# The role of ctDNA testing for HRRm in mCRPC

**Circulating tumor DNA (ctDNA) are small DNA fragments released from cancer cells that are found in blood plasma.[1] Metastatic tissue testing is strongly recommended. When tissue testing is not feasible or available, ctDNA testing may aid in identifying and monitoring HRR alterations in metastatic castration-resistant prostate cancer (mCRPC).[2,3]**

## IN WHAT WAYS CAN ctDNA TESTING COMPLEMENT TISSUE TESTING?

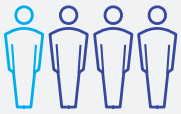
By overcoming some of the technical difficulties and challenges of genomic testing in advanced prostate cancer, ctDNA testing may become a complementary diagnostic, prognostic, monitoring, and predictive tool that[1,2,4-8]:

- Serves as a surrogate for tissue when it is not available[2,9]
- Offers a high-quality DNA alternative to bone biopsy[8,9]
- Captures the heterogeneity of the disease[10]
- Provides prognostic insights[11]
- Allows minimally invasive, easily repeatable monitoring for tumor mutation changes and treatment resistance[8,12]

**See inside for more information on ctDNA and tissue testing.**

## WHY TEST FOR HRRm IN mCRPC?

### HRR mutations are alterations in the homologous recombination repair pathway that is involved in DNA repair.[2]



About 1 in 4 men with mCRPC have an HRRm, which may be associated with a worse prognosis[13-16]

HRR testing can help inform treatment options, including targeted therapies[11]

More than a dozen genes in the HRR pathway can harbor alterations, including *BRCA1*, *BRCA2*, *ATM*, *ATR*, *CDK12*, *CHEK2*, *FANCA*, *MLH1*, *MRE11A*, *NBN*, *PALB2*, *RAD51C*, *FANCL*, *BARD1*, and *RAD51D*[2]

**HRR**, homologous recombination repair; **HRRm**, homologous recombination repair gene-mutated; **NCCN**, National Comprehensive Cancer Network; **NGS**, next-generation sequencing.

NATIONAL COMPREHENSIVE CANCER NETWORK® (NCCN®)

**NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®)** recommend germline and somatic tumor testing for HRR gene alterations in all patients with metastatic prostate cancer with a multigene NGS test. When tissue testing is unsafe or unfeasible, tests can be performed using ctDNA.[3] **Look inside for further details.**

# When and what to test in mCRPC

## TEST FOR GERMLINE AND SOMATIC HOMOLOGOUS RECOMBINATION REPAIR GENE MUTATIONS UPON METASTATIC DIAGNOSIS AND PROGRESSION[3]

| | |
|---|---|
| **NCCN** | **NCCN Guidelines® recommend germline and somatic tumor testing for HRR gene alterations in all patients with metastatic prostate cancer.[3]**<br>• Germline testing is recommended in patients with prostate cancer and a positive family history of certain cancers or familial cancer risk mutation; and high-risk, very high-risk localized, regional (node-positive), or metastatic prostate cancer[3]<br>• Somatic tumor mutation testing for alterations in homologous recombination DNA repair genes, such as *BRCA1*, *BRCA2*, *ATM*, *RAD51D*, *PALB2*, *FANCA*, *CHEK2*, and *CDK12*, is recommended in patients with metastatic prostate cancer[3] |
| **AUA** AMERICAN UROLOGICAL ASSOCIATION® | **The AUA recommends clinicians should offer germline (if not already performed) and somatic genetic testing to identify DNA repair deficiency, MSI status, tumor mutational burden, and other potential mutations that may inform prognosis and familial cancer risk, as well as direct potential targeted therapies, for patients with mCRPC.[17]** |

## NCCN GUIDANCE ON ctDNA

NCCN Guidelines strongly recommend a metastatic biopsy for histologic and molecular evaluation. This could include lymph node biopsy for patients with N1 disease. When unsafe or unfeasible, plasma circulating tumor DNA (ctDNA) assay is an option, preferably collected during biochemical (PSA) and/or radiographic progression in order to maximize diagnostic yield.[3] Caution is needed when interpreting ctDNA-only evaluation due to potential interference from clonal hematopoiesis of indeterminate potential (CHIP), which can result in a false-positive biomarker signal.*[3]

