

Whole-blood RNA Transcript-Based Prognostic Model for Predicting Survival in Men with Castration-Resistant Prostate Cancer (CRPC)

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ABSTRACT

Abstract:

Introduction: Survival for CRPC patients is very variable, from a few months to many years. We developed a whole blood RNA transcript-based diagnostic test as a prognostic biomarker in CRPC.

Methods: From Aug 2006 to June 2008, 62 CRPC patients consented to the collection of whole blood in PAXgene™ blood RNA tubes for gene expression and CellSave™ tubes for circulating tumor cells (CTC). 168 inflammation and prostate cancer-related genes (Source MDx Precision Profiles™) were evaluated using optimized Q-PCR technology to assess biomarkers predictive of survival. Cox type 2 class proportional hazard model was developed from time of CRPC diagnosis and time of blood draw.

Results: A 6-gene model (consisting of ABL2, SEMA4D, ITGAL and C1QA, TIMP1, CDKN1A) correctly predicted 45 of 47 alive CRPC subjects (95.7%) and 14 of 15 dead CRPC subjects (93.3%). The results were similar regardless of the survival time definition (CRPC diagnosis vs. blood draw) and were independent of treatment. The model separated CRPC patients into two groups: high risk men who died within 2.2 yrs of developing castration-resistant disease and low risk men who lived over 2.2 yrs (log rank p=0.00083). Clinical prognostic factors (Halabi nomogram) in 56 patients also predicted low and high risk groups, but the discriminatory value was less than the 6-gene model (p=0.012). CTC counts were not predictive of survival in this cohort.

Conclusions: These data suggest that individual differences in cell-mediated and humoral immunity are associated with survival in men with CRPC. More importantly, using a simple blood assay, we have developed a six-gene model which is strongly associated with survival in these patients. Such an individualized, biologically based test may be a powerful tool to stratify patients for clinical trials and also be useful in the clinical care of CRPC patients. Validation of this model is ongoing.

Patients	N	# Deaths	K-M Mean Survival	p-value
6-Gene Model Risk Group				
all	62	15	76.5 mo	
low risk	48	2	99.1 mo	0.00083
high risk	14	13	21.1 mo	
Clinical Model Risk Group				
all	56	13	79.0 mo	
low risk	44	5	92.1 mo	0.012
high risk	12	8	28.7 mo	

INTRODUCTION

Survival for CRPC patients is variable from several months to over eight years.

Current CRPC clinical trials poorly distinguish patients with more aggressive form of disease.

Biomarkers which independently predict survival can be useful for clinical trial design and for prognostic information for patients and physicians.

METHODS

Source MDx Precision Profiles™, a molecular diagnostic assay based on RNA transcript measurements using a quantitative PCR (QPCR) assay optimized for precision and calibration, was used to analyze CRPC patient whole blood samples.

Key components of the Precision Profile™ assay and associated processes include:

- **PAXgene Blood RNA Tube Sample Processing**

Clinical PAXgene tube whole blood samples processed to total RNA. RNA quality assessed on the Agilent Bioanalyzer 2100.

- **First strand cDNA synthesis**

RNA converted to cDNA in a random hexamer primed reaction with MultiScribe™ reverse transcriptase. cDNA quality checked and used as template in quantitative PCR Precision Profile Assay™.

- **Precision Profile Assay™**

Source MDx proprietary primer probe sets used for target gene specific amplification Individual target genes multiplexed with 18s rRNA endogenous control Assay configured in a 384-well plate formatted for triplicate measures and run on the ABI Prism® 7900HT Sequence Detection System.

- **Precision Profile™ Data Analysis**

Data files are "filtered-by-rule" to ensure all replicate values meet predefined metrics. Normalized gene expression values (delta CT values) for each amplified target gene are calculated (target gene CT - endogenous control CT).

