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ORIGINAL ARTICLE

Transcriptional profiling of primary prostate tumor in metastatic hormone-sensitive prostate cancer and association with clinical outcomes: correlative analysis of the E3805 CHAARTED trial☆

A. A. Hamid[1,2], H.-C. Huang[3], V. Wang[4], Y.-H. Chen[4], F. Feng[5], R. Den[6], G. Attard[7], E. M. Van Allen[1], P. T. Tran[8], D. E. Spratt[9], R. Dittamore[3], E. Davicioni[3], G. Liu[10], R. DiPaola[11], M. A. Carducci[8] & C. J. Sweeney[1*]

[1]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, USA; [2]University of Melbourne, Melbourne, Australia; [3]Decipher Biosciences, San Diego; [4]Department of Data Science, Dana-Farber Cancer Institute, Boston; [5]Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, San Francisco; [6]Department of Radiation Oncology, Sidney Kimmel Medical College at Thomas Jefferson University, Philadelphia, USA; [7]University College London Cancer Institute, London, UK; [8]Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore; [9]Department of Radiation Oncology, University of Michigan, Ann Arbor; [10]University of Wisconsin Carbone Cancer Center, Madison; [11]University of Kentucky Medical Center, Lexington, USA

Available online 12 June 2021

Background: The phase III CHAARTED trial established upfront androgen-deprivation therapy (ADT) plus docetaxel (D) as a standard for metastatic hormone-sensitive prostate cancer (mHSPC) based on meaningful improvement in overall survival (OS). Biological prognostic markers of outcomes and predictors of chemotherapy benefit are undefined.

Patients and methods: Whole transcriptomic profiling was performed on primary PC tissue obtained from patients enrolled in CHAARTED prior to systemic therapy. We adopted an *a priori* analytical plan to test defined RNA signatures and their associations with HSPC clinical phenotypes and outcomes. Multivariable analyses (MVAs) were adjusted for age, Eastern Cooperative Oncology Group status, *de novo* metastasis presentation, volume of disease, and treatment arm. The primary endpoint was OS; the secondary endpoint was time to castration-resistant PC.

Results: The analytic cohort of 160 patients demonstrated marked differences in transcriptional profile compared with localized PC, with a predominance of luminal B (50%) and basal (48%) subtypes, lower androgen receptor activity (AR-A), and high Decipher risk disease. Luminal B subtype was associated with poorer prognosis on ADT alone but benefited significantly from ADT + D [OS: hazard ratio (HR) 0.45; $P = 0.007$], in contrast to basal subtype which showed no OS benefit (HR 0.85; $P = 0.58$), even in those with high-volume disease. Higher Decipher risk and lower AR-A were significantly associated with poorer OS in MVA. In addition, higher Decipher risk showed greater improvements in OS with ADT + D (HR 0.41; $P = 0.015$).

Conclusion: This study demonstrates the utility of transcriptomic subtyping to guide prognostication in mHSPC and potential selection of patients for chemohormonal therapy, and provides proof of concept for the possibility of biomarker-guided selection of established combination therapies in mHSPC.

Key words: metastatic prostate cancer, docetaxel, Decipher, gene expression profiling, biomarker

INTRODUCTION

Most men with metastatic hormone-sensitive prostate cancer (mHSPC) respond to testosterone suppression, commonly referred to as androgen-deprivation therapy (ADT), achieved by medical or surgical castration; however, the durability of response and time to castration resistance are variable. The treatment paradigm of mHSPC has changed rapidly in the last 7 years, with improvements in overall survival (OS) demonstrated first by concurrent use of cytotoxic chemotherapy [docetaxel (D)][1-3] and agents targeting the androgen receptor (AR) axis by inhibition of extragonadal androgen synthesis (abiraterone acetate)[4,5] or direct AR antagonism (enzalutamide; apalutamide),[6,7] with a backbone of ADT. The phase III randomized CHAARTED study was the first trial to demonstrate a marked improvement in time to castration-resistant PC (ttCRPC) and OS with ADT + D versus ADT alone.[1] Subgroup analyses have suggested that the OS benefit from chemohormonal therapy is consistently evident in patients who present with high-volume metastatic disease.[2,8]

*Correspondence to: Prof. Christopher J. Sweeney, Department of Medical Oncology, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215, USA. Tel: +1-617-582-7221

E-mail: Christopher_Sweeney@dfci.harvard.edu (C.J. Sweeney).

☆Note: This study was presented in part at the Oral Abstract Session, *American Society of Clinical Oncology Genitourinary Cancer Symposium*, San Francisco, February 2020.

Currently, there are no validated molecular biomarkers to personalize treatment in mHSPC to guide which men should receive ADT + D or AR-targeted therapy, resulting in a critical unmet need. Biomarker-informed prediction of chemohormonal therapy benefit may offer greater precision than clinical factors such as disease volume. Metastatic PC is associated with increased, but nonetheless modest, DNA mutational burden and the majority of primary tumors do not harbor genomic alterations associated with selective sensitivity to available treatments.[9,10] By contrast, discrete transcriptomic subgroups of PC have been identified as prognostic for a greater risk of metastatic relapse from localized HSPC, namely, intrinsic luminal—basal subtype using the PAM50 classifier (luminal A, luminal B and basal subgroups), the Decipher genomic classifier (GC), and AR activity (AR-A; classified as average versus lower).[11-13] In localized HSPC, luminal B subtype is associated with higher AR-A score and poorer prognosis. Patients with lower AR-A tumors may have an attenuated response to ADT alone in the adjuvant setting. Prior work by our group using gene expression-based models of drug sensitivity (derived from analyses of diverse cancer cell lines) showed that luminal and high AR activity (AR-A) subtypes are predicted to have greater sensitivity to taxane chemotherapy, compared with basal and low AR-A subtypes.[13]

These classifiers represent unique biological profiles of HSPC. Their clinical utility in the context of (chemo)hormonal therapy for metastatic disease remains unknown. We, therefore, leveraged primary PC samples from patients enrolled in the CHAARTED trial and sought to define the transcriptional landscape of mHSPC and the impact of these signatures on outcomes with ADT alone as a prognostic biomarker, and with the addition of docetaxel as a potential predictive biomarker.