*CHIP is an age-related acquisition of somatic mutations that lead to clonal expansion in regenerating hematopoietic stem cell populations.[18]

**MSI**, microsatellite instability; **N1**, metastasis in regional node(s); **PSA**, prostate-specific antigen.

# Role of ctDNA testing and tumor tissue for HRR mutations

## THE POSSIBLE ADVANTAGES AND LIMITATIONS OF ctDNA AND TUMOR TISSUE TESTING[1,2,8,9,19]

### ctDNA TESTING

| SAMPLE TYPES | SAMPLE PREPARATION | ALTERATION TYPES DETECTED | SAMPLE QUALITY | POTENTIAL ADVANTAGES | POTENTIAL LIMITATIONS | TURNAROUND TIME |
|---|---|---|---|---|---|---|
| •Whole blood | Ethylenediamin-etetraacetic acid (EDTA) tubes or cfDNA-stabilizing tubes | •Somatic<br>•Germline | •DNA quantity: low<br>•DNA quality: variable | •Easy to obtain<br>•Represents tumor heterogeneity<br>•Minimally invasive<br>•Easily repeatable<br>•Tool for monitoring resistance and clonal evolution | •Early-stage disease may have low concentrations of ctDNA<br>•Potential for false positive if HRR genes affected by CHIP<br>•Some platforms may have limited ability to detect copy number alterations | ~ 1-3 weeks |

### TISSUE TESTING

| SAMPLE TYPES | SAMPLE PREPARATION | ALTERATION TYPES DETECTED | SAMPLE QUALITY | POTENTIAL ADVANTAGES | POTENTIAL LIMITATIONS | TURNAROUND TIME |
|---|---|---|---|---|---|---|
| •Biopsies<br>•Surgical specimens<br>•Metastatic deposits | Formalin-fixed, paraffin-embedded (FFPE) slides | •Somatic<br>•Germline | •DNA quantity: medium<br>•DNA quality: low | •Considered the "gold" standard<br>•Can use archival tissue | •Bone biopsies difficult to collect<br>•Bone may not have enough quality DNA for sequencing<br>•May not show tumor heterogeneity | 2+ weeks |

**APPROXIMATELY 1 IN 3 TUMOR TISSUE TESTS FAILED MOLECULAR SCREENING** IN LARGE PHASE 3 TRIALS OF PATIENTS WITH mCRPC DUE TO INSUFFICIENT TUMOR TISSUE, LOW DNA QUALITY/QUANTITY, OR DNA EXTRACTION ISSUES.[4,14,20,21]

**ACCURACY OF ctDNA TESTING:** 81-93% CONCORDANCE BETWEEN TISSUE BIOPSIES AND ctDNA IN mCRPC, BASED ON MULTIPLE CLINICAL STUDIES.[4,6-8,12,19]

REPEAT TESTING OF PATIENTS WITH METASTATIC PROSTATE CANCER WITH ctDNA MAY IDENTIFY ADDITIONAL HRR ALTERATIONS OVER TIME.[22]

**cfDNA**, circulating free DNA.

# Selecting and using ctDNA testing for HRRm

**Comprehensive sequencing panels** use capture-based enrichment to sequence many different genes (200 kb-2000 kb DNA). Some comprehensive sequencing panel tests will also report on microsatellite instability and/or tumor mutational burden.[2,8,23]

**Select available ctDNA assays from commercial laboratories include[4,6,8,12,19,24,25]:**

| FoundationOne® Liquid CDx | Guardant360® CDx | Tempus xF+* | Caris Assure™* |
|---|---|---|---|

## HOW TO ORDER A ctDNA TEST

Request a ctDNA test from a laboratory of choice that assesses HRR mutations. HRR mutations include *BRCA1*, *BRCA2*, *ATM*, *ATR*, *CDK12*, *CHEK2*, *FANCA*, *MLH1*, *MRE11A*, *NBN*, *PALB2*, *RAD51C*, *FANCL*, *BARD1*, and *RAD51D*.[2]

ctDNA may preferably be collected during biochemical (PSA) and/or radiographic progression in order to maximize diagnostic yield.[3] Carefully collect and prepare blood samples in the provided tubes according to the test manufacturer's instructions.

