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TITLE: Genomic predictors of clinical outcomes and benefit of (chemo)hormonal therapy in metastatic hormone sensitive prostate cancer

PRINCIPAL INVESTIGATOR: Dr Anis Hamid

CONTRACTING ORGANIZATION: Dana-Farber Cancer Institute, Inc., Boston, MA

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14. ABSTRACT
Despite rapid changes in the therapy landscape of metastatic prostate cancer which has resulted in improved patient survival, there remains a dearth of predictive biomarkers to guide precision patient care. Our knowledge of the genomic drivers that underpin lethal prostate cancer has grown exponentially. Preclinical and clinical data reveal that tumor suppressor gene (TSG) aberrations drive rapid castration resistance and poorer patient survival. There is a critical unmet need to discover, train and validate genomic biomarkers in metastatic prostate cancer treated with hormonal therapy alone or in combination with docetaxel chemotherapy.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. Introduction

Approximately one third of men who die of prostate cancer present with *de novo* metastatic disease, with most of the remaining two thirds relapsing after localized disease. In recent years, large randomized phase III clinical trials have demonstrated significant prolongation in overall survival by combining agents (such as docetaxel, abiraterone, enzalutamide and apalutamide) to the long-held standard of androgen deprivation therapy (ADT) for newly-diagnosed metastatic hormone sensitive prostate cancer (mHSPC). Despite these advances, mHSPC remains a lethal disease with a typical life expectancy of less than five years. Additionally, there remains a dearth of validated, predictive biological markers of therapy benefit. This study will directly address a PCRP Overarching Challenge to (i) define the biology of lethal mHSPC and (ii) identify and validate prognostic and predictive biomarkers to improve precision clinical care and reduce deaths among men with metastatic prostate cancer. By way of leveraging archival patient tumor samples banked from pivotal trials of mHSPC (E3805 CHAARTED; STAMPEDE), this study will be the first comprehensive genomic profiling of mHSPC linked to prospectively-collected therapy outcomes in order to fundamentally define the genomic determinants of adverse-risk clinical features and treatment benefit or non-benefit in mHSPC.

2. Keywords

Prostate cancer; metastatic; lethal; biomarkers; genomics; predictive; prognostic

3. Accomplishments

Major Goals

Aim 1A: To determine whether TSG alterations are prognostic and/or predictive of outcome with ADT or ADT plus docetaxel in the CHAARTED trial.

Aim 1B: To determine whether TSG alterations are associated with clinicopathologic features of mHSPC, and assess both prognostic and predictive associations in multivariable models in the CHAARTED trial**.**

- *Aim 1 Milestone (6 months): Raw whole exome sequencing data (BAM files) from CHAARTED tumors.*
    - *Completion: Ongoing. Ongoing DNA extraction in preparation of exome sequencing.*
- *Aim 1 Milestone (18 months): Prognostic and predictive role of TSG alterations in CHAARTED by whole exome profiling.*
    - *Completion: N/A*

Aim 2: To validate the prognostic and predictive role of TSG alterations in clinicogenomic models of mHSPC in patients treated with ADT or ADT plus docetaxel in the STAMPEDE trial.

- *Aim 2 Milestone (15 months): DNA extracted and bait set designed for validation cohort of STAMPEDE tumors*
    - *Completion: Ongoing. Ongoing DNA extraction in preparation of exome sequencing.*
- *Aim 2 Milestone (27 months): Prognostic and predictive role of TSG alterations in STAMPEDE by targeted tumor profiling.*
    - *Completion: N/A*

Accomplishments with respect to Major Goals and Statement of Work

AIM 1

CHAARTED ADT versus ADT plus Docetaxel diagnostic samples

Despite challenges with extended partial and full shutdowns of wet laboratory facilities over months, due to the ongoing COVID-19 pandemic, we have made important progress in preparing CHAARTED samples for sequencing and translation to clinical outcomes as detailed in the projects aims and statemement of work.

Firstly, we have established a workflow between the ECOG-ACRIN Biorepository in Houston, TX to modify the intent to extract DNA from samples on site, but instead send full blocks of tumor tissue to the Broad Institute in Cambridge, MA for coring and DNA extraction using standard techniques. This was established in light of lower-than-expected DNA yields on a test batch of tumors at ECOG-ACRIN, with few tumors passing a high threshold for confidence for research whole exome sequencing. In deciding so, we sought and have successfully gained approval to modify this workflow by the ECOG-ACRIN Scientific Advisory Committee.

Secondly, we have now completed a first batch of 24 CHAARTED tumor samples which have undergone full sample preparation and DNA extraction, and downstream quantification and quality control analysis. Pleasingly, DNA yields appear significantly improved compared to the initial test batch and all of samples have passed the minimum threshold to push to the exome sequencing platform at the Broad (>20ng input DNA) as detailed in the right column of the table below:

Sample	Concentration (ng/ul)	DNA yield (ng)
1	1.87	87.89
2	0.52	24.44
3	0.43	20.21
4	14.67	689.49
5	0.99	46.53
6	0.44	20.68
7	3.77	177.19
8	12.23	574.81
9	4.03	189.41
10	0.45	21.15
11	77.58	3646.26
12	1.12	52.64
13	8.08	379.76
14	16.83	791.01
15	1.01	47.47
16	1.23	57.81
17	0.87	40.89
18	77	3619
19	1.68	78.96
20	0.67	31.49
21	0.58	27.26
22	0.64	30.08
23	0.97	45.59
24	0.49	23.03

Currently, further batches of samples are being prepared for shipment to Cambridge for bulk DNA extraction and preparation for sequencing. We expect all extractions and sequencing to be complete prior to the next reporting period.

Thirdly, we have obtained permission to access updated clinical data from the CHAARTED trial via ECOG-ACRIN, and this data is now accessible to the investigative team led by Dr Hamid. Once sequencing and bioinformatic methods are completed, we are poised to translate findings to clinical and therapy outcomes in the trial.

AIM 2

<u>STAMPEDE ADT versus ADT plus Docetaxel diagnostic samples</u>

The practice changing STAMPEDE trial randomized 2962 hormone-sensitive prostate cancer patients (HSPC) with metastatic and locally advanced disease, to receive either SOC (ADT+/- radiotherapy) or SOC and docetaxel chemotherapy. 95% of STAMPEDE participants consented to donate surplus diagnostic tissue for research and to-date, we have retrieved formalin fixed and paraffin embedded (FFPE) diagnostic prostate core biopsies from 1418 of men recruited to these trial arms. Many prostate cancer genomic studies are centered around single site recruitment from large urban academic centers. These tissue blocks were retrieved from 136 trial centers in the UK and will enable unique insights into prostate cancer genomics across a heterogeneous mix of rural and urban areas.