METHODS

Trial and correlative study design

The primary objective of the CHAARTED trial was to determine whether docetaxel would improve OS in men with mHSPC commencing ADT. The clinical trial was designed by the Eastern Cooperative Oncology Group-ACRIN Cancer Research Group (ECOG-ACRIN). Sanofi provided docetaxel for study conduct and grant support for pilot correlative studies but had no role in protocol design, data analysis, or preparation of the current manuscript. Decipher Biosciences completed gene expression profiling as in-kind support and aided in data interpretation. This correlative substudy followed a National Clinical Trials Network (NCTN)-approved ancillary project analysis plan, with exploratory components as noted. Patients consented to use of their samples and Institutional Review Board (Dana-Farber Cancer Institute) approval was obtained.

Patients, RNA processing, and microarray profiling

The ECOG-ACRIN biobank retrieved available formalin-fixed, paraffin-embedded (FFPE) biopsy and radical prostatectomy samples from patients enrolled in the CHAARTED trial. Deidentified specimens were sent to Decipher Biosciences (San Diego, CA) for central pathology review. The highest grade tumor focus was identified and underwent RNA extraction after macrodissection by a genitourinary pathologist. At least 0.5 mm^2 of tumor with at least $\geq$60% tumor cellularity was required for the assay. RNA was extracted using the RNeasy FFPE kit (Qiagen, Germantown, MD), converted into cDNA and amplified using the Ovation FFPE kit (TECAN Genomics, Redwood City, CA) and hybridized to the Human Exon 1.0 ST oligonucleotide microarray (Thermo Fisher, Carlsbad, CA), as previously described,[14] in a Clinical Laboratory Improvement Amendments-certified laboratory facility (Decipher Biosciences, San Diego, CA). Quality control was performed using Affymetrix Power Tools, and normalization was performed using the single-channel array normalization (SCAN) algorithm. A total of 198 of 790 patients (25%) had banked FFPE tumor blocks available for profiling. Among the 190 samples with sufficient tumor available for RNA profiling, 160 samples (84%) passed quality control for downstream analysis.

Correlative study design

The NCTN prespecified analysis plan included Decipher GC score and AR-A. With the emergence of data regarding luminal—basal subtyping as a prognostic biomarker in localized PC[12] and as a potential predictive marker of taxane benefit from *in silico* modeling,[15] we expanded our *a priori* analysis plan to include this classifier as a third putative biomarker.

Transcriptomic signatures

PAM50 subtyping consists of three PC-relevant subtypes (luminal A, luminal B, and basal-like). Previously developed cut points were used to call subtypes, based on the 50-gene messenger RNA signature developed in breast cancer,[16] with the exclusion of the Her2-enriched subtype. True Decipher scores (continuous scale of 0-1) were generated based on 22 transcripts as previously described.[14] Categorical GC results are presented by quartile based on the analytic cohort of 160 samples; given that the middle two quartiles have comparable prognoses, the two quartiles are grouped to form three groups: [0, 0.568], (0.568, 0.835], (0.835, 1]. The commercial cut points of the GC were not used as they were optimized in localized PC. The AR-A score comprises nine canonical AR transcriptional target genes (*KLK3, KLK2, FKBP5, STEAP1, STEAP2, PPAP2A, RAB3B, ACSL3,* and *NKX3-1*). The AR-A model was used with the previously locked cut point (score of 11) to define lower versus average AR-A.[13]

Endpoints

The primary endpoint of CHAARTED and this ancillary study was OS, defined as the time from randomization to death from any cause. Secondary endpoints included ttCRPC, defined as the time from randomization to prostate-specific antigen (PSA) and/or clinical progression (excluding death

as an endpoint), with a testosterone level of <50 ng/dL or documentation of gonadal suppression at progression. As the primary analyses, biomarkers were assessed for the ability to independently associate with ttCRPC and OS in the full analytic cohort. Subsequently, the biomarkers were assessed within the ADT arm and the ADT + D arm, to determine whether a differential treatment effect with the addition of docetaxel existed by transcriptomic subgroup.

Statistical analysis

OS and ttCRPC were estimated by the Kaplan–Meier method and the log-rank test was used for comparison, in keeping with the original trial analysis plan. The prognostic ability of biomarker subgroups on OS and ttCRPC was assessed across the analytic cohort using Cox univariable (UVA) and multivariable analyses (MVA) with Firth's penalized method.[17] Covariables in the MVA models were age, ECOG performance status (PS), prior local therapy, volume of disease (as defined by the CHAARTED trial[1]), and treatment arm. In the trial cohort, all patients who did not receive prior local therapy presented with *de novo* metastatic disease, and all patients who received prior local therapy presented with recurrent (metachronous) metastatic disease. Multiple testing adjusted (MT-adj) results using Bonferroni correction for three signatures were performed within each endpoint for the primary analyses. This ancillary study was not powered to detect a treatment–biomarker interaction and was designed as a training set for related mHSPC trials.[3,6] We estimated <30% power to identify a treatment–biomarker interaction on OS with the current sample size when postulating a hazard ratio (HR) of no smaller than 0.6 with a two-sided alpha of 0.05, and thus interaction tests were not performed. Treatment effect in each biomarker subset was illustrated by Cox biomarker-subset UVAs, with HRs and 95% confidence intervals (CIs). Statistical analyses were performed using R, version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). All statistical tests were two-sided and a P value <0.05 was deemed statistically significant.

RESULTS

Biopsy and cohort characteristics

The treatment arms of the final analytic cohort (76 in the ADT arm, 84 in the ADT + D arm; Supplementary Figure S1, available at https://doi.org/10.1016/j.annonc.2021.06.003) were balanced with respect to clinical prognostic variables such as age, ECOG PS, volume of disease, and receipt of prior local therapy (Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2021.06.003). The median follow-up was 4 years. A significant OS improvement favoring ADT + D was observed in the analytic cohort [median OS 53.9 versus 32.4 months; HR 0.58, 95% CI 0.38-0.87; $P = 0.009$]. Compared with the trial cohort, there was a higher proportion of patients with poor prognostic features including *de novo* metastatic (88% versus 73%) and high-volume (78% versus 65%) disease (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2021.06.003).

