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Consensus Statement on Circulating Biomarkers for Advanced Prostate Cancer

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Abstract

Context: In advanced prostate cancer (PC), there is increasing investigation of circulating biomarkers, including quantitation and characterization of circulating tumour cells and cell-free nucleic acids, for therapeutic monitoring and as prognostic and predictive biomarkers. However, there is a lack of consensus and standardisation regarding analyses, reporting, and integration of results into specific clinical contexts. A consensus meeting on circulating biomarkers was held to address these topics.
Objective: To present a report of the consensus statement on circulating biomarkers in advanced PC.
Evidence acquisition: Four important areas of controversy in the field of circulating biomarkers in PC management were identified: known clinical utility of circulating biomarkers; unmet clinical needs for circulating biomarkers in PC care; most pressing blood-based molecular assays required; and essential steps for developing circulating biomarker assays. A panel of 18 international PC experts in the field of circulating biomarkers developed the programme and consensus questions. The panel voted publicly but anonymously on 50 predefined questions developed following a modified Delphi process.
Evidence synthesis: Voting was based solely on panellist opinions of the predefined topics and therefore not on a standard literature review or meta-analysis. The outcomes of the voting had varying degrees of support, as reflected in the wording of this article and in the detailed voting results provided in the Supplementary material.
Conclusions: The expert voting results presented can guide the future development of circulating biomarkers for PC care. Notably, the consensus meeting highlighted the importance of reproducibility and variability studies, among other significant areas in need of trials specifically designed to address them.
Patient summary: A panel of international experts met to discuss and vote on the use of different blood-based prostate cancer tests, and how they can be used to guide treatment and disease monitoring to deliver more precise and better patient care.

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1. Introduction

The urgent need for circulating biomarkers for the care of advanced prostate cancer (PC) patients is well described, but there is a lack of consensus regarding how these should be discovered and developed, with little transformative prospective trial data. Investigations focusing on the utility of blood-based assays including plasma cell-free nucleic acids (eg, cell free DNA [cfDNA]) and circulating tumour cells [CTCs] have generated major interest and could transform patient care. A consensus meeting was held to address these issues and produce a statement on circulating biomarkers in advanced PC, defined as metastatic disease or disease that recurred after local treatment. The panel comprised 18 physicians and scientists from nine countries selected on the basis of their academic track record and involvement in clinical or translational research in the field of advanced PC, with expertise in the clinical qualification of biomarkers. None of the invited experts declined the invitation to participate. Before this meeting, the panel identified four areas of controversy for discussion:

1. Current utility of circulating biomarkers.
2. Unmet clinical needs for circulating biomarkers in PC care.
3. Most pressing blood-based molecular assays required.
4. Essential steps for development of circulating biomarker assays.

2. Evidence acquisition

A modified Delphi process was used for consensus development, following procedures described by Gillessen et al. [1]. The meeting comprised state-of-the-art lectures, presentations, and debates by panellists before voting. Following this, 50 questions that were previously agreed on were presented with options for answers in multiple-choice format. Panellists voted anonymously, with results displayed to all attendees immediately. For all questions, responses were based on idealised assumptions that all diagnostic procedures (including expertise in interpretation and application) mentioned were readily available. Importantly, in an effort to address questions from an evidence-based and clinical utility perspective, panellists were specifically instructed not to consider cost, reimbursement, and access in their deliberations, although clearly these are critical factors in decision-making.

We acknowledge that the results reflect the opinions of a small chosen panel of experts on predefined topics, and therefore are not based on a standard literature review or meta-analysis. The results presented are intended to serve only as a guide to clinicians, researchers, and industry partners. The option “unqualified to answer” (short form: “unqualified”) should have been chosen if a panellist lacked experience for a specific question, and the “abstain” option if a panellist felt unable to vote for any reason. Detailed voting records for all questions are provided in the Supplementary material. The denominator was based on the number of panel members voting on the particular question, excluding those who voted “unqualified” or “abstain”. Consensus was declared if $\geq$75% of the panellists chose the same option and did not abstain or vote “unqualified” [2]. Throughout, the percentage of voting panellists giving a particular response is reported, followed by absolute numbers. All panellists contributed to designing the questions, editing the manuscript, and approving this final document. Importantly, this process was uniquely able to highlight areas of disagreement and identify priorities for future clinical research for which additional data acquisition is warranted.

3. Evidence synthesis

3.1. Current utility of circulating biomarkers

3.1.1. CTC assays

Multiple assays have been described for CTC evaluation; the CellSearch system is the only one with regulatory clearance for monitoring PC and has not been improved since its introduction in 2008. CTC number is robustly associated with poor outcome, with declining counts indicating response to therapy [3,4]. Accurate assessment of the actual number of CTCs is especially important when assessing therapy response, and prospective trials evaluating CTC enumeration as response and surrogate biomarkers of response in PC are ongoing. To eliminate inter- and intra-operator bias, the open source ACCEPT software has been developed, allowing automatic CTC enumeration [5].

For CTC testing/enumeration with any assay, 33% (6/18) of the experts voted that testing was ready for use in daily routine clinical practice, 61% (11/18) that current data support testing in prospective trials, and 6% (1/18) that clinical studies are required before prospective clinical validation trials.