We have established a robust and efficient pipeline for block processing. All blocks are sent by trial site to the Wales Cancer Bank where they are anonymized, accompanied by a histopathology report. The Medical Research Council Clinical Trials Unit (MRC CTU) is responsible for tissue tracking. Tissue blocks are then sent to the UCL Cancer Institute and logged into our audit and tracking system software 'Freezerpro". Diagnostic blocks are cut for one additional H&E which is morphologically assessed by a specialist pathologist, along with 6 immunohistochemistry (IHC) sections at 5 micron, followed by alternate sections for nucleic acid isolation cut at 10 microns (alternate sections are assigned for DNA and RNA extraction). We have scanned all H&Es creating a biorepository and have developed a core labelling standard operating protocol so that all morphological, transcriptomic and genomic data can be integrated. IHC sections are being used for Ki67 scoring and additional markers are being optimized. For each case, we have received a median of 5 diagnostic prostate core biopsies (range 1-12).

The specialist pathologist marks out tumor areas on the H&E and scores tumor cellularity. The tissue sections are then stained with nuclear fast red dye and deparaffinized. The sections are then microdissected under a stereoscope to optimize tumor purity. We tested a number of FFPE DNA extraction kits and found our yield to be highest with the Zymo Quick- FFPE kit. To further optimize DNA yield, we have adapted the protocol by prolonging the proteinase K digestion time, passing the DNA lysate through spin-columns twice and eluting the DNA from the column twice with heated buffer. To maintain DNA nucleic acid quality, all DNA extractions are performed within 24 hours of the tissue sections being cut.

We have extracted DNA from a median of 5 cores per case for 363 control arm cases (ADT) with a median of 233ng of DNA per case (range 0-9922) and a median of 46ng (range 0-2506) per core. The median tumor cellularity across all cores was 60%. To determine feasibility of sequencing this challenging FFPE material (median block age 9 years across 136 sites with variable formalin fixation practices), our first aim was to perform low pass whole genome sequencing (lpWGS) as a material QC step. The cost of lpWGS is significantly cheaper than whole exome sequencing (WES) or targeted next generation sequencing (tNGS). If a case failed lpWGS then we will not continue WES this case. We have generated libraries from an individual core (selected as the highest Gleason grade and cellularity) from each case, with a minimum DNA input amount of 10ng and tumor cellularity 40%. We are using the NEBNext Ultra II assay with FFPE repair mix and Covaris fragmentation. We tested enzymatic DNA fragmentation, however the additional bead clean-up step in this protocol led to DNA loss.

We proceeded with library generation for 306/363 cases (3 cases excluded as post-ADT tissue sample, 54 cases excluded as < 10ng DNA was extracted from a single core or tumour cellularity < 50%). Libraries were successfully generated for lpWGS if 300/306 cases (98%). Libraries were generated for 108 cases manually and 202 processed in collaboration with the Oxford Genomics centre using an automated approach. Over a 6 month period we optimized programming of an automated system for rapid library generation.

Libraries for 300 cases were sequenced across 8 S1 flow cells of an illumine Novaseq achieving a median coverage of 0.4X and acceptable QC metrics. The average library fragment size was 259bp (range 194-357) and therefore after the first two runs, we reduced our sequencing strategy from 100 paired end to 50 paired reads to reduce adapter sequencing. This lpWGS data has been run through the QDNAseq pipeline for copy number calling. This work so far has resulted in an understanding of the sample quality and will enable us to move forward with a WES and/or targeted NGS assay. We will also move on to DNA extraction from docetaxel arm cases.

Opportunities for training and professional development:

I have had outstanding ongoing opportunities in training and professional development related to the project. I continue mentorship with Prof Sweeney and A/Prof Van Allen in weekly progress meetings, laboratory meetings and virtual call with the A/Prof Gert Attard in the UK, along with his research fellow Dr Emily Grist. Ongoing discussion, troubleshooting and refinement of skill has grown my expertise in practical genomics and sample preparation in particular over the current reporting period. Through mentorship, I have had the unique opportunity to interact with leaders in the prostate cancer

field with regular presentations at ASCO Genitourinary Symposium 2020 (in person and Oral Abstract presentation of transcriptomic biomarkers in CHAARTED; manuscript currently under review as attached in the Appendix), ASCO Annual Meeting (2020, virtual), Prostate Cancer Foundation Scientific Retreat (2020, virtual), and monthly Dana-Farber Prostate Cancer SPORE Meetings. I am also a newly-invited Member and regular attendee of the ANZUP Prostate Cancer Subcommittee and ENZAMET Translational Research Steering Committee to grow future aligned validation projects related to the work being done in this current project.

Dissemination of results to communities of interest:
Nothing to report

Plans for the next reporting period:
Our goal is to fully execute Aims 1 and significant progress on Aim 2 by the time of the next reporting period. Specifically, we aim to complete transfer, sample curation, DNA extraction, sequencing, quality control and variant calling of the full CHAARTED cohort within the next year, and in turn address the specific aim regarding role of deleterious tumor suppressor gene variants. In parallel, sampled from the STAMPEDE trial will continue DNA extraction and undergo targeted sequencing which likely continue by the time of next reporting.

4. Impact

Impact on the development of the principal disciplines of the project:
While the work detailed in the project has not yet matured to make a direct impact, closely aligned biomarker research using gene expression (RNA) data from CHAARTED (160 patients) was presented as the 2020 American Society of Clinical Oncology Genitourinary Cancers Symposium and currently under review at a scientific journal. This study was the first to define the gene expression landscape of metastatic hormone sensitive prostate cancer, and nominate subtypes of prostate cancer associated with benefit and non-benefit from chemotherapy. This was, therefore, proof-of-concept that predictive biomarkers can guide chemotherapy selection in metastatic prostate cancer and has influenced the field in pursuing validation of these findings.

Impact on other disciplines:
Nothing to report

Impact on technology transfer:
Nothing to report

Impact on society beyond science and technology:
Nothing to report

5. Changes/Problems

Changes in approach and reasons for change:
As DNA extraction of samples in the CHAARTED and STAMPEDE trials continue, one notable change has been to perform DNA extraction of CHAARTED samples at the Broad Institute, Cambridge, MA rather than the ECOG-ACRIN Core Facility in Houston, TX as described in the Project Narrative of this study. The primary reason for this has been due to significantly limited DNA yields noted on a test batch of samples at the ECOG-ACRIN facility and subsequent discussions about maximizing yield using a standard platform established at the center where sequencing will be performed. We have sought approval from the ECOG-ACRIN Scientific Advisory Committee to have samples sent to the Broad for extraction using their Genomics Platforms. Initial results from a batch of 20 samples show appropriate yields to continue this approach for the rest of the cohort.

Actual or anticipated problems or delays and actions/plans to resolve:
The COVID-19 pandemic has presented a significant challenge in advancing both Aims 1 and 2 with specific respect to wet laboratory work. The pandemic has resulted in a partial and complete extended shutdowns at lab facilities in Houston and Cambridge, resulting in many months of delay behind described timelines and milestones. For example, the Broad Institute paused all sample extraction completely for months due to the demand for COVID-19 PCR testing in Massachusetts. We have worked hard to resolve these issues through careful preparation of samples within the restrictions and open line of communication between teams over the last 9 months. We are now poised to continue sending batched samples from CHAARTED/ECOG-ACRIN biorepository to the Broad Institute to execute full sample preparation for sequencing before the next reporting period.