Landscape of transcriptomic subtypes in primary prostate cancer specimens of patients with mHSPC

The relative frequencies of transcriptomic subtypes were discovered to differ from the frequencies reported in non-mHSPC, consistent with enrichment in mHSPC of transcriptional profiles associated with a higher risk of metastatic progression. The distribution of luminal–basal subtypes in mHSPC were as follows: basal 50%, luminal B 48%, and luminal A 2%, compared with 34%, 33% and 33%, respectively, in localized PC,[12] and 65%, 30% and 5%, respectively, in nonmetastatic CRPC (nmCRPC).[18] The median GC score was 0.72, and 71% was Decipher high risk compared with 0.37 and 16.5%, respectively, in localized PC.[11] About 42% of patients with mHSPC had lower AR-A compared with 58% in nmCRPC,[18] but only 10% in localized PC.[13] All three transcriptomic biomarkers were well-balanced by treatment arm (Supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2021.06.003). Samples with higher Decipher scores tended to have higher luminal B scores, although these interbiomarker correlations were relatively weak, indicating no substantial overlap between subtypes. Furthermore, strong correlation between biomarker scores and volume of disease was not observed, with the exception of AR-A where high-volume disease was significantly associated with lower AR-A scores (median AR-A in low versus high volume: 12 versus 11; $P = 0.042$; Figure 1); 48.6% and 18.4% of low- and high-volume subgroups had AR-A scores in the highest quartile, respectively. AR-A did not correlate strongly with luminal B nor Decipher scores.

Clinical outcomes of patients by luminal–basal (PAM50) subtype

Only three patients (2%) were classified with luminal A disease and all were alive at their last follow-up. Greater than 50% of patients in luminal B and basal groups had died. There were no significant differences between luminal B or basal groups in the overall cohort with respect to OS or ttCRPC in UVA or MVA (OS: $P = 0.298$, MT-adj $P = 0.894$; ttCRPC: $P = 0.399$, MT-adj $P > 0.99$; Table 1 and Supplementary Figure S2A, available at https://doi.org/10.1016/j.annonc.2021.06.003).

Survival in the ADT alone arm and the relative treatment effect of docetaxel differed by luminal–basal subtype. Consistent with a prior report in the localized PC setting,[12] luminal B subtype was associated with poorer OS on ADT alone versus basal subtype (median OS: 29.8 versus 47.1 months; HR 1.75, 95% CI 0.99-3.10; $P = 0.052$; Figure 2A). We then tested the OS benefit associated with the addition of docetaxel split by transcriptomic subtype. Patients with basal disease showed no evidence of a significant OS benefit from docetaxel

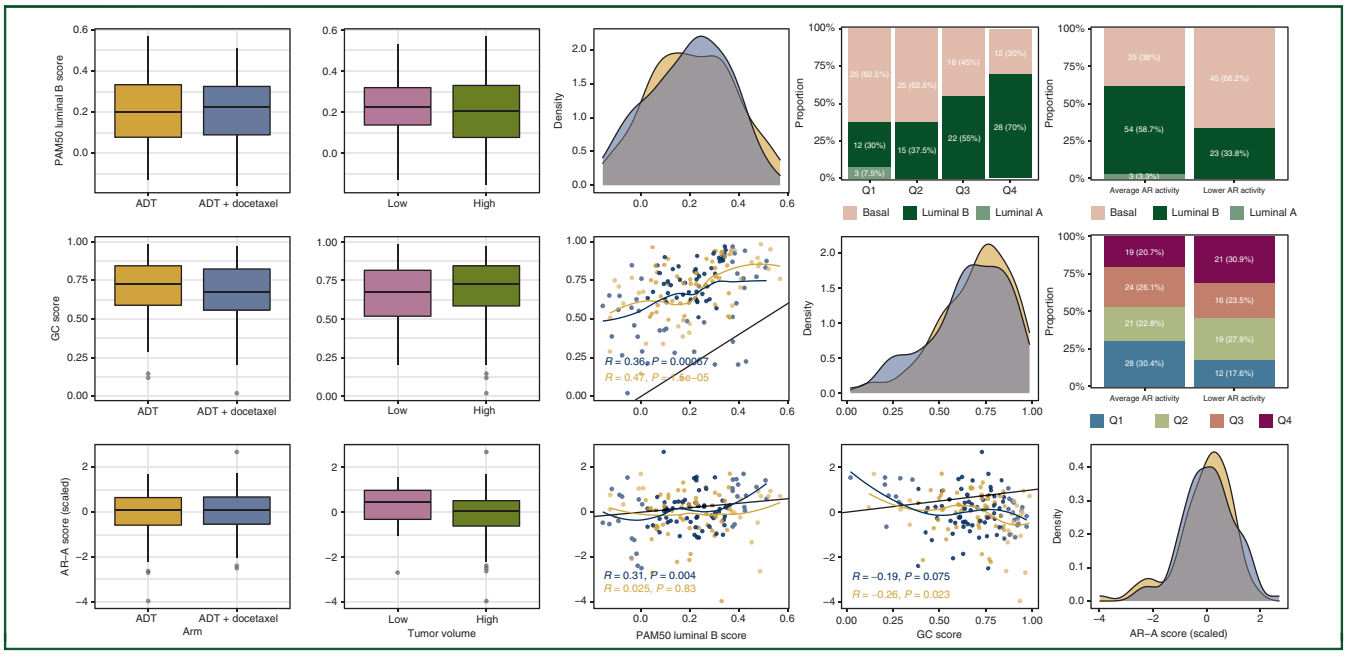

Figure 1. Pairs plot of transcriptomic signatures by treatment arm and volume of disease.
Orange denotes the androgen-deprivation therapy (ADT) arm; blue denotes the ADT plus docetaxel arm. Axes represent the range of the respective biomarker scores; box and whisker plots represent the median, interquartile range, and range of biomarkers scores within a given subgroup (left two columns). Scatterplots and density plots represent continuous interbiomarker correlations and distributions, respectively, with correlation (R) denoting Pearson's coefficient with corresponding *P* value. Bar graphs represent categorical interbiomarker distributions with subtypes defined as described in study methodology.
AR-A, androgen receptor activity; GC, Genomic classifier (Decipher); Q1, lowest quartile; Q2-3, middle quartiles; Q4, highest quartile.