For CellSearch CTC counting specifically, 67% (12/18) of the experts voted that testing was ready for use in daily routine clinical practice, 22% (4/18) that current data support testing in prospective trials, and 11% (2/18) that clinical studies are required prior to prospective, clinical validation trials.

Overall, most of the experts endorsed the utility of CTC counts via CellSearch in clinical practice and trials (given the available data and US Food and Drug Administration [FDA] clearance); however, consensus was not reached regarding routine clinical use.

3.1.2. Alternative CTC detection

The successful development of the CellSearch system prompted the study of alternative CTC detection platforms, with >50 companies currently involved in developing and marketing CTC-based liquid biopsy tools [6]. The use of validated CTC detection methods that minimise false positives and allow molecular analyses is mandated. Limitations in CTC detection have been acknowledged; with several patients having undetectable CTCs despite progressive disease, difficulties in capturing these rare events in those that do, and possible subsequent size-selection bias.

For genomic analyses of CTC, none of the experts voted that testing was ready for use in daily routine clinical practice; 61% (11/18) voted that current data support testing in prospective trials and 39% (7/18) that clinical studies are required prior to prospective, clinical validation trials.

The experts voted, based on current knowledge, on the most appropriate clinical situation for CTC testing if the tests were readily available: 28% (5/18) voted for testing before starting first-line PC treatment, 16.5% (3/18) for testing before starting second-line or greater treatment, 39% (7/18) for testing before starting treatment for advanced disease, and 16.5% (3/18) for no appropriate clinical situation currently.

The experts voted on whether CTC assays are likely to impact patient care by 2020, with 33% (6/18) voting yes, 33% (6/18) likely, 28% (5/18) possibly, and 6% (1/18) no.

Overall, the experts indicated an urgent need for clinical trials to validate and qualify CTC-based genomic biomarkers.

3.1.3. AR-V7 expression in CTCs

mRNA transcripts for many androgen receptor (AR) splice variants have been described and characterized [7]. AR splice variant-7 (AR-V7) has received the most attention [8] because it is:

1. Most abundant, making robust detection more feasible;
2. Constitutively active and functionally relevant (may mediate castration resistance);
3. Detectable by antibodies against a variant-specific 16–amino acid sequence; and
4. Associated with increasing levels with the emergence of castration resistance.

A blood-based AR-V7 test using the Adnagen CTC assay has been described as being reproducible, with pretreatment AR-V7 detection associated with poorer responses to abiraterone/enzalutamide and poor outcome [9–11]. This test has been implemented in a clinical testing laboratory with Clinical Laboratory Improvement Amendments certification and has been used in a biomarker selection trial [12,13]. A number of validation studies are still ongoing [14,15], with the specific context of use yet to be defined. In addition, cross-platform comparisons of various AR-V7 tests have yet to be performed.

For AR-V7 testing, 6% (1/18) voted that testing was ready for use in daily routine clinical practice, 72% (13/18) that current data support testing in prospective trials, and 22% (4/18) that clinical studies are required before prospective clinical validation trials.

Regarding which AR-V7 test should be used in daily routine clinical practice if only one test were funded, 31% (4/13) of the experts voted for the EPIC AR-V7 CTC protein assay, 7.67% (1/13) for the Hopkins/Qiagen AR-V7 RT-PCR Adnagen CTC assay, 7.67% (1/13) for a custom RT-PCR based CTC assay, 7.67% (1/13) for any/either of these tests, and 46% (6/13) for the option that there is currently no appropriate assay.

Overall, the experts required prospective clinical trial data to validate and qualify AR-V7 testing before this is used clinically.

3.1.4. cfDNA assays

cfDNA has prognostic and potentially predictive utility, allowing the identification of tumour genomic aberrations. Studies comparing cfDNA aberrations with matched contemporaneous biopsies indicate that somatic mutations identified in biopsies are detectable in cfDNA. Furthermore, cfDNA analyses can identify additional mutations not represented in a biopsy, as cfDNA comprises genomic material released from multiple metastases [16]. Copy number profiles of matched liquid and solid biopsies are also highly correlated, although detection of copy number changes, particularly deletions, requires higher tumour DNA fractions (>35%). Higher total cfDNA is associated with poorer outcome and may be a biomarker of aggressive disease or disease burden [17,18]; decreases in cfDNA during therapy are associated with response and better outcomes [19,20]. Serial measurement of cfDNA levels and tumour fractions may be useful for monitoring prognosis, disease burden, and response to treatment.

For cfDNA analysis, 6% (1/17) of the experts voted that testing was ready for use in daily routine clinical practice, 59% (10/17) that current data support testing in prospective trials, and 35% (6/17) that clinical studies are required before prospective clinical validation trials.

For quantitative analyses of cfDNA concentrations, none of the experts voted that testing was ready for use in daily routine clinical practice, 39% (7/18) voted that current data support testing in prospective trials, and 61% (11/18) that clinical studies are required before prospective clinical validation trials.

Genomic alterations in *AR* including mutations, amplification, and structural rearrangements are demonstrable in cfDNA and can drive endocrine treatment resistance [17–19,21]. Other aberrations detected in cfDNA include alterations in DNA repair genes, *TP53*, and in PI3K pathway genes [22]. Identifying these may assist in selecting patients benefiting from novel treatment approaches such as PARP inhibitors or platinum-based chemotherapy for DNA repair defects [23] and AKT inhibition for aberrant PI3K pathways [24]. Prospective studies testing the utility of cfDNA profiling for treatment stratification are ongoing.

