Plasma DNA and Metastatic Castration-Resistant Prostate Cancer – the odyssey to a clinical biomarker test

Jayaram A[1], Wetterskog D[1], Attard G[1]

SUMMARY: Comprehensive plasma DNA analyses identifies clinically actionable genomic aberrations.

1. UCL Cancer Institute, Paul O'Gorman Building London UK

Conflicts of Interest

GA is on the Institute of Cancer Research list of rewards to inventors for abiraterone. GA has received honoraria, consulting fees, or travel support from Astellas, Medivation, Janssen, Millennium Pharmaceuticals, Ipsen, Ventana, ESSA Pharmaceuticals, and Sanofi-Aventis and grant support from Janssen, AstraZeneca, and Arno. Other authors have no conflict of interest disclosure

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Corresponding Author:

Prof Gerhardt Attard MD FRCP PhD
John Black Charitable Foundation Endowed Chair for Urologic Research | T: 00 44 20 7679 0891 Research Department of Oncology | UCL Cancer Institute | 1st floor, Room 134 | Paul O'Gorman Building |
72 Huntley Street | London | UK I WC1E 6DD |
E: g.attard@ucl.ac.uk

Genotyping of cell-free DNA (cfDNA) extracted from plasma allows minimally invasive real-time testing for tumor somatic genomic alterations. This approach is now increasingly recognized as clinically valid and in 2016 the Cobas test (Roche Molecular Systems, Inc.), detecting genomic aberrations in the epidermal growth factor receptor *(EGFR*) gene to identify patients with metastatic non-small cell lung cancer for treatment with erlotinib, became the first liquid biopsy assay to receive U. S. Food and Drug Administration approval for treatment selection (http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm504540.htm). Currently, a key treatment decision point in metastatic prostate cancer is the selection of patients progressing after primary castration therapy, when the standard of care is potent AR targeting with abiraterone or enzalutamide, who would derive greater benefit from alternative or potentially combination treatments. Clinico-pathological variables sometimes used to assist this decision are primarily prognostic and have not been shown to have predictive utility.

In this issue of Cancer Discovery, Annala and colleagues highlight this unmet need in their presentation of the first clinical trial to prospectively randomize patients between abiraterone or enzalutamide[1]. In keeping with previous studies, 14% (29/202) of patients had primary resistance (based on no prostate specific antigen [PSA] decline at 12 weeks) and 57% (116/202) had progressed within 12 months. There was no significant difference between either treatment in unselected patients. Given the multiple limitations with studying archival tumor tissue and the challenges in obtaining fresh tumor biopsies in this population, the authors collected plasma for exploratory biomarker analyses. They subjected plasma DNA to targeted capture sequencing of 72 metastatic castration-resistant prostate cancer (mCRPC) genes and for 65 cases with matched cfDNA and germline pairs, whole exome sequencing. Given the relatively low tumor load in this population, the main challenge for biomarker discovery is low circulating tumor DNA (ctDNA) fraction. A low ctDNA fraction limits the detection of less abundant sub-clonal aberrations and accurate estimation of copy number changes, especially mono-allelic deletions. Using the detected presence of somatic mutations for estimation, the authors identified approximately half the population as having a ctDNA fraction greater than 2% (115/201 patients). Patients with a ctDNA fraction 2-30% had a worse outcome than <2% and >30% had the worst. The authors limited genomic analysis to patients with detected ctDNA <2%. This has important implications in interpreting the results as the selection of a worse prognosis cohort could result in differences compared to studies that have tested archival tissue or germline DNA and been more inclusive. The authors identify changes commonly described in tumor biopsies of mCRPC but of note that tumor

biopsy studies are also biased by the feasibility of tumor to biopsy and often enriched for more heavily treated patients or disease presenting with the more aggressive features of soft tissue or visceral metastases.