(median OS: 47.1 versus 49.2 months; HR 0.85, 95% CI 0.47-1.54; $P = 0.584$; Figures 3 and 4A), even in the subgroup of patient with high-volume disease. In the luminal B subgroup, there was an improvement in OS with docetaxel (median OS: 29.8 versus 52.1 months; HR 0.45, 95% CI 0.25-0.81; $P = 0.007$), suggesting a potential treatment—biomarker interaction. No substantial differences in receipt of OS-improving therapies upon disease progression were noted when comparing luminal B and basal subtypes (Supplementary Table S4, available at https://doi.org/10.1016/j.annonc.2021.06.003). No differential treatment benefit by subtype was observed with respect to ttCRPC (Figure 4B and Supplementary Figures S3A and S4A, available at https://doi.org/10.1016/j.annonc.2021.06.003).

Clinical outcomes of patients by Decipher score (GC)

In the overall cohort, GC significantly stratified both ttCRPC and OS, with Q1, Q2-3, and Q4 cut-off subgroups showing 3-year OS rates of 77%, 60%, and 31%, respectively (Supplementary Figure S2B, available at https://doi.org/10.1016/j.annonc.2021.06.003). On MVA, continuous GC scores were independently associated with OS (HR 1.21, 95% CI 1.08-1.36 per 0.1-unit increase; $P < 0.001$, MT-adj $P = 0.002$) and ttCRPC (HR 1.17, 95% CI 1.07-1.29; $P <$

Table 1. Multivariable analysis of OS and time to CRPC.

Model	Variable	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
		PAM50 luminal B—basal B		AR-A score		GC score	
OS	Genomic signature	1.25 (0.82-1.91)	0.298	0.91 (0.84-0.99)	0.024	1.21 (1.08-1.36)	<0.001
	ADT + docetaxel versus ADT	0.63 (0.42-0.95)	0.027	0.59 (0.39-0.89)	0.012	0.59 (0.39-0.89)	0.011
	Age	1.00 (0.98-1.02)	0.929	1.00 (0.98-1.03)	0.902	1.00 (0.98-1.02)	0.844
	ECOG 1-2 versus 0	1.77 (1.15-2.70)	0.010	1.73 (1.13-2.63)	0.013	1.65 (1.06-2.51)	0.025
	Prior local treatment versus none	1.20 (0.60-2.19)	0.580	1.37 (0.68-2.50)	0.354	1.40 (0.70-2.55)	0.319
	Tumor volume high versus low	1.82 (1.05-3.39)	0.032	1.82 (1.06-3.36)	0.030	2.01 (1.16-3.73)	0.012
ttCRPC	Genomic signature	1.18 (0.81-1.72)	0.399	0.93 (0.86-1.00)	0.049	1.17 (1.07-1.29)	<0.001
	ADT + docetaxel versus ADT	0.48 (0.33-0.69)	<0.001	0.46 (0.32-0.67)	<0.001	0.47 (0.32-0.68)	<0.001
	Age	0.98 (0.96-1.00)	0.133	0.99 (0.97-1.01)	0.173	0.98 (0.96-1.00)	0.108
	ECOG 1-2 versus 0	1.51 (1.00-2.24)	0.049	1.43 (0.95-2.12)	0.083	1.47 (0.98-2.18)	0.062
	Prior local treatment versus none	0.90 (0.47-1.60)	0.737	0.96 (0.50-1.70)	0.899	1.06 (0.55-1.88)	0.853
	Tumor volume high versus low	2.41 (1.47-4.18)	<0.001	2.44 (1.49-4.21)	<0.001	2.65 (1.61-4.60)	<0.001

Hazard ratios of luminal—basal classifier are reported for luminal B subtype versus basal subtype (as reference). HRs of GC score are reported per 0.1-unit increase. HRs of AR-A score are reported per 1-unit increase.
ADT, androgen-deprivation therapy; AR-A, androgen receptor activity; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group (performance status); GC, Genomic classifier (Decipher); OS, overall survival; ttCRPC, time to castration resistant prostate cancer.

