive tumours based on PD-L1 expression on tumour and infiltrating immune cells. Tumour tissue for PD-L1 examination was obtained from various locations and time points during treatment of patients in this study. The study was closed after the first stage of 12 accrued non-seminoma patients due to the lack of objective responses. Two patients were deemed to have short lived disease stabilisation although AFP continued to increase in both patients during the interval of radiological stability.

So, why have targeted agents and immunotherapy failed in refractory GCT to date? Are targeted agents and immunotherapy ineffective in these patients? Or are we just not selecting the appropriate patients based on a sound and firm rationale?

The very vast majority of the studies on targeted therapies in GCT as well as the current study testing PD-1 inhibition were based on little preclinical and molecular data. The rationale for the current study was derived from some smaller studies describing various degrees of PD-L1 expression in GCT as well as some case series reporting a few short-lived responses [6, 7]. Similar to other tumour types, the predictive role of PD-L1 expression, by either tumour cells or immune cells in the microenvironment, on checkpoint inhibitor treatment outcomes in patients with GCT is entirely unclear. In addition, it has been shown that PD-L1 expression can be transient, may change over time and under the pressure of various treatments, and intra-patient and even intra-tumour heterogeneity in PD-L1 tumour expression can exist [8]. Therefore, tumour sampling at one time point or at only one tumour site or a portion of one tumour might not accurately reflect the state of the PD-L1 expression in a patient. All studies examining PD-L1 expression in GCT as well as the current study used archival tissue rather than fresh tumour biopsies begging the question whether these results truly reflect the PD-L1 status of refractory patients. Moreover, PD-L1 is constitutively expressed in spermatocytes and spermatids in seminiferous tubules of the testis making the assessment of PD-L1 expression in orchiectomy specimens even more complicated [9].

In addition, tumours constitute a dynamic milieu and integrate numerous reinforcing and antagonistic signals from both local and systemic conditions. Tumour immune response appears to be mediated by a complex combination of factors including not only PD-1/PD-L1 expression but also the mutational