## WHAT TO EXPECT FROM TEST RESULTS

Reports typically include an analysis of the number and types of mutations detected, platform-specific components that may affect results, and clinical insights on approved therapies or clinical trials based on the alterations identified.[23,26] Consider factors like tumor fraction and variant allele fraction when interpreting results.[27]

**Not FDA-approved tests. These are Laboratory Developed Tests (LDTs), which are in vitro diagnostic products (IVDs) that are intended for clinical use and are designed, manufactured, and used within a single clinical laboratory which meets certain laboratory requirements. Although LDTs must be certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) and meet the regulatory requirements under CLIA to perform high complexity testing, these tests are not individually FDA-approved.*

*The diagnostic tests listed are generally commercially available HRR alteration gene panels. List is current as of April 2024. Tests listed may include analysis of genes outside of HRR pathways. NCCN Guidelines recommend a multigene test or panel that assesses a number of HRR germline alterations.*[3]

*This information is intended as educational only, is not intended as a complete list of available testing options, and is not regularly updated. While diagnostic testing may assist providers in identifying appropriate treatment for patients, the decision and action should be decided by a provider in consultation with the patient. Pfizer is not responsible for any test provider and does not endorse or recommend any particular diagnostic test. The accuracy and results of diagnostic tests vary, and Pfizer shall have no liability arising from such testing. The information provided herein should in no way be considered a guarantee of coverage, reimbursement, availability, or patient assistance. Providers should contact third-party laboratories for information.*