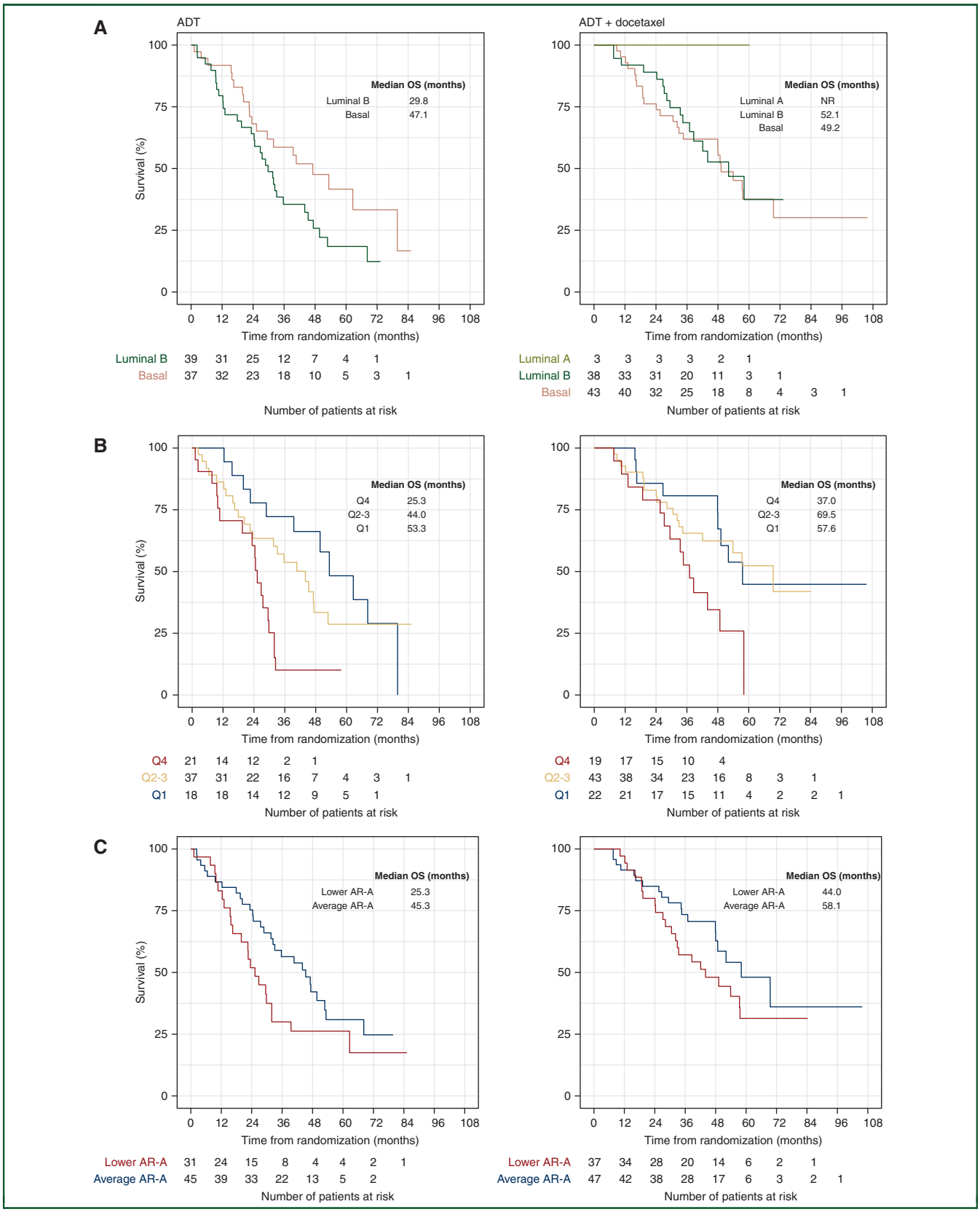

Figure 2. Kaplan-Meier estimates of overall survival (OS) in treatment arms by transcriptomic signatures.
(A) Luminal—basal subtype, (B) genomic classifier (GC; Decipher) subgroup, and (C) androgen receptor activity (AR-A) subtype.
Q1, lowest quartile; Q2-3, middle quartiles; Q4, highest quartile. ADT, androgen-deprivation therapy.

https://doi.org/10.1016/j.annonc.2021.06.003

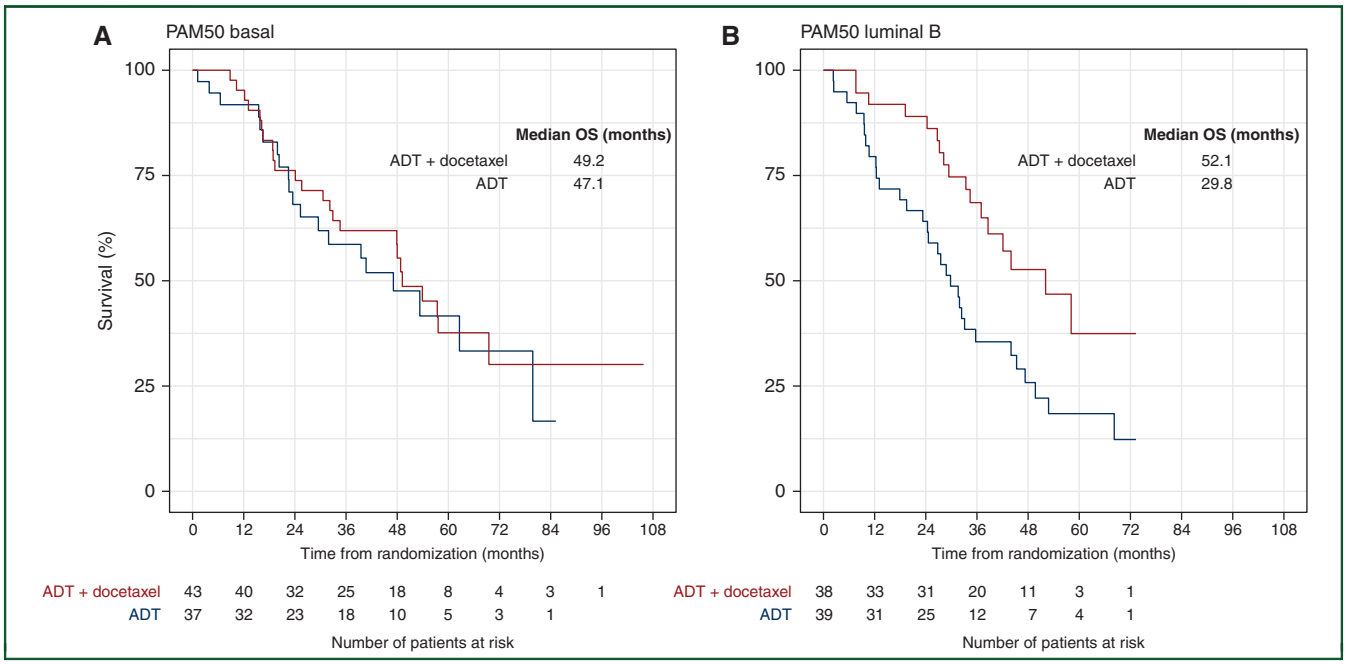

Figure 3. Kaplan-Meier estimates of overall survival (OS) by treatment arm within luminal–basal subtypes.
(A) Basal subtype and (B) luminal B subtype.
ADT, androgen-deprivation therapy.