Changes that has significant impact on expenditures:
Nothing to report

Significant changes in use or care of human subjects, animals, biohazards and/or select agents:
Not applicable.

6. Products

Publications:

Journal publications: Nothing to report

Books or other non-periodical, one-time publications: Nothing to report

Other publications, conference papers and presentations:
Prostate Cancer Foundation Scientific Retreat (poster presentation), virtual, San Diego, CA
Grist et al, "Copy number profiles of primary tumours for risk stratification of advanced prostate cancer: a biomarker study embedded in the multi-centre STAMPEDE trial" (see Appendix)

Websites or internet sites:
Nothing to report

Technologies or techniques:
Nothing to report

Inventions, patent applications and/or licenses:
Nothing to report

Other products:
Nothing to report

7. Participants and Other Collaborating Organizations

Individuals that have worked on the project:

Name:	Anis Hamid
Project Role:	PI
Nearest person month worked:	9.6 CM
Contribution to Project:	Dr Hamid is primarily responsible for execution of aims related to CHAARTED cohort.

Funding Support:	National Health and Medical Research Council Australia (NHMRC) supports salary

Name:	Emily Grist
Project Role:	Research Fellow
Nearest person month worked:	3.0 CM
Contribution to Project:	Dr Grist has been primarily responsible for work related to STAMPEDE samples (Aim 2) in London, UK.
Funding Support:	Cancer Research United Kingdom (CRUK) supports salary

Name:	Xin Victoria Wang
Project Role:	Bioinformatician
Nearest person month worked:	N/A
Contribution to Project:	Dr Wang is responsible for clinical statistical analysis of the CHAARTED cohort.
Funding Support:	Not funded from this grant

Name:	Gerhardt Attard
Project Role:	Significant Contributor
Nearest person month worked:	N/A
Contribution to Project:	Dr Attard is the STAMPEDE PI, key personnel/collaborator and mentor for Dr Grist.
Funding Support:	Not funded from this grant

Name:	Christopher Sweeney
Project Role:	Mentor
Nearest person month worked:	N/A
Contribution to Project:	Dr Sweeney is the Dr Hamid's primary mentor and PI of the CHAARTED trial, and responsible for oversight of clinical/scientific work related to the trial.
Funding Support:	Not funded from this grant

Name:	Eli Van Allen
Project Role:	Co-Investigator
Nearest person month worked:	N/A
Contribution to Project:	Dr Van Allen is the primary bioinformatic collaborator who will help drive analysis of genomic data from the CHAARTED cohort.
Funding Support:	Not funded from this grant

Change in active other support of PI or senior/key personnel since last reporting period:

ANIS HAMID
NEW:

PC190530 PAIR (Hamid) 02/15/20-02/14/22 0 Cal Mos.
DoD/ W81XWH-19-PCRP-EIRA
Genomic Predictors of Clinical Outcomes and Benefit of (Chemo) Hormonal Therapy in Metastatic Hormone Sensitive Prostate Cancer
Role: Co-Investigator
The aims of this project are: Aim 1A: to determine whether TSG alterations are prognostic and/or predictive of outcome with ADT or ADT plus docetaxel in the CHAARTED trial, Aim 1B: to determine whether TSG alterations are associated with clinicopathologic features of mHSPC, and assess both prognostic and predictive associations in multivariable models in the CHAARTED trial, Aim 2: to validate the prognostic and predictive role of TSG alterations in clinicogenomic models of mHSPC in patients treated with ADT or ADT plus docetaxel in the STAMPEDE trial.
POC: Michelle Cromwell, Grants Management Specialist,
USAMRRA, Phone , michelle.l.cromwell.civ@mail.mil
Overlap: None

CHRISTOPHER SWEENEY
NEW:
PC190530 PAIR (Hamid) 02/15/20-02/14/22 0 Cal Mos.
DoD/ W81XWH-19-PCRP-EIRA
Genomic Predictors of Clinical Outcomes and Benefit of (Chemo) Hormonal Therapy in Metastatic Hormone Sensitive Prostate Cancer
Role: Co-Investigator
The aims of this project are: Aim 1A: to determine whether TSG alterations are prognostic and/or predictive of outcome with ADT or ADT plus docetaxel in the CHAARTED trial, Aim 1B: to determine whether TSG alterations are associated with clinicopathologic features of mHSPC, and assess both prognostic and predictive associations in multivariable models in the CHAARTED trial, Aim 2: to validate the prognostic and predictive role of TSG alterations in clinicogenomic models of mHSPC in patients treated with ADT or ADT plus docetaxel in the STAMPEDE trial.
POC: Michelle Cromwell, Grants Management Specialist,
USAMRRA, Phone , michelle.l.cromwell.civ@mail.mil
Overlap: None

R01CA238020 (Sweeney) 05/01/20-04/30/25 2.40 CM

NIH/NCI
Comprehensive genomic profiling of aggressive hormone sensitive prostate cancer
Role: Principal Investigator
Goals/Aims: Aim 1: To define he impact of tumor suppressor gene (TSG) alterations (TP53, PTEN, RB1) on clinical outcomes of patients with mHSPC treated with ADT or ADT plus docetaxel in the CHAARTED trial. Aim 2: To determine whether transcriptional profiles are associated with poor prognostic clinical features and/or TSG alterations and result in more accurate prognostication of outcomes with ADT or ADT plus docetaxel. Aim 3: To determine whether transcriptional profiles result in more accurate predictive models for benefit from adding docetaxel to ADT.
POC: Ashley Salo, Grants Management Specialist,
Email: ashley.salo@nih.gov Phone:
Overlap: None

PC190362 (Ellis) 05/01/20 -04/30/23 0.60 CM
DoD/ W81XWH-19-PCRP-TSA (Sweeney)
Defining over-expression of MYBL2 as a Driver of Lethal Prostate Cancer
Role: Co- Investigator
Goals/Aims: The overall goal is to identify novel genomic/epigenomic mechanisms which will lead to discovery of biomarkers and therapeutic targets for clinical testing, with a specific focus on aggressive variant prostate cancer (AVPC).
POC: Michelle Cromwell, Grants Specialist, Phone:
Email: michelle.l.cromwell.civ@mail.mil
Overlap: None

PC190730 (Sharifi) 6/1/2020 – 5/31/2023 0.36 CM
DoD/W81XWH-19-PCRP-TSA (DFCI)
Genetically Directed HSD3B1 Therapeutics for Metastatic Prostate Cancer
Role: Co-Investigator
Goals/Aims: This proposal has two overarching goals: 1) to determine whether the “permissive” and “restrictive” forms of the HSD3B1 gene can help determine which patient requires what therapy, and 2) to identify a drug that effectively blocks HSD3B1. This genetic factor is a clear driver of prostate cancer in about half of all patients.
POC: Joshua D. McKean, joshua.d.mckean3.civ@mail.mil
Overlap: None