**References: 1.** Cimadamore A, Cheng L, Massari F, et al. Circulating tumor DNA testing for homology recombination repair genes in prostate cancer: from the lab to the clinic. Int J Mol Sci 2021;22(11):5522. **2.** Catalano M, Generali D, Gatti M, et al. DNA repair deficiency as circulating biomarker in prostate cancer. Front Oncol 2023;13:1115241. **3.** Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Prostate Cancer V.4.2024. © National Comprehensive Cancer Network, Inc. 2024. All rights reserved. Accessed May 20, 2024. To view the most recent and complete version of the guideline, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way. **4.** Chi KN, Barnicle A, Sibilla C, et al. Detection of *BRCA1*, *BRCA2*, and *ATM* alterations in matched tumor tissue and circulating tumor DNA in patients with prostate cancer screened in PROfound. Clin Cancer Res 2023;29(1):81-91. **5.** Kwan EM, Wyatt AW, Chi KN. Towards clinical implementation of circulating tumor DNA in metastatic prostate cancer: opportunities for integration and pitfalls to interpretation. Front Oncol 2022;12:1054497. **6.** Azad A, Fizazi K, Matsubara N, et al. Use of circulating tumor DNA (ctDNA) to complement tumor tissue homologous recombination repair (HRR) gene alteration testing in TALAPRO-2, a phase 3 study of talazoparib (TALA) + enzalutamide (ENZA) versus placebo (PBO) + ENZA as first-line (1L) treatment in patients (pts) with castration-resistant prostate cancer. Poster 5056. J Clin Oncol 2023;41(16_suppl):5056. **7.** Clarke NW, Armstrong AJ, Thiery-Vuillemin A, et al. Abiraterone and olaparib for metastatic castration-resistant prostate cancer. NEJM 2022;1(9):EVIDoa2200043. **8.** Tukachinsky H, Madison RW, Chung JH, et al. Genomic analysis of circulating tumor DNA in 3,334 patients with advanced prostate cancer identifies targetable BRCA alterations and AR resistance mechanisms. Clin Can Res 2021;27(11):3094-105. **9.** Kostos L, Fettke H, Kwan EM, Azad AA. Utility and clinical application of circulating tumor DNA (ctDNA) in advanced prostate cancer. SIUJ 2023;4(4):273-86. **10.** Herberts C, Annala M, Sipola J, et al. Deep whole-genome ctDNA chronology of treatment-resistant prostate cancer. Nature 2022;608(7921):199-208. **11.** Fonseca NM, Maurice-Dror C, Herberts C, et al. Prediction of plasma ctDNA fraction and prognostic implications of liquid biopsy in advanced prostate cancer. Nat Commun 2024;15(1):1828. **12.** Schweizer MT, Sivakumar S, Tukachinsky H, et al. Concordance of DNA repair gene mutations in paired primary prostate cancer samples and metastatic tissue or cell-free DNA. JAMA Oncol 2021;7(9):1378-82. **13.** Chung JH, Dewal N, Sokol E, et al. Prospective comprehensive genomic profiling of primary and metastatic prostate tumors. JCO Precis Oncol 2019;3:PO.18.00283. **14.** de Bono J, Mateo J, Fizazi K, et al. Olaparib for metastatic castration-resistant prostate cancer. N Engl J Med 2020;382(22):2091-102. **15.** Plym A, Dióssy M, Szallasi Z, et al. DNA repair pathways and their association with lethal prostate cancer in African American and European American men. JNCI Cancer Spectr 2021;6(1):pkab097. **16.** Castro E, Romero-Laorden N, del Pozo A, et al. PROREPAIR-B: a prospective cohort study of the impact of germline DNA repair mutations on the outcomes of patients with metastatic castration-resistant prostate cancer. J Clin Oncol 2019;37(6):490-503. **17.** Lowrance W, Dreicer R, Jarrard DF, et al. Updates to advanced prostate cancer: AUA/SUO guideline (2023). J Urol 2023;209(6):1082-90. **18.** He W, Xiao Y, Yan S, Zhu Y, Ren S. Cell-free DNA in the management of prostate cancer: current status and future prospective. Asian J Urol 2023;10:298-316. **19.** McFarland TR, Mathew Thomas V, Nussenzveig R, et al. Detection of *BRCA1*, and *BRCA2* alterations in matched tumor tissue and circulating cell-free DNA in patients with prostate cancer in a real-world setting. Biomedicines 2022;10(12):3170.567. **20.** Abida W, Patnaik A, Campbell D, et al. Rucaparib in men with metastatic castration-resistant prostate cancer harboring a *BRCA1* or *BRCA2* gene alteration. J Clin Oncol 2020;38(32):3763-72. **21.** Sweeney C, Bracarda S, Sternberg CN, et al. Ipatasertib plus abiraterone and prednisolone in metastatic castration-resistant prostate cancer (IPATential150): a multicentre, randomised, double-blind, phase 3 trial. Lancet 2021;398(supp):1-30. **22.** Park JJ, Chu A, Li J, et al. Repeat next-generation sequencing testing on progression in men with metastatic prostate cancer can identify new actionable alterations. JCO Precis Oncol 2024;8:e2300567. **23.** Friedlander TW, Pritchard CC, Beltran H. Personalizing therapy for metastatic prostate cancer: the role of solid and liquid tumor biopsies. Am Soc Clin Oncol Educ Book 2017;37:358-69. **24.** Giri VN, Morgan TM, Morris DS, Berchuck JE, Hyatt C, Taplin ME. Genetic testing in prostate cancer management: considerations informing primary care. CA Cancer J Clin 2022;72:360-71. **25.** Kato T, Matsubara N, Shiota M, et al. IMAGENE trial: multicenter, proof-of-concept, phase II study evaluating the efficacy and safety of combination therapy of niraparib with PD-1 inhibitor in solid cancer patients with homologous recombination repair genes mutation. BMC Cancer 2022;22(1):1292. **26.** Schmid S, Jochum W, Padberg B, et al. How to read a next-generation sequencing report—what oncologists need to know. ESMO Open 2022;7(5):100570. **27.** Vandekerkhove G, Giri VN, Halabi S, et al. Toward informed selection and interpretation of clinical genomic tests in prostate cancer. JCO Precis Oncol 2024;8:e2300654.

Pfizer Oncology