Using cox regression univariate analysis for a series of specific pre-determined gene aberrations or pathways, the authors did not identify associations with neither *SPOP* mutations nor WNT pathway defects. This is in contrast to a recent study on 75 bone and soft tissue biopsies from patients treated with abiraterone that found enrichment for aberrations in the Wnt/β-catenin pathway in non-responders[2]. Annala et al identified significant hazard ratios for *TP53* alterations, *BRCA2/ATM* mutations, *AR* gain, *RB* defects and *PI3K* pathway defects. They then proceeded to perform multivariate analysis including clinical prognostic factors, ctDNA fraction and each individual genomic aberration in turn as a covariate and report significant associations between shorter time to progression and deleterious alterations in *TP53* and mutations in either of the homologous recombinant repair (HRR) genes *BRCA2* or *ATM*. Loss of *TP53* occurring concurrently with loss of *RB* was recently shown in preclinical models to promote differentiation to a neuroendocrine phenotype that associates with resistance to AR targeting[3]. The TP53 aberrant population included here could therefore be enriched for this sub-type of AR indifferent cancers. Due to the limitations of detecting genomic losses at ctDNA fractions <30%, comprehensive quantitation of *Rb* loss in this cohort was not reliable. Integration with distinct methylation changes recently shown to associate with a neuroendocrine phenotype could further improve on genomic-based detection of this sub-type[3]. Men with localized prostate cancer harboring a germline *BRCA2* variant have a shorter time to development of metastases but several retrospective studies have recently suggested no worse outcome with AR targeting therapies or taxanes in mCRPC with aberrations in DNA damage repair genes [4,5]. The PRO-REPAIR prospective observational study has also not identified worse outcomes for mCRPC patients on first-line targeted AR therapy harboring *BRCA* or *ATM* germline variants[6]. These seemingly discordant reports highlight the challenges that underlie potential differences in outcome for distinct aberrations within the same gene or different genes within the same class, germline carriers that do not suffer a second hit, technologies that have differential sensitivities and analysis in a worse prognosis group selected by virtue of being amenable to tumor biopsy or circulating DNA tumor fraction. Moreover, given the prevalence of HRR defects is <20%, the numbers of affected patients in these cohorts are relatively small and this could also account for these variances. Importantly, detection of patients with HRR gene defects in plasma, potentially in combination with germline analysis, introduces the opportunity to target treatment, for

example with PARP inhibitors, especially in this patient population with low volume metastases where tissue access is challenging. The study also detected 2 patients with mismatch repair defects who could be selected for immune checkpoint blockade. Finally, the detection of deletions could be supported by using allele specific information at germline heterozygous SNPs within the region of interest as has been performed for other areas of truncal gene loss[7].

The study confirms the lack of clinical utility of *AR* ligand binding mutations in this setting where L702H and T878A are rarely detected at allelic frequencies above 2%[8]. The clinical relevance of detecting mutations at lower allelic frequencies is unclear. *AR* copy number gain has previously been associated with worse outcome independently of clinical variables on multivariate analysis[8]. Differences in cut-offs for defining amplification, such as *AR* gain, could lead to studies including patients with different outcomes. *AR* on chromosome X is present as one copy in normal cells but recent studies have suggested that two *AR* copies in plasma most accurately splits the population into two prognostically-defined groups[8]. This would also account for differences in reported prevalence: 15% previously and approximately double in the current study. The authors implement an elegant approach to correct *AR* copy number for ctDNA fraction. The interpretation of this adjustment is however limited due to not all the cells within the tumor compartment harboring a uniform *AR* copy number as has been previously shown in circulating tumor cells and tissue (Fig 1). This challenge could be in-part circumvented by tracking specific *AR* genomic structural rearrangements (*AR*-GSRs). These have been associated with being enriched in cancers harboring *AR* amplification and/or disruptive events leading to loss of transcription of the ligand-binding domain[1]. Detection of AR splice variants particularly *AR*-V7 has been correlated with poor responses to abiraterone or enzalutamide [9]. Annala et al's exploratory analysis of *AR*-GSRs in plasma from a limited number of patients suggests they associate with primary resistance and could be used to comprehensively identify AR aberrant patients with genomic perturbations and a range of genomically-driven splicing variants. This alone may however be insufficient with recent studies also suggesting that nuclear protein expression more strongly associates with worse outcome than solely detection of cellular splice variants[10]. This could require composite tests that incorporate a variety of blood-based assays.

Overall, interpretation of plasma DNA analysis addressing predictive questions needs to be recognized in the context of detection of an aberration being more likely with higher tumor fractions that in themselves are prognostic. Moreover, we need to increasingly utilize contemporary mathematical tools to evaluate

associations with outcome from cutting-edge molecular data, for example modern Bayesian approaches that maintain effectiveness with 1000s of variables. Finally, the discoveries in this study are but the start of the long road to implementation of a biomarker test for mCRPC. Putative biomarkers will need to be confirmed in additional cohorts' representative of the target population and then fixed in an analytically validated assay prior to clinical qualification in prospective trials where the biomarker test is used for treatment decisions. Same as for Odysseus whose homecoming was successful but not as expected, the treatment decision point for abiraterone, enzalutamide or docetaxel will shift over the next few years with many patients increasingly receiving these treatments at start of primary androgen deprivation therapy. Nonetheless the strategy presented here allowing broad, real-time and accurate genomic characterization of a high proportion of metastatic prostate cancer patients without the need for tissue biopsies at a stage in the disease when multiple treatment options are available introduces a vision of future patient management that is likely to become routine.

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Figure 1). The odyssey of a plasma biomarker test. A) The Biomarker Road Map, B) Overview of factors that can give rise to the observed copy number gains and losses in plasma across different ctDNA fractions and challenges in identifying what is associated with poor clinical outcome. C) The effect of estimated ctDNA fraction and observed copy number in interpreting the average copy number change in assessed ctDNA fraction. Copy number (CN), Mutant Allele Frequency (MAF). Circulating tumor DNA (ctDNA).