For genomic analyses of gene panels in cfDNA, 6% (1/18) of the experts voted that testing was ready for use in daily routine clinical practice, 72% (13/18) that current data support testing in prospective trials, and 22% (4/18) that clinical studies are required before prospective clinical validation trials.

The experts voted on the most appropriate clinical situation, based on current knowledge, for cfDNA testing if the tests were readily available: 6% (1/16) voted for testing before starting treatment for metastatic PC, 6% (1/16) for testing before starting first-line treatment, 38% (6/16) for testing before starting ≥second line PC treatment, 31% (5/16) for before all of the three options while 19% (3/16) voted for no appropriate clinical situation currently.

Overall, expert consensus was that cfDNA genomic analyses should not yet be utilized in clinical practice based on currently available data, with 94% (17/18) of the panel requiring further prospective clinical trial validation and/or qualification.

3.1.5. AR genomic aberrations

Serial targeted next-generation sequencing of cfDNA has identified *AR* somatic point mutations (2632A > G, p. T878A; and 2105T > A, p.L702H) that develop on treatment with abiraterone acetate and prednisolone [17]. These mutations result in promiscuous AR activation by progesterone or prednisolone, respectively [18,25]. Detection of cfDNA *AR* copy gain before starting enzalutamide and gain of these mutations before abiraterone is associated with lower response rates and shorter progression-free and overall survival [19,26].

Besides *AR* mutations, overexpression, amplification, and expression of splice variants, genomic structural rearrangements (GSRs) in *AR* have been identified as potential predictive biomarkers in the context of castration resistance. GSRs are defined as the presence of at least one breakpoint in the *AR* gene [26–28]. *AR* GSRs have been identified in tumour and liquid biopsies from patients with advanced PC, but have not been detected in localised androgen-dependent disease [29]. Limitations and future challenges in detecting *AR* GSRs in cfDNA include the presence of highly repetitive regions within the *AR* gene that are difficult to sequence, and the possibility of missing GSR events in these regions. The clinical relevance and functional effects of different *AR* GSRs have not yet been established.

For *AR* copy gain/mutations in cfDNA testing, none of the experts voted that testing was ready for use in daily routine clinical practice, 83% (15/18) that current data support testing in prospective trials, and 17% (3/18) that clinical studies are required before prospective clinical validation trials.

The experts voted on the most appropriate clinical situation, based on current knowledge, for AR-V7/*AR* copy gain/*AR* mutation testing if the tests were readily available: 18% (3/17) voted for testing before starting first-line PC treatment, 29% (5/17) for testing before starting second-line or greater PC treatment, 29% (5/17) for before all three options, and 24% (4/17) for no appropriate clinical situation currently.

The experts voted on whether cfDNA assays are likely to impact patient care by 2020: 67% (12/18) voted yes, 17% likely (3/18), 11% possibly (2/18), and 5% (1/18) no.

Overall, the expert consensus indicated that further evaluation in prospective clinical trials is merited before *AR* genomic aberration testing can be implemented in clinical practice. Most of the expert panel voted for cfDNA biomarkers impacting patient care by 2020.

3.1.6. MicroRNAs

MicroRNAs (MiRs) are 20–25-bp noncoding RNAs regulating gene expression through interaction with complementary binding sites of target mRNAs. MiR expression is deregulated in tumours and MiRs are released into the circulation. The correlation between MiR expression and specific cancer types, combined with MiR stability in blood, makes these important biomarkers for evaluation [30,31]. Published studies indicate that high baseline levels of some MiRs, perhaps most notably MiR-375, are associated with poorer prognosis [32–34]. Although prospective validation and qualification through clinical trials are required to confirm these associations, MiRs show promise as prognostic, predictive, and therapy-monitoring biomarkers.

The experts voted on the clinical need for PC-focused targeted MiR profiling: 81% (13/16) voted that this was of low priority and 19% (3/16) that this was a high (relevant) clinical need.

Overall, the experts recommended that more data on MiR PC disease biology are needed to support the pursuit of clinical circulating biomarker studies.

3.2. Unmet clinical needs for circulating biomarkers for monitoring PC care

Clinical trials for men with PC require clinically meaningful endpoints that are valid measures of response and survival [35]. Many patients have bone-only disease, which poses particular challenges to disease monitoring [36,37], and PSA and symptoms commonly drive treatment-switch decisions. The need for superior circulating biomarkers in this setting is of paramount importance to better identify patients not benefiting from treatment before worsening of their clinical condition precludes the use of alternative, potentially active agents. The development and validation of surrogate biomarkers of survival can also improve the identification of active agents in phase 2 trials and allow alternative phase 3 trial endpoints to facilitate the approval and incorporation of novel agents into daily clinical practice.

Regarding the clinical need for circulating response biomarkers, 72% (13/18) of the experts voted for this as a very high need (development urgently needed), 17% (3/18) as a high/relevant clinical need, and 11% (2/18) as a low clinical need.

Regarding the clinical situation for which the development of circulating response biomarkers is most relevant, 100% (17/17) of the experts voted for metastatic PC.