ENDED
Johns Hopkins University (Lotan) 09/30/17-08/31/20 0.36 CM
Department of Defense / PCRP Idea Award
Prospective-Retrospective Analysis of PTEN Immunohistochemistry Assay for Prediction of Outcomes in Recurrent and Metastatic Prostate Cancer: PC160783
Role: Co-Investigator
Goals/Aims: Determine whether loss of PTEN in the primary is associated with poorer survival with ADT and whether this poor prognosis is overcome by adding in early docetaxel.
POC: Andrew Byrd, Administrative Manager, Johns Hopkins University
Divisions of GI Pathology & Gyn Pathology
CRBII Room#302, 1550 Orleans Street
Baltimore, MD 21231, Phone: 410-614-7707
Overlap: None

NIH/NCI (Sweeney) 08/15/16-07/31/20 R01CA208254 2.22 CM

Analysis of Blood Borne Markers in Hormone Sensitive Metastatic Prostate Cancer
Role: Principal Investigator

Goals/Aims: The aims of this project are 1) Assess the prognostic value of blood borne markers of bone biology in patients treated with ADT or ADT plus docetaxel; 2) Assess the prognostic value of blood androgen levels in patients treated with ADT and ADT plus docetaxel; 3) Assess the prognostic and predictive value of blood borne markers of inflammation in patients treated with ADT or ADT plus docetaxel; 4) Assess the prognostic and predictive value of blood borne markers of metabolism in patients treated with ADT or ADT plus docetaxel; 5) Evaluate which circulating blood borne biomarkers emerge at time of CRPC; 6) Evaluate whether blood borne biomarkers can identify extreme responders
POC: Tawnya Mckee, NIH/NCI Program Officer, Phone: 240.276.5719, Email: mckeeta@mail.nih.gov
Overlap: None

Prostate Cancer Foundation (Halabi/Sweeney) 10/12/18-10/12/20 0.60 CM
2018 VAlor Challenge Award (DFCI only)
Surrogate Endpoints of Overall Survival in Men with Metastatic Hormone Sensitive Prostate Cancer
Role: Partnering PI
Goals/Aims:
Aim 1: To assess whether intermediate clinical endpoints (ICEs) are surrogate for OS in men with mHSPC.
a) We will assess whether PSA <0.2 ng/ml at 6-7 months is a surrogate of OS. We will also assess whether PSA<0.2 ng/ml at 6-7 months is a surrogate of time to castration-resistant prostate cancer.
b) We will assess whether time to castration-resistant (PSA, radiographic PD) is a surrogate of OS.
c) We will assess if time to clinical progression (symptomatic or radiographic PD) is surrogate of OS.
Aim 2: To validate the ICEs that are identified in specific aim 1.
POC: Howard R. Soule, PhD, Executive Vice President
Prostate Cancer Foundation, Discovery and Translation
Phone: 310.570.4596, Email: hsoule@pcf.org
Overlap: None

XIN VICTORIA WANG
ENDED:
R01 CA193541 (Shanafelt, Tait) 5/19/2015 – 4/30/2020 1.20 cal
NIH/NCI (10% Effort)
(Subcontract from: Mayo Clinic)
Title: **Predicting Clinical Outcome After Traditional and Ibrutinib-based Therapy In Chronic Lymphocytic Leukemia**
Role: Statistician
Project Goals: The major goal of this project to leverage the platform of the phase III clinical trial to conduct critical correlative scientific studies designed to rapidly impact the treatment of CLL.
Specific Aims: The primary aims are 1) Determine if MRD can be used as a surrogate marker of clinical outcome after ibrutinib-based therapy and define the optimal timing and thresholds of assessment.
2) Assess the impact of somatic genetic abnormalities on response to CIT and non-CIT.
3) Characterize immune function after CIT and non-CIT and assess its relationship to clinical outcome.
Funding Agency's Contacts:
John Jessup, Program Official; Email: jessupj@mail.nih.gov; Phone: 301-435-9010
Executive Plaza North (EPN), Room 6040; Rockville, MD 20892-7420
Overlap:
None

R01CA208254 (Sweeney, Christopher) 8/1/2016-7/31/2020 0.96 cal

NIH/NCI (8% effort)
Title: **Analysis of Blood Borne Markers in Hormone Sensitive Metastatic Prostate Cancer**
Role: Statistician
Project Goals and Specific Aims: The aims of this project are 1) Assess the prognostic value of blood borne markers of bone biology in patients treated with ADT or ADT plus docetaxel; 2) Assess the prognostic value of blood androgen levels in patients treated with ADT and ADT plus docetaxel; 3) Assess the prognostic and predictive value of blood borne markers of inflammation in patients treated with ADT or ADT plus docetaxel; 4) Assess the prognostic and predictive value of blood borne markers of metabolism in patients treated with ADT or ADT plus docetaxel; 5) Evaluate which circulating blood borne biomarkers emerge at time of CRPC; 6) Evaluate whether blood borne biomarkers can identify extreme responders.
Funding Agency's Contacts:
Tawnya McKee, Program Official; Email: tawnya.mckee@nih.gov; Phone: 240-276-5719
Overlap:
None

NEW:
PC190530 PAIR (Hamid) 03/01/20-02/28/22 0.0 CM
DoD/ W81XWH-19-PCRP-EIRA
Genomic Predictors of Clinical Outcomes and Benefit of (Chemo) Hormonal Therapy in Metastatic Hormone Sensitive Prostate Cancer
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Role: Bioinformatician and Statistician
Funding Officer: Michelle Cromwell, Grants Management Specialist,
USAMRRA, Phone (301)619-4024, michelle.l.cromwell.civ@mail.mil
Overlap: None

R01CA238020-01A1 (Sweeney, Christopher) 5/1/2020 – 4/30/2025 0.48 cal
NIH Current year TC (4% effort)
Title: **Comprehensive Genomic Profiling of Aggressive Hormone-Sensitive Prostate Cancer**
Role: Biostatistician
Project Goals and Specific Aims: The aims of this project include: 1) To define the impact of tumor suppressor gene (TSG) alterations (TP53, PTEN, RB1) on clinical outcomes of patients with mHSPC treated with ADT or ADT plus docetaxel in the CHAARTED trial. 2) To determine whether transcriptional profiles are associated with poor prognostic clinical features and/or TSG alterations and result in more accurate prognostication of outcomes with ADT or ADT plus docetaxel. 3) To determine whether transcriptional profiles result in more accurate predictive models for benefit from adding docetaxel to ADT.
Funding Agency Contacts:
Program Official - Tawnya McKee
Email: mckeeta@mail.nih.gov
Phone:

ELIEZER VAN ALLEN
Previous:

Ended since last submission

Movember Foundation 03/01/18 – 02/28/20 0.06 CM
Movember GAP5 Award
Testicular Cancer Translational Research Project (GAP5)
Aim #1: To establish a secure platform for identifying, collecting and analysing clinical and biospecimen data to answer the above clinical question and to ensure this resource is available for future translational use. Aim#2:To determine if there are germline genetic features associated with resistance to cisplatin-based chemotherapy. Aim #3: To determine the association between putative molecular tumour tissue markers (DNA, mRNA, miRNA) and resistance to cisplatin-based chemotherapy.
Role: PI
POC: Paul Villanti; International:
Overlap: N/A

