

Patient Name: John Doe	Requesting Physician: David A. Smith, M.D.	Collected: 03/13/2009
Sex: Male	Client/Group Facility: Urology Associates, PA	Received: 03/20/2009
Date of Birth (Age): 10/10/1958 (50 years)	Additional Recipient: Mark Howard, M.D.	Reported: 03/23/2009
SSN: 212-22-2234	Submitting Pathologist: Tim Jackson, M.D.	Case #: S09-123445
Patient ID: 23445921	Specimen ID: S09-2311	Req #:

Prostate BioStrat™ Assay

CLINICAL HISTORY

PSA: 4.5 ng/mL	Clinical Stage: cT1c	Gleason Score: 7(3+4)	# Positive Cores: 2 of 12
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RESULTS

Positive for loss of LPL (8p22) and concurrent additional increase of C-MYC (8q24).

INTERPRETATION

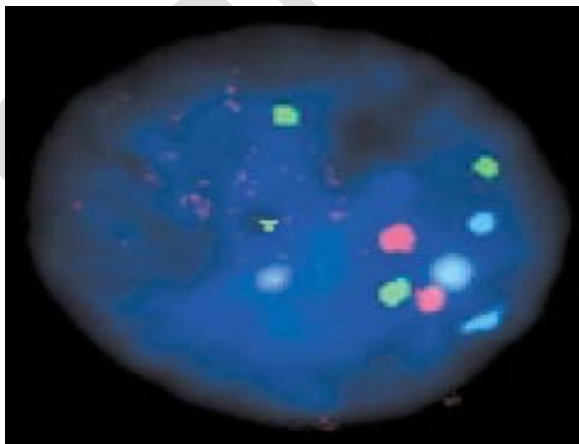
While the clinicopathological parameters (PSA 4.5 ng/mL, Gleason score 7(3+4), clinical stage T1c) of this tumor appear to represent an intermediate risk of clinical recurrence (D'Amico et al, 1998), the cytogenetic profile of this tumor supports an unfavorable-risk stratification, with the tumor positive for 8p22 loss concurrent with 8q24 additional increase.

Studies using fluorescence in situ hybridization (FISH) analysis, have demonstrated that 8q24 overrepresentation, especially concurrent with 8p22 loss, is associated with disease progression and poor survival in organ-confined prostate cancer.¹⁻²

Prostate cancer patients with an unfavorable-risk profile are less likely to achieve high rates of disease control with monotherapy and warrant consideration of alternative modes of therapy and/or neoadjuvant or adjuvant treatment.

The data reported above should be considered along with all other relevant clinical information in reaching a conclusion regarding the most appropriate prostate cancer management strategy.

IMAGE FROM ARIOL® SYSTEM



Abnormal result observed in an interphase prostate tissue cell obtained from a biopsy sample showing four copies of CEP 8 (aqua), four copies of LSI MYC (green), and two copies of LSI LPL (orange) after the Prostate BioStrat™ Assay hybridization.

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ENUMERATION SUMMARY

CHROMOSOME 8, LPL (8p21-22), and C-MYC (8q24)

Total number of cells analyzed: 100

DNA Target	Probe Description	Result	Mean # of Signals/Nuclei	LSI/CEP 8 Ratio	Percent Nuclei with Number of Signals		
					Nuclei w/ 0-1 Signals	Nuclei w/ 2 Signals	Nuclei w/ ≥3 Signals
Chromosome 8	CEP 8 SpectrumAqua	Gain	1.7	N/A	20%	60%	20%
LPL	LSI (8p22) SpectrumOrange	Loss	1.6	0.70	60%	40%	0%
C-MYC	LSI (8q24) SpectrumGreen	Additional Increase	1.6	1.8	20%	50%	30%

Criteria for categorization of chromosome 8, LPL, and C-MYC copy number status.

- Normal (CEP 8, LSI LPL or LSI C-MYC):** Less than 10% of nuclei with ≥3 signals and <55% of nuclei with ≤1 signal.
- Loss (CEP 8):** Fifty-five percent or more of nuclei with ≤1 signal.
- Loss (LSI LPL or LSI C-MYC):** Fifty-five percent or more of nuclei with ≤1 signal or LSI/CEP 8 ratio <0.85.
- Gain (CEP 8):** Ten percent or more of nuclei with ≥3 signals.
- Gain (LSI LPL or LSI C-MYC):** Ten percent or more of nuclei with ≥3 signals or LSI/CEP 8 ratio >1.3.
- Additional Increase (LSI C-MYC):** Ten percent or more of nuclei with ≥3 signals and LSI C-MYC/CEP 8 ratio >1.3.

INTENDED USE

The Prostate BioStrat™ Assay is a fluorescence *in situ* hybridization (FISH) assay that was designed to help assess the severity of prostate cancer at the time of diagnosis. It consists of fluorescently labeled DNA probes to the pericentromeric region of chromosomes 8 (aqua) and to the 8p21-22 (orange) and 8q24 (green) loci containing the lipoprotein lipase (LPL) and C-MYC genes, respectively.

Results from the assay can be used as an aid to distinguish patients with indolent disease from those with clinically relevant cancers thereby allowing for either conservative management (active surveillance and deferred intervention) or more aggressive primary and/or neoadjuvant or adjuvant treatment.

BACKGROUND

Prostate cancer is a complex heterogeneous disease and can have strikingly different clinical behaviors and responses to therapy. Accurate prognostication is a prerequisite for accurate therapeutics and management of prostate cancer because indolent tumors may require no intervention, whereas aggressive tumors lead to patient mortality.