PATIENT POPULATION

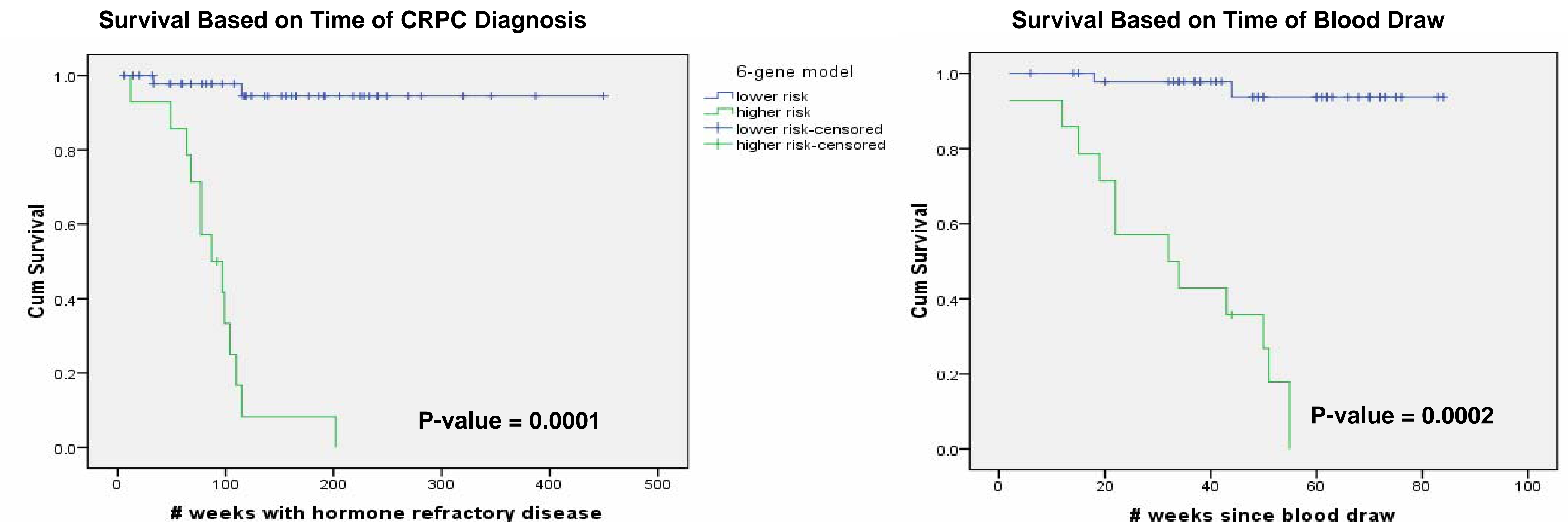
- All patients approached with metastatic CRPC at the time of blood collection

- Primary endpoint: overall survival from time of CRPC onset and from blood collection

Number of patients (n)	62
Age (years) [range]	68 [49-86]
Gleason score at diagnosis, median	7 [5-10]
PSA, median (ng/ml)	21 [0.04-3905]
Alkaline phosphatase, median (IU/L)	93 (27-1565)
Metastatic disease	54 (87%)
Alive at time of analysis	47 (76%)
Duration since CRPC at blood draw	16 months [0-93 mo]

