AR-V7 Transcripts in Whole Blood RNA of Patients with Metastatic Castration Resistant Prostate Cancer Correlate with Response to Abiraterone Acetate

Tilman Todenhöfer,* Arun Azad,* Craig Stewart, Jian Gao, Bernhard J. Eigl, Martin E. Gleave, Anthony M. Joshua, Peter C. Black and Kim N. Chi†

From the Vancouver Prostate Centre, University of British Columbia (TT, CS, JG, MEG, PCB) and British Columbia Cancer Agency (AA, BJE), British Columbia (KNC), Vancouver and Princess Margaret Cancer Centre (AMJ), Toronto, Ontario, Canada, Department of Urology, University Hospital (TT), Tübingen, Germany, and Department of Medicine, Monash University (AA), Melbourne, Victoria, Australia

Purpose: The expression of AR-V7 (androgen receptor splice variant) 7 in circulating tumor cells has been associated with resistance to abiraterone and enzalutamide in patients with metastatic castration resistant prostate cancer. We used a sensitive, whole blood reverse transcriptase-polymerase chain reaction assay that does not require circulating tumor cell enrichment to correlate outcomes of abiraterone with whole blood expression of AR-V7 and other prostate cancer associated transcripts.

Materials and Methods: We assessed the expression of AR-V7, FOXA1, GRHL2, HOXB13, KLK2, KLK3 and TMPRSS2:ERG mRNA in 2.5 ml whole blood from each of 27 patients with metastatic castration resistant prostate cancer and 33 controls without cancer as the discovery cohort. Cycle threshold values of controls with the highest gene expression were set as the threshold for a positive test. Thresholds were then applied to a validation cohort of 37 patients with metastatic castration resistant prostate cancer who were commencing abiraterone. Gene expression was correlated with the prostate specific antigen response rate using the chi-square test, and with time to prostate specific antigen progression and overall survival using the log rank test.

Results: In the discovery cohort 3 of 27 patients (11.1%) with metastatic castration resistant prostate cancer were AR-V7 positive vs 4 of 37 (10.8%) in the validation cohort. In the validation cohort patients with a positive AR-V7 test had a lower prostate specific antigen response rate (0% vs 42%, $p = 0.27$) together with shorter median prostate specific antigen progression (0.7 vs 4.0 months, $p < 0.001$) and median overall survival (5.5 vs 22.1 months, $p < 0.001$).

Abbreviations and Acronyms

ALP = alkaline phosphatase
AR = androgen receptor
CRPC = castration resistant prostate cancer
Ct = cycle threshold
CTC = circulating tumor cell
mCRPC = metastatic CRPC
PCa = prostate cancer
PCR = polymerase chain reaction
PFS = progression-free survival
PSA = prostate specific antigen
qPCR = quantitative PCR

Accepted for publication June 24, 2016.

No direct or indirect commercial incentive associated with publishing this article.

The corresponding author certifies that, when applicable, a statement(s) has been included in the manuscript documenting institutional review board, ethics committee or ethical review board study approval; principles of Helsinki Declaration were followed in lieu of formal ethics committee approval; institutional animal care and use committee approval; all human subjects provided written informed consent with guarantees of confidentiality; IRB approved protocol number; animal approved project number.

Supported by the CUA-CUOG (Canadian Urological Association-Canadian Urological Oncology Group) Astellas Research Grant Program funded by Astellas Pharma Canada, Inc., and jointly established by Astellas Pharma Canada, Inc., CUA and CUOG.

* Equal study contribution.

† Correspondence: Department of Medical Oncology, British Columbia Cancer Agency, 600 West 10th Ave., Vancouver, British Columbia, V5Z 4E6, Canada (telephone: +1-604-877-6000; FAX: +1-604-877-0585; (e-mail: kchi@bccancer.bc.ca).

See Editorial on page 8.

0022-5347/17/1971-0135/0
THE JOURNAL OF UROLOGY®

http://dx.doi.org/10.1016/j.juro.2016.06.094
Vol. 197, 135-142, January 2017
Printed in U.S.A.

Conclusions: Reverse transcriptase-polymerase chain reaction detection of AR-V7 transcripts in whole blood was associated with inferior outcomes in patients treated with abiraterone. These results reinforce the potential usefulness of AR-V7 as a prognostic and predictive biomarker for metastatic castration resistant prostate cancer.