There is a critical need to define these subgroups of patients with prostate cancer differing in clinical outcome. Previous efforts to predict extent of disease and natural history of prostate cancer have focused on Gleason score of the needle biopsy sample, serum prostate-specific antigen (PSA) concentration, and clinical stage.³ Published nomograms based on these three parameters provide useful predictions of clinical states and outcomes,⁴⁻⁵ but they need further refinements to improve accuracy and universality. More accurate prognostic markers for this patient group would permit assignment of more aggressive treatment to those most likely to fail and would justify expectant management (deferral of active treatment) in those likely to have indolent disease.

Occurrence of a variety of chromosomal and genetic alterations has been discovered in prostate cancer. Two significant genetic alterations identified in prostate cancer include gain of the band 8q24 and loss of heterozygosity of 8p21-22.⁶⁻⁸ Gain of 8q24 has been shown to be a common event in prostate cancer, frequently accompanied by 8p21-22 loss of heterozygosity.⁹⁻¹⁰ It is believed that genes mapped to 8p may be involved in the early stage of tumorigenesis.⁸ Over-representation of C-MYC has been associated with prostate cancer progression.¹¹ Multiple probe analysis using DNA FISH probes for 8p22 (LSI LPL), C-MYC (LSI C-MYC) and the centromere of chromosome 8 (CEP 8) has shown that prostate carcinomas have frequent genetic abnormalities of chromosome 8, loss of 8p22 and gains of chromosome 8. Use of multiple FISH probes within a single reagent provides an excellent tool for determining the occurrence of these multiple genetic events on a cell by cell basis in a variety of tissue specimen preparations.

METHODOLOGY

Prostate BioStrat™ analyses are performed on formalin-fixed paraffin embedded tissue sections containing the most characteristic area of prostate cancer cells (i.e., maximum amount of carcinoma and highest Gleason score). Tissue specimens are prepared in sections between 4 and 6 microns thick and mounted on glass slides. Following deparaffinization, protease-digestion and fixation, the DNA is denatured to its single-stranded form and subsequently allowed to hybridize with the Prostate BioStrat™ probes. The probe mixture consists of the CEP 8 probe labeled with SpectrumAqua, LSI LPL labeled with SpectrumOrange and LSI C-MYC labeled with SpectrumGreen. Following hybridization, the unbound probe is removed by a series of washes, and the nuclei are counterstained with DAPI (4,6 diamidino-2-

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phenylindole), a DNA-specific stain that fluoresces blue.

Determination of hybridization results is conducted by enumeration of CEP 8, LSI LPL, and LSI C-MYC signals through automated interphase FISH analysis using the Ariol® digital image analysis system (Genetix, New Milton, UK) equipped with appropriate excitation and emission filters allowing visualization of the aqua, orange, and green fluorescent signals. A minimum of 100 non-overlapping nuclei are analyzed. CEP 8 alpha satellite DNA probe hybridizes to the centromere region of chromosome 8 and provides a mechanism for identification of copy number of chromosome 8 by enumeration of the number of aqua colored probe signals. LSI LPL hybridizes to the LPL gene located at 8p22. Loss of the LPL gene can be determined by enumeration of the orange LPL probe signal. LSI C-MYC hybridizes to the C-MYC gene located at 8q24 and provides information on C-MYC copy number through enumeration of the number of green signals observed in each nucleus. Copy numbers of more or less than two of any of the three probes indicates chromosome or gene gain or loss, respectively. Copy numbers of the LSI LPL or LSI C-MYC relative to the CEP 8 copy number indicate loss of the LPL region or additional gain of the C-MYC region relative to the centromere.

An LSI or CEP 8 is classified as normal if less than 10% of nuclei have 3 or more signals and less than 55% of nuclei have 0 or 1 signal. CEP 8 is classified as loss if 55% or more nuclei have 0 or 1 signal. An LSI is classified as loss, either if 55% or more nuclei have 0 or 1 signal or if the LSI/CEP 8 ratio is less than 0.85. CEP 8 is classified as gain if 10% or more nuclei have 3 or more signals. An LSI is classified as gain if 10% or more nuclei have 3 or more signals or if the LSI/CEP 8 ratio is more than 1.3. An LSI is classified as additional increase (AI) if 10% or more nuclei have 3 or more signals and the LSI/CEP 8 ratio is more than 1.3.

PROSTATE FISH FOR DETERMINING PROSTATE CANCER PROGNOSIS

Prostate BioStrat™ analysis provides information on molecular targets present in a prostate cancer for which are believed to be of prognostic significance based on the published literature.¹⁻² Specifically, assessing loss or gain of the 8p22 locus of chromosome 8, the centromere of chromosome 8, and the 8q24 locus provides a prognostic indicator for prostate cancer.

Given this information, Prostate BioStrat™ results, along with standard clinicopathologic parameters, can be used as an aid to help stratify patients into favorable-, and unfavorable-risk groups for the likelihood of prostate cancer progression. Accordingly, prognosis of a patient is determined to be unfavorable when the hybridization pattern indicates loss of the 8p22 locus, gain of chromosome 8, and additional increase of 8q24 copy number relative to centromere copy number.

Patients stratified into the unfavorable-risk group are more likely to benefit from more aggressive and/or additional therapy as opposed to those patients in the favorable-risk group who may respond to monotherapy or choose active surveillance.

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If you have any questions on this report please do not hesitate to contact the BioVantra client support center at (866) 627-8221.

Peter A. Tsivis, M.D.
Pathologist Electronic Signature

03/23/2009
Date

This test was developed and its performance characteristics determined by BioVantra, LLC. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). However, the FDA has determined that such approval is not necessary. This test is permitted for clinical purposes and should not be regarded as purely investigational or for research. BioVantra is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing.

Photomicrograph is a symbolic representation of the key findings of the Prostate BioStrat™ Assay report. The photomicrograph is not intended to replace a complete review and reading of the final diagnostic report provided.