Article type: Editorial

Title**: PARP inhibitors for Advanced Prostate Cancer: Validating predictive biomarkers.**

Authors: Joaquin Mateo[1], Suzanne Carreira[2], Johann S. de Bono[2]

Affilitations:

1. Vall d´Hebron Institute of Oncology (VHIO) and Vall d'Hebron University Hospital, Barcelona, Spain.
2. The Institute of Cancer Research and The Royal Marsden NHS Foundation Trust, London, United Kingdom

Prostate cancer (PC) is a highly heterogeneous disease. Many PC have DNA repair gene aberrations in germline DNA or, more commonly, tumour DNA usually as early, truncal, events in prostate carcinogenesis. In advanced PC, BRCA2 is most commonly aberrant; other reported defects involve ATM and CDK12. Unlike BRCA2, loss of ATM or CDK12 does not generate typical genomic signatures of homologous recombination DNA repair (HRD) defects, although other HRD genes are deleteriously aberrant in PC including BRCA1, PALB2, RAD51 and FANCA. Preclinical studies have shown that total loss of function (bi-allelic loss) of one of these genes sensitizes to poly(ADP)-ribose polymerase inhibition (PARPi). The degree of sensitization differs from one gene to another with loss of BRCA2 being most sensitizing. Multiple PARP inhibitors are being evaluated in clinical trials for prostate cancer including olaparib, niraparib, talazoparib and rucaparib. An analytically valid and clinically qualified predictive biomarker identifying tumours sensitive to these agents is urgently needed, as some tumours are sensitive and have evidence of HRD loss through genomic scar detection (high HRD score) without identifiable gene aberrations.

In this issue of European Urology, Marshall et al report on 23 men with mCRPC with BRCA2 or ATM alterations treated with off-label olaparib 300mg twice-daily, although overall 46 patients received off-label olaparib; 13/17 with germline or somatic BRCA defects had a 50% PSA decline following olaparib with a median PFS of >1 year while none of 6 men with ATM alterations had a >50% PSA fall. These BRCA1/2 data are in keeping with published data and supports the use of PARP inhibitors for this subgroup. The olaparib dose

utilised in this retrospective analysis was 300mg BID, the approved dose for ovarian cancer which is lower than the 400mg BID dose used in the TOPARP-A trial. While olaparib antitumour activity is highly dose dependent (in ovarian cancer with BRCA1/2 aberrations the response rate to maximum tolerated dose of 400 mg BID was 33% but only 13% at the minimum biologically active dose of 100 mg BID), these data indicate that 300mg BID dose may be sufficient to impact outcome from this disease although it is impossible to determine if their durations of benefit differ. Dose may be more critical for less sensitizing aberrations like ATM; the lack of biochemical responses in these 6 patients with ATM alterations differs from the data we reported in TOPARP-A with olaparib 400 mg BID where ATM deleterious aberrations were detected in five different tumours, with a 6th patient having an ATM mutation in plasma cfDNA not detectable in biopsies. Overall, 2/6 (33%) had a 50% PSA response, 2 a circulating tumor cell (CTC) count fall but no PSA fall, and 2 no discernible benefit in TOPARP-A. A phase II trial of rucaparib has also recently reported no confirmed responses in 18 men classified as having ATM altered tumours, although several had significant PSA declines. Overall, however, no RECIST responses were reported in any of these three series of ATM altered cancers, although most had bone-only disease.

ATM is a large gene on chromosome 11 encoding for ATM serine/threonine kinase, a 350 kDa protein of 3056 amino acids found in its inactive form as a homodimer. ATM activation occurs primarily after the recognition of double-strand break (DSB) by the MRE11-RAD50-Nbs1 (MRN) complex; ATM activation after DSB sensing involves Serine-1981 auto-phosphorylation and

dissociation into active monomers with ATM-dependant stabilization of p53 regulating pro/anti-apoptotic signalling responses to DNA damage. ATM protein loss impacts DNA damage response quite differently to dysfunctional ATM protein as a result of a truncating mutation encoding a kinase-dead protein; mouse models ATM loss are viable, but mouse embryos expressing kinase-dead expressed ATM are not. ATM loss of function induces PARP inhibition sensitivity in multiple preclinical models including chronic lymphocytic leukaemia, lung and breast models, although with most of these having dual ATM and p53 loss. ATM loss can also result in sensitivity to platinum-based chemotherapy, but associates with a different mutational signature to BRCA2/HRD tumours, reflecting differing biological functions. Unlike BRCA2, the genomic mutation landscape of the large ATM gene remains poorly mapped and interpreting the genomic assays being used to report these alterations remains challenging. Interestingly in the reported series by Marshall et al 1 of 6 ATM alterations, compared to 12 of 17 BRCA aberrations, was of germline origin. Patient selection for off label olaparib 300 mg BID utilised germline or somatic DNA sequencing data, different sequencing platforms including either panel testing or exome sequencing, and either plasma cfDNA or tumor biopsy DNA. Moreover, mutation allele frequency and loss-of-heterozygosity status, which supports characterisation of mono- from bi-allelic loss is not reported. Assay performance may therefore have impacted these data; it remains critically important to identify loss of ATM function, that is absence of functional protein, and the optimal way to do this may involve immunohistochemistry assays complementing genomics data.

In conclusion, how do these data impact the field? They clearly confirm that olaparib, even at the lower dose of 300mg BID, has impressive antitumour activity against BRCA2 loss prostate cancer. The jury, however, is still be out on ATM loss. Future trials studying ATM loss, and indeed other key DNA repair genes, need to analyse this separately to BRCA2 with better validated and preferably multiple orthogonal assays such as next generation sequencing (of both germline and tumour DNA) and immunohistochemistry. Overall, however, it is clear that loss of ATM is a major player in prostate carcinogenesis. Studies are now warranted into the biology of these ATM aberrations in prostate cancer, and how biological and genomic contexts alter their impact on sensitivity to DNA repair inhibitors and platinum chemotherapy.