Key Words: prostatic neoplasms, castration-resistant; neoplasm metastasis; receptors, androgen; biomarkers, tumor; abiraterone

THE treatment of CRPC has changed dramatically in the last decade. New drugs targeting the AR axis, such as abiraterone acetate (abiraterone) and enzalutamide, are able to prolong overall survival and improve health related quality of life.[1–6] However, for optimal treatment selection and sequencing models are urgently needed that predict the response to abiraterone, enzalutamide and taxanes.

Clinical models were recently introduced with modest prognostic and predictive value.[7–9] In addition, there has been intense recent research in the field of circulating biomarkers in an effort to improve the prediction of outcome and response.[10,11] We and others recently identified associations between AR and other gene aberrations in circulating cell-free DNA with resistance to enzalutamide and abiraterone.[11–14]

Because AR-Vs are an important mechanism for the development and progression of CRPC, they represent an attractive potential biomarker.[15–17] AR-Vs are isoforms coding only for the DNA binding and transactivation domains. This leads to a truncated AR that is constitutively active and, due to the lack of the normal ligand binding domain, is also impervious to inhibition by conventional AR targeted agents. In patients the detection of AR-V7, the most commonly expressed AR-V, has been shown to be associated with resistance to abiraterone and enzalutamide.[18] PCa associated transcripts such as ARV7 have been assessed as predictive markers mainly through molecular characterization of CTCs.[18–20] Most of these CTC platforms are relatively expensive and require CTC enrichment and analysis within a few days or earlier after blood collection.

Using specific collection tubes such as the PAXgene® system, whole blood RNA can be stabilized and stored for several years with minimal degradation.[21] Importantly, these platforms provide protocols for standardized extraction of RNA with no need for specialized laboratory equipment. This system has been used for the discovery of whole blood RNA gene expression profiles that correlate with prognosis in patients with mCRPC.[22,23]

The aim of the current study was to correlate the expression of select PCa associated transcripts, particularly AR-V7, with the response to abiraterone in patients with mCRPC.

PATIENTS AND METHODS

Patients

A discovery cohort comprising 27 heavily pretreated patients with mCRPC and 33 male controls without PCa was used to analyze the presence of PCa associated transcripts and determine thresholds for a positive test. Men with mCRPC in the discovery cohort were recruited at British Columbia Cancer Agency. They were required to have received abiraterone, enzalutamide, docetaxel and/or cabazitaxel. Healthy controls were recruited from volunteers and from patients without prostate cancer attending the urology clinic at Vancouver Prostate Centre. All healthy controls older than 50 years were required to have serum PSA less than 1 ng/ml and a negative digital rectal examination.

A validation cohort was constructed of 37 patients with mCRPC receiving abiraterone in a prospective biomarker clinical study (ClinicalTrials.gov NCT01857908) performed at 2 Canadian centers, including Princess Margaret Cancer Center and British Columbia Cancer Agency. The study was approved by the clinical research ethics board at each center. Patients were permitted to enroll in this clinical study before and after receiving docetaxel. PSA and other laboratory tests for monitoring were performed monthly in line with provincial guidelines. Imaging was done at the discretion of the participating investigators.

Gene Selection

Our marker panel included FOXA1, GRHL2, HOXB13, KLK2 and KLK3 because these genes were previously described to be significantly over expressed in the whole blood of patients with mCRPC and to correlate with outcome.[23] Moreover, due to potential predictive relevance in mCRPC we assessed the expression of AR-V7 and TMPRSS2:ERG.[24]

Blood Collection and RNA Isolation

Peripheral blood (2.5 ml) was collected in a PAXgene RNA blood tube and stored at −80C according to the manufacturer protocol. Blood was thawed overnight and RNA extraction was performed using the PAXgene blood RNA kit according to the manufacturer protocol. The supplementary material (http://jurology.com/) shows RNA quality control.

Reverse Transcription

Reverse transcription was performed with the SuperScript® Vilo™ cDNA Synthesis Kit according to the manufacturer protocol. RNA (10 μl) was used in a total reaction volume of 20 ul.