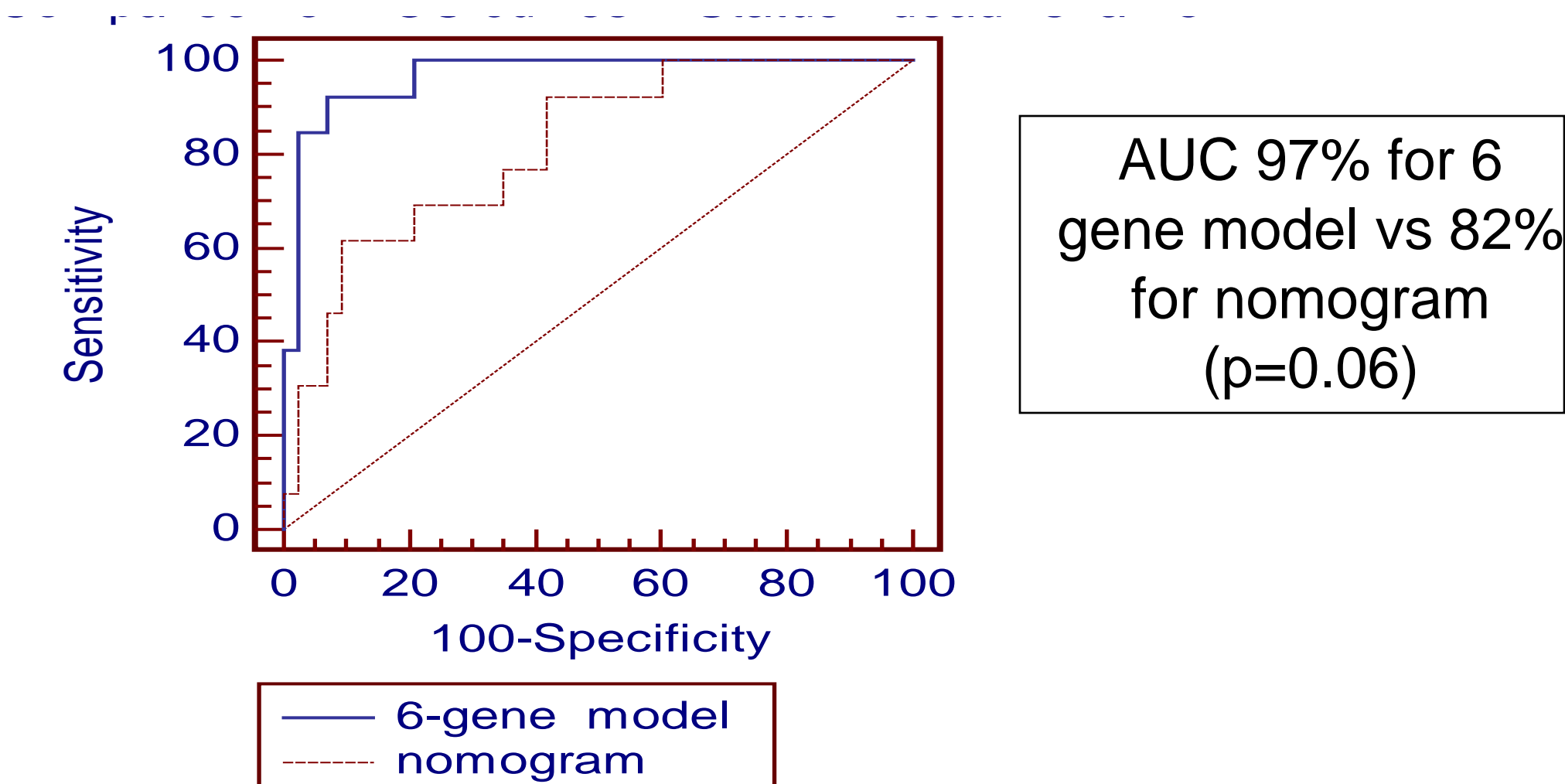
RESULTS

Kaplan Meier Survival Assessment Prediction Using 6-Gene Model



Lower risk: 2*ABL2 + SEMA4D + ITGAL -C1QA -TIMP1 -CDKN1A < 21.21
Higher risk: 2*ABL2 + SEMA4D + ITGAL -C1QA -TIMP1 -CDKN1A > 21.21

ROC Curves Predict Survival for 6 Gene Model and Halabi Nomogram



CTCs and Survival

- CTC counts ranged from 0 to 931.
- When CTCs are included in a Cox model as a continuous predictor of survival, p-value > 0.4 (N = 53; 9 missing).
- If the missing group is excluded and CTCs are divided into 3 groups (0, 1-10 and >10), CTCs are significant predictors of survival (p=.01).
- However, when included as an additional covariate with the 6-gene model risk score, the 6-gene model risk score is highly significant (p=.000001) but the grouped numeric CTC count is not significant (p=.22).

The Data Suggest a "Biasing" of the Immune System Towards Macrophage Differentiation and a Decrease in BOTH Cell-Mediated and Humoral Immunity in Patients with Higher Mortality

Gene	Protein	Effect of Observed Change			Discussion
		Cellular Immunity	Humoral Immunity	Mortality	
C1QA	Complement C1q subunit A	↓	↓	↑	The up-regulation of these genes in patients with increased mortality is indicative of a process that is driving monocyte differentiation (CDKN1A) towards the production of tissue macrophages (C1Qa and TIMP1) and, by default, away from dendritic cells. This process will result in a relative down-regulation of cellular and humoral immunity in these patients.
CDKN1A	Cyclin-dependent kinase inhibitor 1A	↓	↓	↑	
TIMP1	Metalloproteinase inhibitor 1	↓	↓	↑	
ABL2	Tyrosine-protein kinase ABL2	↓	↓		The down-regulation of these genes in patients with increased mortality is indicative of a down-regulation of cellular and humoral immunity. ABL2, semaphorin-4D and LFA-1 are components of an integrated system required for T-cell motility, antigen surveillance and T-helper cell activity with respect to B-cell activation.
ITGAL	Integrin alpha-L (LFA-1)	↓	↓		
SEMA4D	Semaphorin-4D	↓	↓		

CONCLUSIONS

- Individual differences in cell-mediated and humoral immunity are associated with survival in men with CRPC.
- Using a simple blood assay, a six-gene model is strongly associated with survival in CRPC patients.
- An individualized, biologically based test could stratify patients for clinical trials and be useful in the clinical care of CRPC patients.
- Validation of this model is ongoing.