Leidos Biomedical Research, Inc (Boehm/Ligon) 12/1/18- 05/31/20 0.60 CM
Broad Institute/National Cancer Institute
Phase II: Broad Institute Cancer Model and Development Center
The Broad CMDC has three goals: 1) Achieve industry scale: Produce 100 patient-derived models per year with a focus on cancer types currently lacking precision therapies Gastroesophageal, Pancreas, Glioblastoma and Pediatric Solid Tumors); 2) Innovate to maximize efficiency: Iteratively optimize and refine workflows to improve success rates, maximize efficiencies and reduce costs; 3) Succeed for rare cancers: Demonstrate proof-of-concept for how to overcome key bottlenecks in rare and underrepresented cancers.
Overlap: N/A

Bristol Myers Squibb (PI: Haq) 03/01/17 – 02/28/20 0.12 CM

Evaluating the requirement of programmed cell removal in PD-1/CTLA-4 inhibitor response
Aim 1: To evaluate if inhibition of programmed cell removal confers resistance to PD-1 inhibitors Aim 2: To evaluate if anti-CD47 antibodies overcomes resistance to PD-1 inhibitors in FBXW7 and EIF2AK3-mutated melanomas Aim 3: To evaluate if programmed cell removal pathoways are recurrently mutated in melanomas that are resistant to PD-1 inhibitors
POC: Diane Pernice; diane.pernice@bmc.com
Overlap: N/A
Clinical Investigator Award 07/01/15-12/31/20 0.06 CM
Damon Runyon Foundation
Dissecting response to conventional and emerging DNA damage and repair therapies
Specific Aims: Aim 1. To develop computational algorithms that characterize genomic mechanisms of response to platinum-based chemotherapy. Aim 2. To characterize the role of nucleotide excision repair pathway mutations in mediating cisplatin sensitivity. Aim 3. To evaluate the efficacy of PARP inhibition in patients with prospectively identified genomic deficits in DNA damage repair.
Role: PI
POC: Yung S. Lie, Ph.D., CSO;
Overlap: N/A

Current:
New since last submission
W81XWH2010057 (Choudhury/Mouw) 02/15/20-2/14/23 0.12 CM
DoD
Molecular and genetic determinants of response to carboplatin with or without an ATR inhibitor (M6620) in mCRPC

Aims: 1) to correlate genetic and molecular features from pre-treatment tumor biopsy and cfDNA with clinical outcomes for M6620+carboplatin and docetaxel+carboplatin; 2) to discover genetic correlates of resistance to therapy from end-of-study cfDNA specimens and optional tumor biopsies; 3) to functionally characterize novel genetic alterations identified in pre- and post-treatment specimens using pre-clinical model systems.
Role: Co-Investigator
POC:Lauren Conte, Department Grants Management Specialist
Email: LaurenM_Conte@dfci.harvard.edu

(This Award)
PC190530 PAIR (Hamid) 03/01/20-02/28/22 0.0 CM
DoD/ W81XWH-19-PCRP-EIRA
Genomic Predictors of Clinical Outcomes and Benefit of (Chemo) Hormonal Therapy in Metastatic Hormone Sensitive Prostate Cancer
The aims of this project are: Aim 1A: to determine whether TSG alterations are prognostic and/or predictive of outcome with ADT or ADT plus docetaxel in the CHAARTED trial, Aim 1B: to determine whether TSG alterations are associated with clinicopathologic features of mHSPC, and assess both prognostic and predictive associations in multivariable models in the CHAARTED trial, Aim 2: to validate the prognostic and predictive role of TSG alterations in clinicogenomic models of mHSPC in patients treated with ADT or ADT plus docetaxel in the STAMPEDE trial.
Role: Co-Investigator
Funding Officer: Michelle Cromwell, Grants Management Specialist, USAMRRA, Phone , michelle.l.cromwell.civ@mail.mil
Overlap: None

New since last submission
U2C CA252974-01 7/1/20-6/30/25 0.60 CM
NIH (TC All Years –Van Allen)
Rare cancers comprise over 25% of tumors in U.S. adults, but due to low incidence and geographically dispersed patient populations, they are challenging to study, leading to significant unmet clinical needs. This proposal will form a Research Center that will directly engage patients with two such rare cancers – osteosarcoma and leiomyosarcoma – as partners in research in order to generate a large shared database of clinical, genomic, molecular, and patient reported data. We hope that this work can accelerate discoveries that drive novel treatment strategies, new clinical trials, and new standards of care, and also serve as a model for patient partnered research in other cancer types and patient communities.
Role: Co-Investigator
POC: Sharmarke Osman
Email: sosman@broadinstitute.org

New since last submission
NIH/NCI (Sweeney) 05/01/20 –4/30/25 0.60 CM
R01CA238020
Comprehensive genomic profiling of aggressive hormone sensitive prostate cancer
Role: Principal Investigator
Aims: Aim 1: To define he impact of tumor suppressor gene (TSG) alterations (TP53, PTEN, RB1) on clinical outcomes of patients with mHSPC treated with ADT or ADT plus docetaxel in the CHAARTED trial. Aim 2: To determine whether transcriptional profiles are associated with poor prognostic clinical features and/or TSG alterations and result in more accurate prognostication of outcomes with ADT or ADT plus docetaxel. Aim 3: To determine whether transcriptional profiles result in more accurate predictive models for benefit from adding docetaxel to ADT.
Role: Co-Investigator
POC: Bonnie MacEachern

Email: Bonnie_MacEachern@DFCI.HARVARD.EDU

New since last submission
NIH / NCI CTEP UM1 CA186709 03/01/20 – 02/28/23
0.24 CM
(Shapiro, G., Kufe, D., Flaherty, K.)
Dana-Farber/Harvard Cancer Center Experimental Therapeutics Clinical Trials Network Site (DF/HCC ETCTN Site)
Specific Aims are: (1) to propose novel clinical trials based on sound preclinical evidence and rationale that advance the clinical development of CTEP IND agents; (2) to develop early phase clinical trials of CTEP IND agents as monotherapies or in rational combinations that include safety, pharmacokinetic, translational and efficacy endpoints across a broad range of cancer types; (3) to incorporate validated integral and integrated biomarker assays and exploratory biomarkers in clinical study designs that examine proof-of-principle evidence of therapeutic activity in selected patient populations, proof-of-mechanism evidence of target engagement, as well as determinants of response, pathway adaptation and intrinsic resistance along with mechanisms of acquired resistance; (4) to activate trials with CTEP IND agents in compliance with guidelines established by the Operational Efficiency Working Group; (5) to efficiently conduct, complete and report on clinical trial outcomes in a timely fashion by working with other ETCTN sites; (6) to collaborate with other NCI-supported programs, including DF/HCC SPOREs; (7) to utilize NCI resources including the Molecular Characterization Laboratory (MoCha), the Pharmacodynamic Assay Development and Implementation Section (PADIS), the Drug Resistance and Sensitivity Network (DRSN), Cancer Immune Monitoring and Analysis Centers (CIMACs), the Patient-Derived Xenograft Development and Trial Centers Research Network (PDXNet), and the National Clinical Laboratory Network; (8) to extend ETCTN trials to rare and underserved populations; and (9) to provide mentorship to early career clinical and translational investigators in developmental therapeutics and in early phase clinical trial design and conduct.
Role: Co-Investigator
POC: David Mahoney
Email: David_Mahoney@dfci.harvard.edu