0.001, MT-adj $P = 0.002$; Table 1). Similar results were seen when GC was analyzed categorically (not shown). The effect of docetaxel on OS was observed across all GC groups; however, the relative benefit of chemohormonal therapy varied by GC group was significant with higher GC (higher risk) disease (Q1: HR 0.72, 95% CI 0.29-1.73; Q2-3: HR 0.57, 95% CI 0.30-1.05; and Q4: HR 0.41, 95% CI 0.19-0.84; Figure 4A). This can be represented as an absolute benefit in OS for addition of docetaxel to ADT for men with tumors in GC Q1 versus GC Q4 of 9% versus 25% at 3 years, respectively (Supplementary Figure S5, available at https://doi.org/10.1016/j.annonc.2021.06.003).

Clinical outcomes of patients by AR activity (AR-A)

The transcriptional signature of AR-A was prognostic. Lower AR-A exhibited both shorter ttCRPC and OS; in the overall cohort, 3-year OS was 45% versus 65% and 1-year CRPC-free survival was 47% versus 58% in lower compared with average AR-A subtypes, respectively (Supplementary Figure S2C, available at https://doi.org/10.1016/j.annonc.2021.06.003). As a continuous variable, a 1-unit increase in AR-A score had a multivariable HR of 0.91 and 0.93 for OS and ttCRPC ($P = 0.024$ and 0.049; MT-adj $P = 0.072$ and 0.147), respectively (Table 1).

Consistent with prior studies in localized PC, lower AR-A was associated with rapid development of CRPC compared with average AR-A patients treated with ADT alone; the 6-month CRPC-free rates were 40.7% versus 73.0%, respectively [Supplementary Figures S3C and S4C (left panel), available at https://doi.org/10.1016/j.annonc.2021.06.003]. By contrast, there was no association with AR-A and differential benefit from chemohormonal therapy in decreasing the rate of castration resistance or death. A similar magnitude of survival benefit from the addition of docetaxel was seen in both lower AR-A (HR 0.56, 95% CI 0.31-0.98; $P = 0.042$) and average AR-A (HR 0.55, 95% CI 0.30-0.99; $P = 0.048$) subgroups (Figure 4A and Supplementary Figure S6, available at https://doi.org/10.1016/j.annonc.2021.06.003).

DISCUSSION

In this study, we demonstrate that comprehensive gene expression profiling of primary prostate tumors obtained prior to ADT in men with mHSPC has the potential to prognosticate outcomes on ADT alone and predict benefit from chemohormonal therapy. To our knowledge, this is the first published study of whole transcriptome profiling of mHSPC using primary PC specimens and is also the only report linked to clinical outcomes on ADT and chemohormonal therapy from a randomized clinical trial. Furthermore, we have uniquely described the landscape of key transcriptomic PC subtypes as biomarkers in mHSPC.

Much of our knowledge on the molecular landscape of PC lies at the clinical bookends of disease: on one end, localized tumors, which may be associated with later development of mHSPC, and on the other, metastatic CRPC, which is associated with lethal outcomes. Both exhibit transcriptional heterogeneity among tumors of the same disease stage.[19-21] The former, however, has proven the most active area for the development of expression-based biomarkers to stratify prognosis independent of traditional predictors such as stage, PSA, and Gleason grade. Some tools have undergone incorporation in prospective clinical