Regarding the clinical need for circulating biomarkers as surrogate endpoints for clinical trials, 72% (13/18) of the experts voted for a very high need, 6% (1/18) for a high/relevant clinical need, and 22% (4/18) for a low clinical need.

Regarding the clinical situation for which the development of circulating biomarkers as surrogate endpoints for clinical trials is most relevant, none of the experts voted for population-based screening, 5.5% (1/18) voted for localised/locally advanced PC, 5.5% (1/18) for recurrence after radical treatment, and 89% (16/18) for metastatic PC.

Overall, there was expert consensus that there is a very high or high need for circulating response and surrogate endpoint biomarkers, with all the experts voting for these being most relevant for metastatic PC.

3.3. Most pressing blood-based molecular assays required

There is an urgent unmet clinical need for biomarkers that can predict treatment benefit and allow a precision medicine approach to care. Predictive biomarkers measurable in the circulation in cfDNA or CTC were discussed (Table 1). These included genes commonly aberrant in PC, such as genes involved in DNA repair that are associated

Table 1 – Additional predictive biomarkers that may be measurable in the circulation either in ctDNA or CTCs.

Predictive biomarker	Context of use	Mechanism	Therapies linked to predictive biomarker	Novel strategic approaches
AR variants (AR-V7) in CTCs (EPIC AR-V7 protein, Qiagen/ Hopkins Adnatest RT-PCR)	Second-line mCRPC following enzalutamide or abiraterone failure	Lack of AR LBD and drug target of abiraterone or enzalutamide (ligand independent signalling)	Lack of benefit with abiraterone or enzalutamide (requires validation) Not predictive of taxane benefit clinically	N-terminal or DNA-binding domain AR inhibitors, BRD4 inhibitors, novel strategies
AR copy gain (amplification)	mCRPC	High AR levels may lead to altered splicing decisions, activity despite low testosterone levels	Possible lack of benefit with abiraterone or enzalutamide (unclear, requires validation)	Novel AR pathway inhibitors
AR mutations (F876L, T878A, H875Y, L702H) in ctDNA, biopsies, CTCs	mCRPC	Agonistic mutations for anti-androgens, glucocorticoids, progesterone	May be associated with resistance to bicalutamide, enzalutamide, abiraterone/ prednisone	Novel AR pathway inhibitors
3βHSD1 mutations, N367T	mHSPC/CRPC	Gain-of-function mutation promoting DHT synthesis from DHEA	Resistance to ADT, early CRPC development	Early use of AR pathway inhibition in mHSPC
Homologous DNA repair defects (*BRCA2*, *BRCA1*, *FANCA*, *PALB2*, *ATM*)	mCRPC	Sensitivity to PARP inhibition synthetic lethality	May be associated with greater benefit to PARP inhibitors, platinum-compounds	PARP inhibitors or platinum-based chemotherapy
DNA mismatch repair defects (Lynch syndrome genes)	mCRPC	High mutational load, neoantigen generation, immune responsiveness and infiltration, PDL-1 upregulation	PD-1 or PDL-1 inhibition possibly based on small trials in MMR deficient	Requires prospective validation of PD-1/ PDL-1 inhibition
PTEN loss, PI3K/AKT pathway activation	mCRPC	Activation of PI3K/AKT/mTOR pathway	Possible benefit to PI3K or AKT inhibition, ideally in combination with AR inhibition given reciprocal feedback of pathways	PI3K/AKT inhibition with abiraterone or enzalutamide
MAPK activation (*RAF1* mutations, MEK activation)	mCRPC	MAPK signalling, survival, metastasis	MEK or BRAF inhibitors potentially	Trametinib, regorafenib, others
Intact RB, gain in CDK4/6 or cyclin-D1	mCRPC	Intact cell-cycle pathway checkpoints	Susceptibility to CDK4/6 inhibitors	CDK4/6 inhibitors ± AR-directed therapies
Wnt pathway alterations	mCRPC	β-Catenin activation and Wnt canonical or noncanonical pathway activation	Wnt pathway inhibition under study	Porcupine inhibition, immunotherapy

ctDNA = circulating tumour DNA; CTCs = circulating tumour cells; AR = androgen receptor; PC = prostate cancer; mCRPC = metastatic PC; mHSPC = metastatic hormone-sensitive PC; LBD = ligand-binding domain; DHT = dihydrogen-testosterone; DHEA = dehydroepiandrosterone; ADT = androgen deprivation therapy.

with PARP inhibitor sensitivity, and *PTEN* loss to predict PI3K/AKT pathway inhibition efficacy. These were considered promising, but with circulating assays needing further development and validation.

The experts voted on a number of blood-based molecular assays and the clinical need for their development, as shown in Table 2.

Regarding the clinical need for predictive circulating biomarkers, 94% (17/18) of the experts voted for a very high need (development urgently needed), 6% (1/18) for a high/ relevant clinical need, and none for a low clinical need (not a key priority for this purpose).

Regarding the clinical situation for which the development of predictive circulating biomarkers is most relevant, none of the experts voted for population-based screening for PC, 11% (2/18) for localised/locally advanced PC, none for recurrence after radical treatment, and 89% (16/18) for metastatic PC.