OTHER AFFILIATIONS AND RESOURCES:

Trainee: New since last submission
Imam Adbulrahman Bin Fisal University (Aldubayan) 01/01/2020-09/31/21 0.0 CM
Saudi Arabia
Gift to support breast cancer research.
Role: no financial support for Dr. Van Allen

GERHARDT ATTARD

No change reported

Other organizations involved as partners:
- **Organization Name:** University College London
- **Location of Organization:** United Kingdom
- **Partner's contribution to the projec**t:
  Collaboration: academic collaboration between investigators of CHAARTED (based at Dana-Farber Cancer Institute) and STAMPEDE (based at University College London).
  Facilities: Project work related to prostate cancer samples from STAMPEDE trial performed in Attard Laboratory, University College London.

8. **Special Reporting Requirements:**
   Not applicable

9. Appendices:

A. Prostate Cancer Foundation Scientific Retreat 2020: STAMPEDE exome profiling poster
B. Manuscript under review: Hamid *et al*. Primary prostate cancer transcriptome and clinical outcomes in metastatic hormone sensitive prostate cancer: correlative analysis of E3805 CHAARTED

Copy number profiles of primary tumours for risk stratification of advanced prostate cancer: a biomarker study embedded in the multi-centre STAMPEDE trial

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[1]UCL Cancer Institute, London, UK ; [2]Barts Cancer Institute, London, UK; [3]MRC Clinical Trials Unit, London, UK; [4]The Christie NHS Foundation Trust, Manchester, UK; [5]Guy's Hospital, London, UK; [6]Institute of Cancer Research, London, UK

No conflicts of interest to declare

Introduction

- Men with advanced hormone-sensitive prostate cancer (HSPC) starting long-term androgen deprivation therapy (ADT) follow a highly variable clinical course
- Treatment intensification with docetaxel or AR targeted therapies improves outcomes, but there is a risk of overtreatment, especially in non-metastatic (M0) or metastatic (M1) low volume disease
- The multi-arm multi-stage STAMPEDE trial has recruited men with advanced HSPC (M0 and M1) to standard-of-care (SOC) versus SOC plus additional combination treatment arms since 2005

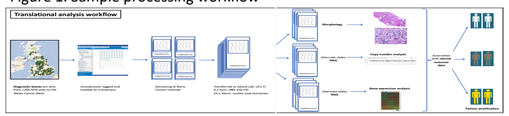
We established a **framework and pre-specified analyses plan** for **biomarker discovery within STAMPEDE** to improve risk stratification in advanced HSPC and here present our **first analyses of copy number burden in the first 108 cases**

Aims

1. Evaluate **feasibility and prognostic utility** of assessing the **burden of copy number (CN) aberrations** in advanced HSPC patients that received SOC (ADT) within the STAMPEDE trial
2. Implement a **scalable strategy** using **low coverage whole genome sequencing (lpWGS) of FFPE diagnostic core biopsies**

Methods

Figure 1. Sample processing workflow



- 95% STAMPEDE participants consented to donate tissue
- DNA (>10ng) was extracted from individual FFPE diagnostic cores and lpWGS and CN profiles generated using QDNAseq
- Percentage genome altered (PGA)-number of genome segments containing more than or fewer reads than the median for that sequenced sample divided by the total number genome segments within the autosome

Results

95% of cases were successfully lpWGS sequenced

Figure 2. Trial sites from which diagnostic tissue has been retrieved from participants

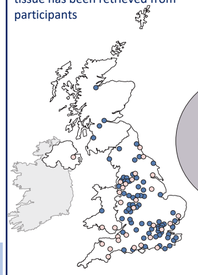
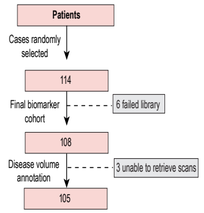


Figure 3. Sample flow chart. Disease volume in MI annotated as per CHAARTED



- 3106 patients randomized to SOC from 2005 (start of STAMPEDE) to 2016 (end of randomisation to the "enza+abi comparison"), referred to here as "full trial comparison"
- FFPE blocks have been retrieved for 1306 SOC cases recruited within that period (block retrieval ongoing)
- 114 cases randomly selected ("biomarker cohort") for first analyses

No significant difference in characteristics between biomarker cohort and full trial comparison

Characteristic		Biomarker (n=108)	Trial comparison (n=3106)	p-value*
Age at randomisation (years)	Median (IQR)	67.5 (62.5 - 72.0)	67.0 (62.0 - 72.0)	0.61
	Range	50.0 - 82.0	37.0 - 86.0	
Pre-ADT PSA (ng/ml)	Median (IQR)	478.0 (21.6 - 145.6)	59.7 (20.4 - 171.5)	0.29
	Range	3.7 - 3300.0	0.1 - 20590.0	
WHO Performance Status	0	87 (81%)	2374 (76%)	0.60
	1	20 (19%)	703 (23%)	
	2	1 (1%)	29 (1%)	
Disease burden	M0N0	36 (34%)	798 (28%)	0.15
	M0N+	19 (18%)	501 (18%)	
	M1 Low	28 (27%)	650 (23%)	
	M1 High	22 (21%)	884 (31%)	
	*Missing^*	3	273	
Tumour stage	T0	0 (0%)	9 (<1%)	0.23
	T1	2 (2%)	36 (1%)	
	T2	14 (13%)	249 (9%)	
	T3	71 (67%)	2073 (71%)	
	T4	19 (18%)	554 (19%)	
	Tx	2	185	
Grade Group*	1	3 (3%)	66 (2%)	0.47
	2	10 (9%)	227 (8%)	
	3	15 (14%)	363 (12%)	
	4	31 (29%)	700 (23%)	
	5	49 (45%)	1626 (55%)	
	Missing	0	*124*	
RT x planned at randomisation	No	66 (61%)	2040 (66%)	0.33
	Yes	42 (39%)	1066 (34%)	
Recurrent vs de novo	Recurrent	5 (5%)	132 (4%)	0.85
	De novo	103 (95%)	2974 (96%)	
Pain	Absent	93 (86%)	2661 (86%)	0.93
	Present	15 (14%)	418 (14%)	
	Missing	0	27	

Table 1. Cohort characteristics

PGA is higher in this cohort of advanced disease compared to reports in lower risk disease

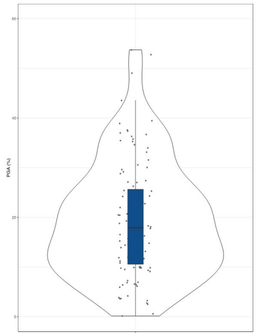


Figure 4. violin plot PGA n=108

Table 2. Sample details

	Median	Range
Coverage	0.3X	0.1-0.9 X
Cellularity	70%	40-100%
PGA	18%	0-54%

FFPE diagnostic core containing tumour enriched region of highest Gleason score with a tumour cellularity > 40% selected for lpWGS.