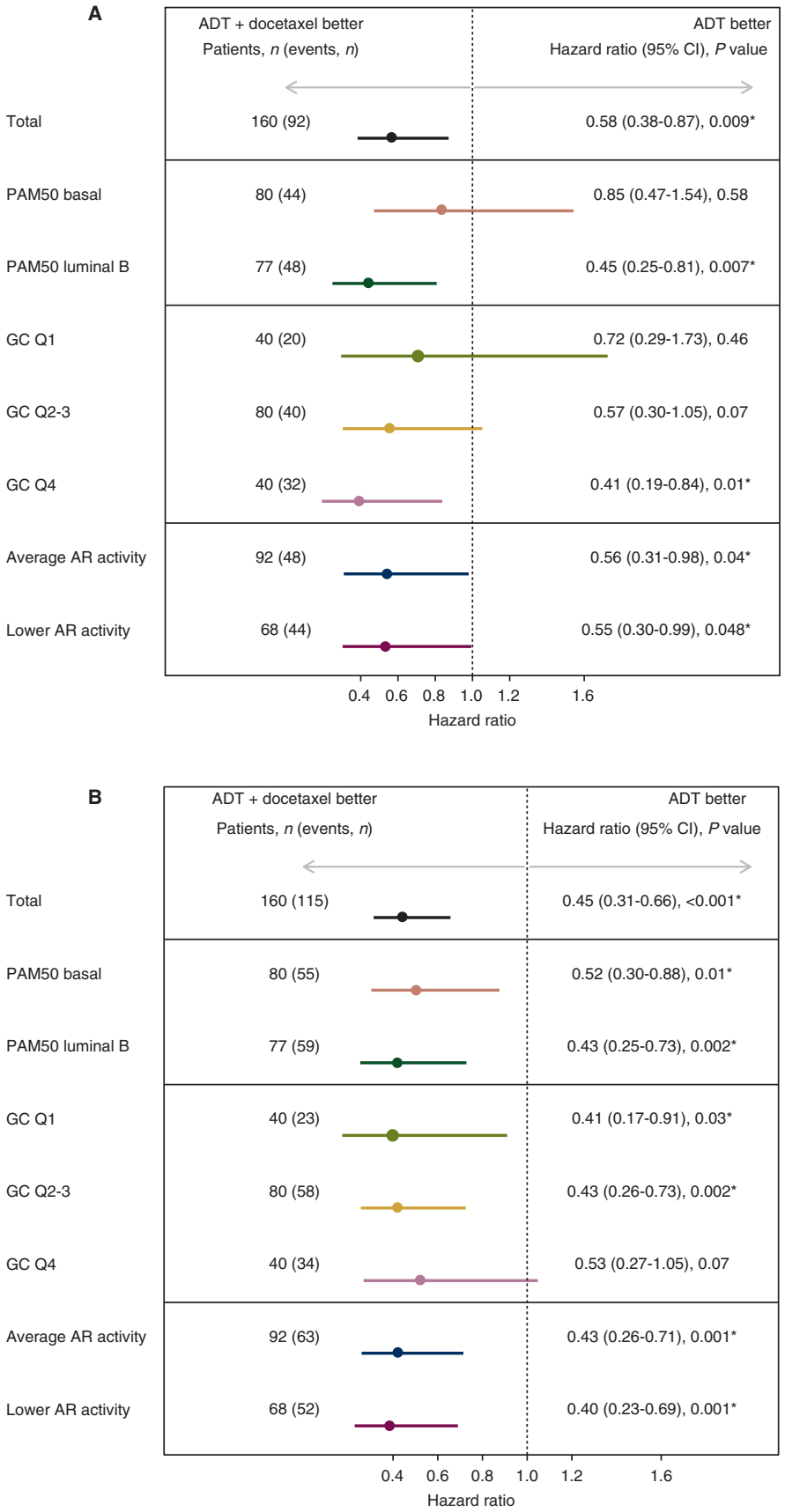

Figure 4. Forest plot of overall survival (OS) and time to castration-resistant prostate cancer (CRPC) by transcriptomic subgroups.
(A) OS; (B) time to CRPC. Univariable hazard ratios and 95% confidence intervals (95% CIs) of treatment arms are represented. *Denotes *P* value <0.05. AR-A, androgen receptor activity; GC, Genomic classifier (Decipher); Q1, lowest quartile; Q2-3, middle quartiles; Q4, highest quartile.

https://doi.org/10.1016/j.annonc.2021.06.003

trials, mirroring the development of gene expression classifiers in other tumor types, most notably breast cancer.

The clinical impact of molecular alterations in mHSPC remains largely undefined despite significant advances in therapy. Limited data of the mutational profile of mHSPC reveal recurrent aberrations in *AR*, *PTEN*, *TP53*, *RB1*, *BRCA2*, and *SPOP*, with frequencies that lie intermediately between localized PC and metastatic CRPC.[9,10,22] Our study has shed first light on the mHSPC transcriptome, with specific focus on subtyping tied to clinical outcomes. We observed a marked difference in the distribution of luminal—basal subtypes compared with localized PC,[12] with very few luminal A tumors and an increasing predominance of AR-low, basal, and GC-high subtypes akin to a previous report in CRPC.[18] Similarly, >40% of tumors had low AR-A compared with 10% in independent cohorts of localized PC.[13] These findings suggest that diverse transcriptional programs in primary tumors of mHSPC, whether related to intrinsic cell subtype or AR signaling, are closer in spectrum to primary tumors from patients with CRPC and are dominated by subtypes associated with aggressive biology and poorer prognosis. Our study cohort predominantly comprised patients with high volume and *de novo* metastatic disease, allowing a unique opportunity to correlate biological (RNA) features with aggressive, lethal PC and study treatment effects that may be pronounced in a poor-prognostic cohort. Even in the setting of profiling only a single focus of primary tumor, transcriptomic subtypes still held clear prognostic value despite known genomic heterogeneity between primary tumors and metastases.[23,24] Whether more indolent mHSPC evidenced by relapsing with low-volume disease years after a prostatectomy or radiation for apparently localized disease has similar features remains an area of active investigation, so too is the transcriptional reprogramming that may occur during evolution from a localized tumor to hormone-naïve metastasis.

We found that luminal B subtype was associated with poorer survival on ADT alone, consistent with previous reports in localized PC, but this lies in contrast to pan-cancer analyses, which generally associated basal disease with shorter OS, with the analysis being agnostic to the type of therapy.[12,25] However, luminal B subtype in another hormone-dependent cancer, early breast cancer, also portends poorer long-term outcomes similar to our findings.[26] It remains challenging to extrapolate clinical and biological features of luminal—basal subtype between cancers. However, luminal B tumors highly express proliferative markers in breast cancer[27] and PC[12] which may in part account for poorer survival on ADT alone for mHSPC. Similarly, GC score, which includes proliferation and cell cycle genes, had an association with prognosis. The association of low AR-A with poorer prognosis (independent of disease volume) parallels similar findings in localized PC and suggests AR-independent drivers. In metastatic CRPC, low AR-A subgroup is associated with early enzalutamide resistance and lineage plasticity;[28] however, our data indicate that a low AR-A subtype does not abrogate significant clinical benefit associated with early chemotherapy.

The observation that luminal B subtype (and not basal subtype) retained OS benefit from docetaxel may have two possible explanations. First, and more simplistically, poor-prognostic disease profiles may preferentially benefit from early treatment intensification with docetaxel as reflected by the greater magnitude of benefit from chemohormonal therapy seen in patients with *de novo* high-volume presentation and the GC Q4 (highest) subgroup. Second, unique biological features of luminal B versus basal mHSPC may govern response to docetaxel. Preclinical drug response models suggest that luminal B PC is associated with increased taxane sensitivity versus basal subtype; however, the reasons for this remain unclear. Nonetheless, an initial report from the randomized phase III TITAN trial in mHSPC of ADT versus ADT plus apalutamide (an AR inhibitor shown to improve OS in this setting) demonstrated a greater benefit in radiographic progression-free survival from combination therapy in basal, compared with luminal subtype.[29] Together, these findings raise the first possibility in mHSPC of precision decision making regarding docetaxel versus novel AR inhibition driven by gene expression classification, specifically luminal—basal subtype.