Regarding the systemic therapy in greatest need of a predictive circulating biomarker in men with PC, 11% (2/18) of the experts voted for abiraterone/enzalutamide, 11% (2/18) for PARP inhibition or platinum-based chemotherapy, 28% (5/18) for immunotherapy, and 50% (9/18) for all systemic treatments.

Regarding the systemic therapy with the least need of a predictive circulating biomarker in men with PC, 6% (1/17) of the experts voted for abiraterone/enzalutamide, 18% (3/ 17) for taxane chemotherapy, 35% (6/17) for radium-223, and 41% (7/17) for none of the systemic treatments.

Regarding the clinical need for prognostic circulating biomarkers, 17% (3/18) of the experts voted for a very high need, 28% (5/18) for a high/relevant clinical need, and 55% (10/18) for a low clinical need.

Regarding the clinical situation for which the development of prognostic circulating biomarkers is most relevant, none of the experts voted for population-based screening for PC, 59% (10/17) for localised/locally advanced PC, 18% (3/ 17) for recurrence after radical treatment, and 23% (4/17) for metastatic PC.

The overwhelming expert consensus was that there is an urgent clinical need for circulating predictive biomarkers and that the greatest need is for metastatic PC.

Table 2 – Expert voting on the clinical need for predictive circulating biomarkers that may be present in circulating nucleic acids or CTCs

Test	Votes on clinical need, % (*n*/*N*) [a]		
	Very high	High	Low
CTC phenotyping and genotyping	61% (11/18)	28 (5/18)	11 (2/18)
Androgen receptor variant assays	33% (6/18)	56 (10/18)	11 (2/18)
Neuroendocrine biomarker analyses	24 (4/17)	41 (7/17)	35 (6/17)
PTEN loss analyses	19 (3/16)	25 (4/16)	56 (9/16)
DNA repair defect analyses	94 (16/17)	6 (1/17)	0
RB1 loss	12 (2/16)	50 (8/16)	38 (6/16)
Mismatch repair/microsatellite instability signatures	71 (12/17)	23 (4/17)	6 (1/17)
Immunological biomarker studies (eg, PD-L1, PD-L2)	39 (7/18)	33 (6/18)	28 (5/18)
PC-focused targeted NGS gene panel	67 (12/18)	33 (6/18)	0
PC-focused targeted microRNA profiling	0	19 (3/16)	81 (13/16)

CTC = circulating tumour cell; PC = prostate cancer; NGS = next-generation sequencing.
[a] Very high = high clinical need, development urgently needed; high = relevant clinical need; low = not a key priority for this purpose.

3.4. *Essential steps for development of circulating biomarker assays*

Biomarker development consists of two separate components: analytical and clinical validation [38]. Analytic validation establishes assay reproducibility and estimates both error and utility in the clinical setting [39]. Many variables can impact analytical validation of an assay. Initial biomarker studies must establish the methodology with the best reproducibility and precision, including sample handling, storage, and processing; evaluation of technical and biological replicates; key quality control measures including high and low limits of detection; intraobserver and interobserver reproducibility; and the availability of adequate controls [40]. After analytical validity has been established, clinical qualification involves evaluation of biomarker utility through clinical studies, frequently in a multistep fashion involving multiple consecutive studies or a single study with different stages to determine biomarker fitness to impact a medical decision in a specific context of use [41]. Each of the FDA-established biomarker contexts (prognostic, predictive, response-indicator, efficacy-response [surrogate]) requires adequately designed and powered studies to establish clinical validity. In addition to demonstrating clinical validation in a context, it is also important to address the clinical utility of a biomarker by establishing whether use of the biomarker result informs a medical decision and improves patient outcomes.

The panel voted on what it considered to be critically important steps in the development of circulating biomarkers for PC:

- Regarding the need for healthy volunteer data in circulating biomarker validation, 65% (11/17) of the experts voted for very high, 29% (5/17) for high, and 6% (1/17) for low importance.
- Regarding reproducibility studies, the experts voted unanimously (100%, 17/17) for very high importance.
- Regarding variability studies, 94% (15/16) of the experts voted for very high importance and 6% (1/16) for high importance.
- Regarding comparison of different platforms, 44% (7/16) of the experts voted for very high importance, 31% (5/16) for high importance, 19% (3/16) for low importance, and 6% (1/16) for not important.
- Regarding qualification involving prospective clinical trials, 82% (14/17) voted for very high importance and 18% (3/17) for high importance.

The experts voted whether a tumour biopsy–based assay is preferable to a blood-based assay when both are feasible: 29% (5/17) voted yes while 71% (12/17) voted no.

Regarding the clinical need for circulating biomarkers for diagnostic purposes, 44% (8/18) of the experts voted for a very high need (development urgently needed), 28% (5/18) for a high/relevant clinical need, and 28% (5/18) voted for a low clinical need.

Regarding the clinical situation for which the development of circulating biomarkers for diagnostic purposes is most relevant, 50% (9/18) of the experts voted for population-based PC screening, 11% (2/18) for localised/locally advanced PC, 6% (1/18) for recurrence after radical treatment, and 33% (6/18) for metastatic PC.

Regarding the clinical setting with the greatest utility for a circulating biomarker of DNA homologous repair deficiency, 6% (1/17) voted for localized disease, 47% (8/17) for diagnosis of metastatic disease, 23% (4/17) for diagnosis of metastatic castration-resistant disease, 6% (1/17) for PC following progression on abiraterone/enzalutamide, and 18% (3/17) for PC following progression on all proven therapies.