Ref for PGA in lower risk disease

1. Lalonde *et al.* Lancet oncol 2014
2. Hieronymous *et al.* PNAS 2014
3. Hieronymous *et al.* elife 2018

PGA is significantly associated with disease burden

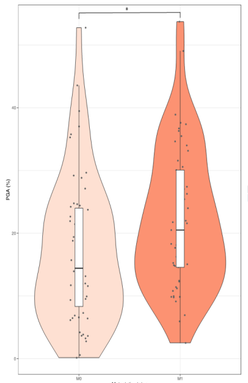


Figure 5. PGA significantly higher in M1 compared to M0 (n=108), p = 0.01

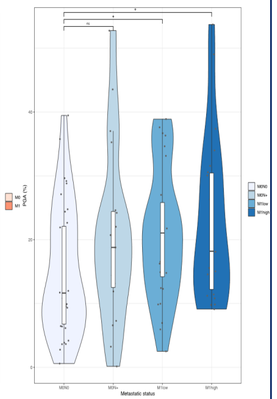


Figure 6. PGA significantly associated with disease burden (n=105), p = 0.04

Increased PGA is associated with poorer FFS and PFS in univariable and multivariable models

Figure 7. KM estimates of FFS

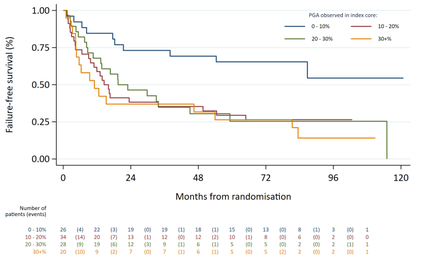
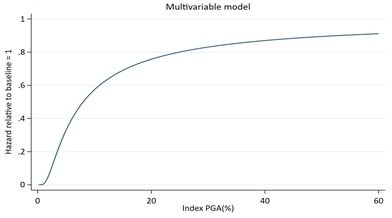


Figure 8. Estimated impact of index PGA on hazard of FFS



Likelihood Ratio (LR) test p-value = 0.003 from fractional polynomial model adjusted for PSA pre-ADT, age, grade group, disease burden, tumour cellularity, number of assessable cores.

Figure 9. KM estimates of PFS

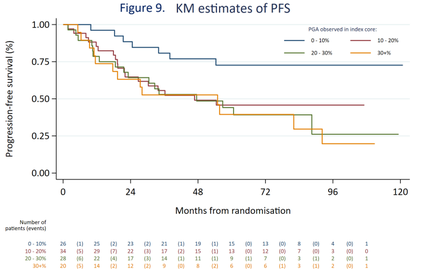
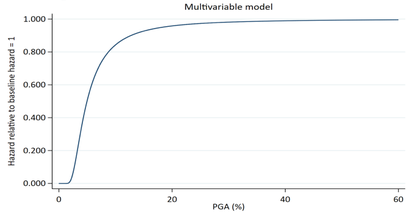


Figure 10. Estimated impact of PGA on hazard of PFS



Likelihood Ratio (LR) test p-value = 0.012 from fractional polynomial model adjusted for PSA pre-ADT, age, grade group, disease burden, tumour cellularity, number of assessable cores.

Conclusions

- Evaluation of **PGA** using **scalable lpWGS** in **archival, poor quality FFPE diagnostic tissue** from men randomised in the STAMPEDE trial is **feasible**
- There is a **significant association of PGA with FFS and PFS** in univariable and multivariable models
- **CN burden may have clinical utility** to identify patients with advanced disease who have a good prognosis and who may not require treatment intensification

We thank the STAMPEDE trial participants and their families. The translational research aspects of the protocol have been discussed and developed with the help of two patient representatives on the STAMPEDE Trial Management Group (TMG) (Millman, Matheson). We thank all UK STAMPEDE participating clinical sites for retrieving and transferring patient FFPE blocks to the Wales Cancer Bank.

Primary prostate cancer transcriptome and clinical outcomes in metastatic hormone sensitive prostate cancer: correlative analysis of E3805 CHAARTED

Authors:

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*Correspondence: Christopher_Sweeney@dfci.harvard.edu

Summary

Despite significant therapeutic advances, clinical-grade biomarkers that guide patient prognostication and predict survival benefit from chemohormonal therapy in metastatic hormone sensitive prostate cancer (mHSPC) are not defined. Through whole transcriptomic analysis of primary prostate cancer specimens obtained prior to therapy in E3805 CHAARTED, we report profiles associated with mHSPC reflecting luminal-basal identity and biological pathways occur at different frequencies compared to non-metastatic prostate cancer, and demonstrate divergent prognoses and differential benefit from combination therapy. A predefined high-risk gene classifier, luminal B and androgen receptor-low profiles all define distinct subgroups of mHSPC associated with shorter survival on testosterone suppression alone and patients with luminal B tumors retain a survival benefit with addition of docetaxel to testosterone suppression, in contrast to basal subtype. Collectively these findings are the start of a strategic plan to define gene expression profiles of primary prostate tumors prior to therapy initiation for biomarker-guided selection of established combinations in mHSPC.

Keywords

prostate cancer, hormone sensitive, metastatic, chemotherapy, Decipher, gene expression profiling, RNA profiling, transcriptome, docetaxel, biomarker.

Introduction

Most men with metastatic hormone-sensitive prostate cancer (mHSPC) respond to testosterone suppression (TS), however the durability of response and time to emergence of castration resistance is 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)[1–3] and agents targeting the androgen receptor (AR) axis by inhibition of extragonal androgen synthesis (abiraterone acetate)[4,5] or direct AR antagonism (enzalutamide; apalutamide)[6,7], with a backbone of TS termed androgen deprivation therapy (ADT). The phase III, randomized CHAARTED study was the first

trial to demonstrate a marked improvement in time to castration resistant prostate cancer (CRPC) and OS with ADT plus docetaxel, versus ADT alone[1]. Subgroup analyses have suggested the OS benefit from chemohormonal therapy is consistently evident in patients who present with high-volume metastatic disease[2,8].

Currently, there are no validated molecular biomarkers to personalize treatment in mHSPC and guide which men should receive TS alone or with docetaxel or with AR-targeted therapy, resulting in a critical unmet need. 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]. In 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, the Decipher genomic classifier (GC), and androgen receptor activity (AR-A)[11–13]. In localized HSPC, luminal B subtype is associated with higher AR-A score and poorer prognosis and patients with low 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 subtypes are predicted to be more sensitive to taxane chemotherapy, compared to those with basal or low AR activity[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 transcriptomic 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.