In comparison to OS, docetaxel was associated with improved ttCRPC across all transcriptomic subtypes including luminal B and basal. It is possible that the luminal—basal lineage may predict the effectiveness of subsequent therapies after upfront docetaxel, as we did not observe differences in receipt of life-prolonging therapies that could account for OS differences after progression to mCRPC. It may be that luminal B tumors undergo transcriptional plasticity with upfront treatment intensification, with a shift to a more sensitive phenotype for sequential mCRPC therapies. In addition, PSA-based endpoints may not be the most reliable marker for therapy resistance in the mHSPC setting, as intrinsic expression of *KLK3* which encodes PSA is lower in basal tumors[13] and 'harder' endpoints of radiographic progression-free survival and OS may represent the cumulative effect of the biological differences better than PSA alone.

Our study has some limitations. First, the study has a smaller sample size due to the availability of specimens and represents a subset of the trial cohort, although we observed a clear treatment effect in the analytic cohort which was consistent with the overall cohort. The sample size reduces the power to detect potentially significant treatment—biomarker interactions. Second, the possibility of significant heterogeneity between primary prostate and metastatic tumors is noted, yet the former represents the most frequent site of tumor biopsy at diagnosis of mHSPC and hence is clinically relevant. The use of validated classifiers such as Decipher risk remains unoptimized for clinical translation in mHSPC; however, GC score estimation has provided valuable biological insight and clearly holds prognostic value. The clinical impact of PAM50 and AR-A classifiers that we observed in CHAARTED requires validation. In short, this cohort provides a robust basis to support our approach of testing the utility of transcriptomic classifiers in independent randomized phase III trials of ADT and ADT +

D (STAMPEDE; ENZAMET) employing the Decipher Biosciences microarray platform. These efforts remain critical to meet a threshold of evidence to support translation in the clinical setting as potential prognostic and predictive tools employing an available clinical-grade assay with strong potential for generalizability. Our planned parallel effort to perform RNA profiling of specimens from trials of novel AR-targeted therapy in mHSPC and compare findings with those from chemohormonal therapy trials may well inform the selection of optimal combination treatment when analyzed collectively.

In conclusion, gene expression profiling of mHSPC in the CHAARTED trial reveals a distinct transcriptional landscape with profiles that serve as potential prognostic biomarkers for survival outcomes on ADT as well as profiles that provide predictive information regarding survival benefit from upfront chemohormonal therapy. These findings hold the promise of ushering in an era of improved prognostication and greater precision in selecting therapy for mHSPC.

ACKNOWLEDGEMENTS

This study was conducted by the ECOG-ACRIN Cancer Research Group (Peter J. O'Dwyer, MD and Mitchell D. Schnall, MD, PhD, Group Co-Chairs) and supported by the National Cancer Institute of the National Institutes of Health under the following award numbers: U10CA180820, U10CA180794, UG1CA233160, UG1CA233180, UG1CA233196, UG1CA233277, UG1CA233341. The study was also supported by Sanofi. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Mention of trade names, commercial products, or organizations does not imply endorsement by the US government.

FUNDING

Decipher Biosciences: In-kind support for Decipher microarray profiling and data generation. AAH: supported by Prostate Cancer Foundation Young Investigator Award [grant number 18YOUN07]; National Health and Medical Research Council Australia (no grant number), Department of Defense Early Investigator Research Award [grant number W81XWH2010055]. CJS: Supported by National Institutes of Health Research Project Grant Program [grant number NIH R01 CA238020-01A1].

DISCLOSURE

A.A.H reports consulting fees from Merck Sharp & Dohme. H-C.H., R.D. and E.D. are employees of Decipher Biosciences. F.F. reports receiving fees for serving as a consultant from Janssen during the conduct of the study, Celgene, Blue Earth Diagnostics, Astellas, Myovant, Roivant, Genentech, and Bayer; being a co-founder having stock options in PFS Genomics; and having stock options and serving on the scientific advisory board of SerImmune Stock outside the submitted work. G.A. reports personal fees, research support and travel support from Janssen during the conduct of the study; personal fees and/or travel support from Astellas, Pfizer, Millennium Pharmaceuticals, Ipsen, Ventana, Veridex, Novartis, Abbott Laboratories, ESSA Pharmaceuticals, Bayer Healthcare Pharmaceuticals, Takeda and Sanofi-Aventis and research funding from AstraZeneca, Innocrin Pharma and Arno Therapeutics outside the submitted work; in addition, G.A.'s former employer, The Institute of Cancer Research (ICR), receives royalty income from abiraterone acetate and GA receives a share of this income through ICR's Rewards to Discoverers scheme. E.M.V.A reports advisory/consulting role: Tango Therapeutics, Genome Medical, Invitae, Enara Bio, Janssen, Manifold Bio, Monte Rosa; research support: Novartis, BMS; equity: Tango Therapeutics, Genome Medical, Syapse, Enara Bio, Manifold Bio, Microsoft, Monte Rosa; travel reimbursement: Roche/Genentech; patents: Institutional patents filed on chromatin mutations and immunotherapy response, and methods for clinical interpretation. P.T.T. reported receiving grants from RefleXion Medical, the Prostate Cancer Foundation, and Movember Foundation; personal fees from Noxopharm, Janssen-Taris Biomedical, Myovant, AstraZeneca, and RefleXion; grants from Astellas and Bayer Healthcare; and owning patent No. 9114158 (with royalties from Natsar Pharmaceuticals) outside the submitted work. D.E.S reports personal fees: Janssen, AstraZeneca, Bayer, Boston Scientific, and Blue Earth; funding: Janssen. G.L. is a co-founder and chief medical officer of AIQ Solutions (Madison, WI). M.A.A. reports past consultation for Pfizer, Astellas, and Exelixis, and current research funding from Arcus, Pfizer, and Merck. C.J.S. report consulting or advisory role: Sanofi, Janssen, Astellas Pharma, Bayer, Genentech, Pfizer, Lilly; research funding: Janssen Biotech (Inst), Astellas Pharma (Inst), Sanofi (Inst), Bayer (Inst), Sotio (Inst), Dendreon (Inst); patents, royalties, other intellectual property: Pathenolide (Indiana University): dimethylaminoparthenolide (Leuchemix); Exelixis: Abiraterone plus cabozantinib combination; stock or other ownership: Leuchemix.

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