Supplementary methods

BEAMing technology

The BEAMing technology is well validated. The assay is College of American Pathologists (CAP) certified and performed in a Clinical Laboratory Improvement Amendments (CLIA) setting with methodology well characterized by Vogelstein and colleagues and cited in the supplementary references [1–3]. These studies showed BEAMing to be sensitive and capable of detecting mutations present in 1 in 10,000 DNA molecules. Higgins and colleagues [4] cite 100% agreement in PIK3CA mutation status determined by BEAMing when it is compared with standard sequencing done on the same tumor samples. More important clinically, there was 100% concordance between mutations detected in paired tumor samples and plasma ctDNA when the samples were obtained from the patient concurrently.

Blood samples were collected using supplied prelabeled K2EDTA evacuated collection tubes. After the blood was obtained, collection tubes were mixed thoroughly by slowly inverting several times. Within 30 minutes of collection, blood tubes were spun down in a refrigerated centrifuge (2–8°C) at approximately 2000 × g for 15 minutes. Within 90 minutes of collection, both plasma aliquot samples were transferred to a freezer set to maintain a temperature of –70°C and a 2 ml frozen vial was shipped on dry ice to analysis labs.

BEAMing detection and quantification was accomplished by magnetic flow cytometry with fluorescent-tagged probes specific for the androgen receptor ligand-binding domain (AR-LBD) mutations. A plasma sample was deemed positive for a given mutation if the percentage of mutant beads was above the cutoff (0.02%) and the number of mutant copies was estimated to be 0.5 or more (no. genome equivalents in plasma × mutant bead fraction ≥0.5). Three technical replicates were conducted. Biological replicates were not performed as

they are typically not necessary for CLIA assays. Mutations detected in the current study were not confirmed by alternative assay methodology due to limited sample availability.

In order for a BEAMing assay to be scored positive (mutant), it has to meet the following criteria:

- The determined relative fraction of mutant to wild-type DNA has to be higher than the anticipated cutoff of the respective BEAMing method.
- The frequency has to be above the total amount of genome equivalents used per assay. For example, if in a sample, 1000 genomic equivalents (GE) are present, yet the calculated fraction of mutant DNA molecules is 0.02% (1 mutant allele in 5000 wild-type alleles), the sample is scored as wild-type.

In the current analysis, DNA was partitioned into reactions each containing approximately 1000 GE, from which a mutation was called if present in at least 2 independent PCR and flow cytometry. Genomic equivalents were calculated by the established human LINE-1 qPCR assay [5]. ctDNA fractions were quantified by taking the total mutant observations divided by the total GE analyzed. The lower limit of sensitivity for this assay was dependent on the total GE analyzed, and ranged from 1603 to 15,308. The cutoff was determined during analytical validation experiments at Sysmex Inostics. A minimum of 30,000 total beads was required. Accordingly, a minimum of 6 mutant beads was required to reach a cutoff of 0.02% for a positive event. Furthermore, each positive event was additionally checked by multiplying the mutant fraction with the DNA input amount (in genome equivalents). At least 1 mutant DNA molecule was required to deem a sample positive.

Table S1. Eleven AR ligand-binding domain mutants selected for evaluation

Gene	**Amplicon (exon)**	**Nucleotide position**		**Nucleotide change**	**Codon position**		**Amino acid change**
		Previous	Current [a]		Previous	Current[a]	
AR	4	2507	2148	G > A	715	716	V > M
AR	5	2585	2226	G > T	741	742	W > C
AR	5	2584	2225	G > T	741	742	W > L
AR	8	2982	2623	C > T	874	875	H > Y
AR	8	2991	2632	A > G	877	878	T > A
AR	8	2992	2633	C > G	877	878	T > S
AR	8	3046	2687	T > C	895	896	M > T

AR	8	3045	2686	A > G	895	896	M > V
AR	8	2988	2629	T > C	876	877	F > L
AR	8	2990	2631	C > A	876	877	F > L
AR	8	2990	2631	C > G	876	877	F > L

[a]Current AR nomenclature per AR Gene Mutations Database [6].

Table S2. Baseline demographics and disease characteristics by cohort

	nmCRPC	**AAP-naïve mCRPC**	**Post-AAP mCRPC**	**Total**
	***n* = 51**	***n* = 25**	***n* = 21**	***N* = 97**
Median age, y (range)	71 (51–88)	68 (53–91)	67 (48–83)	69 (48–91)
Race, *n* (%)				
White	47 (92)	25 (100)	19 (90)	91 (94)
Black	3 (6)	0	2 (10)	5 (5)
Asian	1 (2)	0	0	1 (1)
Ethnicity, *n* (%)				
Hispanic	2 (4)	0	0	2 (2)
Not Hispanic	49 (96)	25 (100)	21 (100)	95 (98)
Baseline PSA, ng/ml	10.7	14.7	58.4	14.9
Median (range)	(0.5–201.7)	(1.1–2552.1)	(1.1–6074.3)	(0.5–6074.3)
ECOG PS, *n* (%)				
0	39 (76)	13 (52)	13 (62)	65 (67)
1	12 (24)	12 (48)	8 (38)	32 (33)
Gleason score, *n* (%)				
≤7	29 (57)	7 (28)	14 (67)	50 (52)
8–10	18 (35)	18 (72)	6 (29)	42 (43)
Missing	4 (8)	0	1 (5)	5 (5)

Metastases, *n* (%)				*N* = 46
Bone	–	11 (44)	8 (38)	19 (41)
Soft tissue	–	9 (36)	5 (24)	14 (30)
Prior first-generation androgen receptor antagonist[a]	41 (80)	19 (76)	20 (95)	80 (82)
Bicalutamide	8 (16)	19 (76)	20 (95)	80 (82)
Flutamide	6 (12)	1 (4)	4 (19)	11 (11)
Nilutamide		0	2 (10)	10 (10)

[a]Patients may have been treated with more than 1 first generation androgen receptor antagonist.

AAP, abiraterone acetate plus prednisone; ECOG PS, Eastern Cooperative Oncology Group performance status; mCRPC, metastatic castration-resistant prostate cancer; nmCRPC, nonmetastatic CRPC; PSA, prostate-specific antigen.

Supplementary references

1. Diehl F, Li M, Dressman D et al. Detection and quantification of mutations in the plasma of patients with colorectal tumors. Proc Natl Acad Sci U S A 2005; 102: 16368-16373.
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3. Diehl F, Schmidt K, Choti MA et al. Circulating mutant DNA to assess tumor dynamics. Nat Med 2008; 14: 985-990.
4. Higgins MJ, Jelovac D, Barnathan E et al. Detection of tumor PIK3CA status in metastatic breast cancer using peripheral blood. Clin Cancer Res 2012; 18: 3462-3469.
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