The experts also voted on circulating biomarker tests in specific clinical situations and had to choose the best performing assay (if all tests were available):

- For men with high-risk localised/locally advanced PC, 34% (5/17) of the experts voted for cfDNA quantification and sequencing, 13% (2/17) for CTC enumeration, and 53% (8/17) for none of the tests.
- For men with rising PSA after radical local treatment (biochemical recurrence), 12% (2/17) of the experts voted for cfDNA quantification and sequencing, 6% (1/17) for CTC enumeration, and 82% (14/17) for none of the tests.
- For men with newly diagnosed metastatic disease, 65% (11/17) of the experts voted for cfDNA quantification and

sequencing, 12% (2/17) for CTC enumeration, and 23% (4/17) for none of the tests.
- For men with metastatic PC, 67% (12/18) of the experts voted for cfDNA quantification and sequencing, 22% (4/18) for CTC enumeration, 5.5% (1/18) for AR-V7 testing, and 5.5% (1/18) for none of the tests.

Overall, there was expert consensus that reproducibility (same sample and time point) and variability (different samples and different time points) studies, healthy volunteer analyses as negative controls, and prospective clinical trials are necessary to analytically validate circulating biomarkers. Consensus was not reached on the need for comparing different biomarker platforms. Surprisingly, the majority of the panel preferred a liquid biopsy to a tumour biopsy, with cfDNA being the preferred single test for the majority, although consensus was not reached.

3.5. *Counting the costs*

Treatment of PC places a huge financial burden on health systems [38]. Liquid biopsies can play a pivotal role in limiting these costs, decreasing the administration of ineffective drugs via earlier discontinuation by using response biomarkers and identifying cancers unlikely to respond to expensive anticancer drugs. The health economic benefits of liquid biopsies must now be ascertained in prospective clinical trials focusing not only on single biomarker assays but also on the potential of more advanced multiplex and multipurpose assays.

With regard to health economic analyses, 65% (11/17) of the experts voted that this was of very high importance, 29% (5/17) of high importance, and 6% (1/17) of low importance.

The overwhelming consensus of this expert panel was that health economic analyses are of very high or high importance in blood-based biomarker clinical trials.

4. Conclusions

PC has some of the highest cfDNA and CTC levels of all solid tumours, allowing serial tumour genomic analyses during treatment. This could transform clinical care of PC, as this is a noninvasive and practical approach to selecting and monitoring treatment, and elucidating drug resistance and clonal evolution.

Overall, this meeting highlighted the urgent need for prospective, bespoke, clinical trials to clinically qualify circulating biomarkers for PC to deliver better and more precise patient care. This paper summarises the current knowledge on circulating biomarkers as guidance for future assay and research/clinical protocol development, since none of these tests are widely available or reimbursed in clinical practice. Many questions are still unanswered in the field of biomarker development, and the voting results of the panel reflect the great need for circulating biomarkers (mainly for prediction of treatment response and as surrogate endpoints), while also highlighting the lack of data, validation studies, and regulatory approval for the majority of the tests discussed.

The consensus of this expert panel was that such assays are highly likely to impact patient care in the near-term, with most experts (67%) recommending the use of CellSearch CTC counts for clinical care. Expert consensus indicated that prospective clinical trials to validate circulating biomarkers are of paramount importance, with the highest need being for predictive biomarkers for metastatic PC. Metastatic PC was also established as the setting for which circulating biomarkers are required as response biomarkers and surrogate endpoints. Expert consensus was also reached on the importance of variability and reproducibility studies in biomarker validation. Figure 1 demonstrates the varying opinions on the current utility of the biomarkers discussed.

Identification, analytical validation, and prioritisation of circulating biomarkers intended to guide patient care represent a major endeavour that must follow strict experimental rules, being as complex and costly as drug development. This should only be attempted by qualified investigators, with biomarker integration into prospective clinical trials requiring teams of highly trained and experienced experts. With few exceptions, corporate sponsors are often reluctant to fund biomarker development; obtaining traditional grants for such work is also frequently outside usual hypothesis-driven grant proposals, making evidence development difficult. A solution to this conundrum is for physicians and regulatory agencies to reject clinical trials lacking biomarkers for patient selection and/or treatment response. Industry partners, government agencies, and investigators must understand that “all comers” clinical trials for PC should only be performed if they incorporate tissue and blood collection and adequate patient consent to the qualification of predictive or response biomarkers. Experts must lobby traditional funders and regulatory agencies to support these endeavours, with co-ordinated efforts needed. The outcomes of this expert consensus can help guide the development of circulating biomarkers for PC care, identifying key areas for prioritisation.

Author contributions: Johann S. de Bono had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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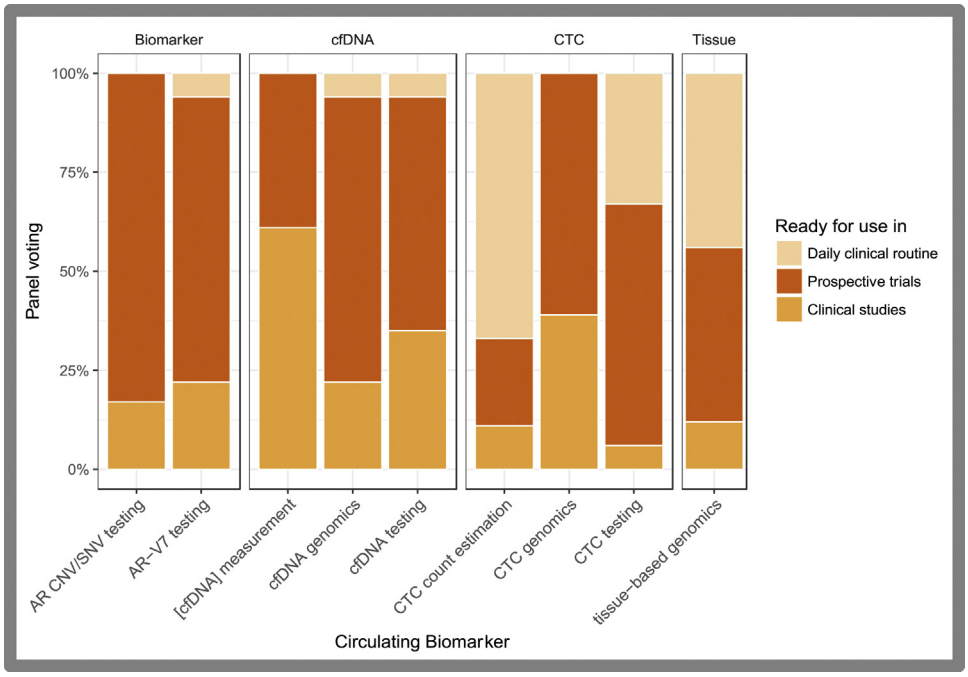

Fig. 1 – Expert voting on circulating biomarkers for advanced prostate cancer and their current utility in clinical practice. AR = androgen receptor; CNV = copy number variation; SNV = single-nucleotide variation; AR-V7 = AR splice variant 7; cfDNA = cell-free DNA; CTC = circulating tumor cell.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.euo.2018.02.009.

References

[1] Gillessen S, Omlin A, Attard G, et al. Management of patients with advanced prostate cancer: recommendations of the St Gallen Advanced Prostate Cancer Consensus Conference (APCCC) 2015. Ann Oncol 2015;26:1589–604.
[2] Diamond IR, Grant RC, Feldman BM, et al. Defining consensus: a systematic review recommends methodologic criteria for reporting of Delphi studies. J Clin Epidemiol 2014;67:401–9.
[3] Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 2004;351:781–91.
[4] Lorente D, Olmos D, Mateo J, et al. Decline in circulating tumor cell count and treatment outcome in advanced prostate cancer. Eur Urol 2016;70:985–92.
[5] Leonie Z. The ACCEPT image analysis algorithm made for the Cancer-ID project. http://github.com/LeonieZ/ACCEPT.
[6] Business Wire. Circulating tumor cell (CTC) diagnostics market 2015-2022. Global strategic business report 2017—Research and Markets. www.businesswire.com/news/home/20170329005642/en/Circulating-Tumor-Cell-CTC-Diagnostics-Market-2015-2022.
[7] Lu C, Luo J. Decoding the androgen receptor splice variants. Transl Androl Urol 2013;2:178–86.
[8] Antonarakis ES, Armstrong AJ, Dehm SM, Luo J. Androgen receptor variant-driven prostate cancer: clinical implications and therapeutic targeting. Prostate Cancer Prostatic Dis 2016;19:231–41.

[9] Antonarakis ES, Lu C, Wang H, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N Engl J Med 2014;371:1028–38.
[10] Antonarakis ES, Lu C, Luber B, et al. Androgen receptor splice variant 7 and efficacy of taxane chemotherapy in patients with metastatic castration-resistant prostate cancer. JAMA Oncol 2015;1:582.
[11] Scher HI, Lu D, Schreiber NA, et al. Association of AR-V7 on circulating tumor cells as a treatment-specific biomarker with outcomes and survival in castration-resistant prostate cancer. JAMA Oncol 2016;52:1593–601.
[12] Lokhandwala PM, Riel SL, Haley L, et al. Analytical validation of androgen receptor splice variant 7 detection in a Clinical Laboratory Improvement Amendments (CLIA) laboratory setting. J Mol Diagn 2017;19:115–25.
[13] Taplin M-E, Antonarakis ES, Ferrante KJ, et al. Clinical factors associated with AR-V7 detection in ARMOR3-SV, a randomized trial of galeterone (Gal) vs enzalutamide (Enz) in men with AR-V7+ metastatic castration-resistant prostate cancer (mCRPC). J Clin Oncol 2017;35(15 Suppl):5005.
[14] Del Re M, Biasco E, Crucitta S, et al. The detection of androgen receptor splice variant 7 in plasma-derived exosomal RNA strongly predicts resistance to hormonal therapy in metastatic prostate cancer patients. Eur Urol 2017;71:680–7.
[15] Zhu Y, Sharp A, Anderson CM, et al. Novel junction-specific and quantifiable in situ detection of AR-V7 and its clinical correlates in metastatic castration-resistant prostate cancer. Eur Urol 2018; 73:727–35.
[16] Wyatt AW, Annala M, Aggarwal R, et al. Concordance of circulating tumor DNA and matched metastatic tissue biopsy in prostate cancer. J Natl Cancer Inst 2018;110:78–86.
[17] Romanel A, Tandefelt DG, Conteduca V, et al. Plasma AR and abiraterone-resistant prostate cancer. Sci Transl Med 2015;7: 312re10.
[18] Carreira S, Romanel A, Goodall J, et al. Tumor clone dynamics in lethal prostate cancer. Sci Transl Med 2014;6, 254ra125.
[19] Wyatt AW, Azad AA, Volik SV, et al. Genomic alterations in cell-free DNA and enzalutamide resistance in castration-resistant prostate cancer. JAMA Oncol 2016;2:1598.
[20] Goodall J, Mateo J, Yuan W, et al. Circulating free DNA to guide prostate cancer treatment with PARP inhibition. Cancer Discov 2017;7:1006–17.
[21] De Laere B, van Dam P-J, Whitington T, et al. Comprehensive profiling of the androgen receptor in liquid biopsies from castration-resistant prostate cancer reveals novel intra-AR structural variation and splice variant expression patterns. Eur Urol 2017;72:192–200.
[22] Khalaf D, Annala M, Beja K, et al. Circulating tumor DNA (ctDNA) and correlations with clinical prognostic factors in patients with metastatic castration-resistant prostate cancer (mCRPC). J Clin Oncol 2017;35(6 Suppl):186.
[23] Mateo J, Carreira S, Sandhu S, et al. DNA-repair defects and olaparib in metastatic prostate cancer. N Engl J Med 2015;373:1697–708.
[24] de Bono JS, De Giorgi U, Massard C, et al. PTEN loss as a predictive biomarker for the Akt inhibitor ipatasertib combined with abiraterone acetate in patients with metastatic castration-resistant prostate cancer (mCRPC). Ann Oncol 2016;27(Suppl 6):7180.
[25] Chen EJ, Sowalsky AG, Gao S, et al. Abiraterone treatment in castration-resistant prostate cancer selects for progesterone responsive mutant androgen receptors. Clin Cancer Res 2015;21: 1273–80.
[26] Li Y, Alsagabi M, Fan D, Bova GS, Tewfik AH, Dehm SM. Intragenic rearrangement and altered RNA splicing of the androgen receptor in a cell-based model of prostate cancer progression. Cancer Res 2011;71:2108–17.
[27] Li Y, Hwang TH, Oseth LA, et al. AR intragenic deletions linked to androgen receptor splice variant expression and activity in models of prostate cancer progression. Oncogene 2012;31:4759–67.
[28] Nyquist MD, Li Y, Hwang TH, et al. TALEN-engineered AR gene rearrangements reveal endocrine uncoupling of androgen receptor in prostate cancer. Proc Natl Acad Sci U S A 2013;110:17492–7.
[29] Abeshouse A, Ahn J, Akbani R, et al. The molecular taxonomy of primary prostate cancer. Cell 2015;163:1011–25.
[30] Sita-Lumsden A, Dart DA, Waxman J, Bevan CL. Circulating microRNAs as potential new biomarkers for prostate cancer. Br J Cancer 2013;108:1925–30.
[31] Lawrie CH, Gal S, Dunlop HM, et al. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol 2008;141:672–5.
[32] Huang X, Yuan T, Liang M, et al. Exosomal miR-1290 and miR-375 as prognostic markers in castration-resistant prostate cancer. Eur Urol 2015;67:33–41.
[33] Zhang H-L, Yang L-F, Zhu Y, et al. Serum miRNA-21: elevated levels in patients with metastatic hormone-refractory prostate cancer and potential predictive factor for the efficacy of docetaxel-based chemotherapy. Prostate 2011;71:326–31.
[34] Lin H-M, Mahon KL, Spielman C, et al. Phase 2 study of circulating microRNA biomarkers in castration-resistant prostate cancer. Br J Cancer 2017;116:1002–11.
[35] Scher HI, Heller G, Molina A, et al. Circulating tumor cell biomarker panel as an individual-level surrogate for survival in metastatic castration-resistant prostate cancer. J Clin Oncol 2015;33:1348–55.
[36] Armstrong AJ, Eisenberger MA, Halabi S, et al. Biomarkers in the management and treatment of men with metastatic castration-resistant prostate cancer. Eur Urol 2012;61:549–59.
[37] Khleif SN, Doroshow JH, Hait WN. AACR-FDA-NCI Cancer Biomarkers Collaborative consensus report: advancing the use of biomarkers in cancer drug development. Clin Cancer Res 2010;16:3299–318.
[38] Dougherty ER. Biomarker development: prudence, risk, and reproducibility. BioEssays 2012;34:277–9.
[39] Cummings J, Raynaud F, Jones L, Sugar R, Dive C. Fit-for-purpose biomarker method validation for application in clinical trials of anticancer drugs. Br J Cancer 2010;103:1313–7.
[40] McShane LM, Altman DG, Sauerbrei W, et al. REporting recommendations for tumour MARKer prognostic studies (REMARK). Br J Cancer 2005;93:387–91.
[41] Norum J, Nieder C. Treatments for metastatic prostate cancer (mPC): a review of costing evidence. Pharmacoeconomics 2017; 35:1223–36.