Results

Biopsy and cohort characteristics

The treatment arms of the final analytic cohort (76 in ADT arm, 84 in ADT+D arm; **Supplementary Figure 1**) were balanced with respect to clinical prognostic variables such as age, ECOG PS, volume of disease and receipt of prior local therapy (**Supplementary Table 1**). Median follow-up was 4 years. A significant OS improvement favoring ADT+D was observed in the analytic cohort (median OS 53.9 vs 32.4 months, HR 0.58, [95% CI 0.38-0.87], p=0.009). Compared to the trial cohort there was a higher proportion of patients with the high risk features of *de novo* metastatic (88% vs 73%) and high volume (78% vs 65%) disease (**Supplementary Table 2**).

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

The relative frequencies of transcriptomic subtypes were discovered to differ to the frequencies reported in non-metastatic prostate cancer, consistent with enrichment in mHSPC of molecular profiles associated with higher risk of relapse from localized disease. The distribution of luminal-basal subtypes in mHSPC were: basal 50%, luminal B 48% and luminal A 2% compared with 34%, 33% and 33%, respectively, in localized prostate cancer[12]. The median GC score was 0.72 and 71% were Decipher high-risk compared with 0.37 and 16.5%, respectively, in localized prostate[11] and 42% of patients with mHSPC had lower AR-A compared with only 10% in localized prostate cancer[13]. All three molecular biomarkers were well-balanced by treatment arm (**Supplementary Table 3**). Samples with higher Decipher scores tended to have higher Luminal B scores, though these inter-biomarker correlations were relatively weak indicating no substantial overlap between subtypes. Furthermore, biomarker scores did not correlate with volume of disease (a known clinical predictor of docetaxel benefit in mHSPC), with the exception of AR-A where high volume disease was significantly associated with lower AR-A scores (median AR-A in low vs high volume: 12 vs 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 or Decipher scores.

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

Only 3 patients (2%) were classified with luminal A disease and all were alive at last follow-up. Greater than 50% of patients had died in luminal B and basal subtypes. There were no significant differences between luminal B or basal in the overall cohort with respect to OS or ttCRPC on univariable or multivariable analyses (OS: p=0.298, MT-adj p=0.894; ttCRPC: p=0.399, MT-adj p=1) (**Table 1 & Supplementary Figure 2A**).

However, 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 vs 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 addition of docetaxel split by molecular subtype. Patients with basal disease showed no evidence of a significant OS benefit from docetaxel (median OS: 47.1 vs 49.2 months, HR 0.85 [95% CI 0.47-1.54], p=0.584, **Figure 3 & 4A**), even in the subgroup of patient with high volume disease (data not shown). In contrast, luminal B subtype showed a pronounced improvement in OS with docetaxel (median OS: 29.8 vs 52.1 months, HR 0.45 (95%CI 0.25-0.81), p=0.007), suggesting a potential treatment-biomarker interaction. No differential treatment benefit by subtype was observed with respect to ttCRPC (**Figure 4B** and **Supplementary Figure 3A**).

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 2B**). On multivariable analysis, continuous GC scores was 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<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 for OS of addition of docetaxel to ADT, for men with tumors in GC Q1 versus GC Q4 of 9% vs 25% at 3 years (**Supplementary Figure 4**).

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

The transcriptional signature of AR activity was prognostic. Lower AR-A exhibited both shorter ttCRPC and OS; in the overall cohort, 3-year OS was 45% vs 65% and 1-year CRPC-free survival was 47% vs 58% in lower vs average AR-A tumors, respectively (**Supplementary Figure 2C**). 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 to average AR-A patients treated with ADT alone; the 6-month CRPC-free rates were 40.7% vs 73.0% respectively (**Figure 5C [left panel] & Supplementary Figure 3C**). In contrast there was no association with AR-A and altered benefit from chemohormonal therapy in decreasing risk of castration resistance or death. A similar magnitude of survival benefit from 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 & Supplementary Figure 5**)

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 primary PC specimens in mHSPC, 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 molecular PC subtypes as biomarkers in mHSPC.

Much of our knowledge of 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. On the other, metastatic CRPC associated with lethal outcomes. Both exhibit transcriptional heterogeneity among tumors of the same disease stage[14–16]. The former, however, has proven the most active area for development of expression-based biomarkers to stratify the risk of recurrence and death independent of traditional predictors such as stage, PSA and Gleason grade. Some tools have undergone incorporation in prospective clinical 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 reveals recurrent aberrations in *AR*, *PTEN*, *TP53*, *RB1*, *BRCA2* and *SPOP* with frequencies that lie intermediate between localized PC and metastatic CRPC[9,1017]. 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 to localized PC[12], with very few luminal A tumors and a predominance of luminal B, basal and GC high subtypes akin to a previous report in CRPC[18]. Similarly, over 40% of tumors had low AR activity compared to 10% in independent cohorts of localized PC[13]. These findings suggest that diverse transcriptional programs in primary tumors of mHSPC, whether it is related to intrinsic cell subtype as well as AR signaling, are closer in spectrum to primary tumors from patients with CRPC and are dominated by subtypes associated with aggressive biological features and poorer prognosis when found in the tumors of patients with localized PC. Our study cohort was predominantly comprised of patients with high volume and *de novo* metastatic disease, allowing a unique opportunity to correlate biological (RNA) features with aggressive/lethal PC. Indeed we profiled a single foci of primary tumor which was associated with development of metastatic disease and molecular subtypes held clear prognostic value despite known genomic heterogeneity between primary tumors and metastases[19,20]. Whether more indolent mHSPC evidenced by relapsing with low volume disease years after a prostatectomy or radiation for apparently localized disease have similar features remains an area

of active investigation, so too is the transcriptional reprograming 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 associate basal disease with shorter OS with the analysis being agnostic to type of therapy[12,21]. In early breast cancer, luminal B subtype portends poorer long-term outcomes similar to our findings[22]. 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[23] and prostate cancer[12] which may in part account for poorer survival on ADT alone for mHSPC. Similarly, GC score, which includes proliferation and cell cycle genes, stratified prognosis. The association of low AR activity 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[24], however our data indicates 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. Firstly, and more simplistically, poor-prognostic disease profiles may preferentially benefit from early treatment intensification as reflected by the greater magnitude of benefit from chemohormonal therapy seen in patients with *de novo* high volume presentation and the GC Q4 subgroup. Secondly, unique biological features of luminal B versus basal mHSPC may govern response to docetaxel. Pre-clinical 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 (AR inhibitor shown to improve OS in this setting) demonstrated a greater benefit in radiographic progression-free survival from combination therapy in basal, compared to luminal subtype[25]. 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 time to CRPC across all molecular subtypes including luminal B and basal. It is possible that luminal-basal classification predicts the effectiveness of subsequent therapies after docetaxel given at time of ADT start, which in most cases would include AR-targeted therapy for metastatic CRPC. 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 may represent the cumulative effect of the biological differences better than PSA alone.

Our study has the limitations of a smaller sample size due to availability of specimens and represents a subset of the trial cohort, though we observed a clear treatment effect in the analytic cohort which was consistent with the overall cohort. The sample size reduces power to detect potentially significant treatment-biomarker interactions. Secondly, 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 ADT arm of the analytic cohort had a short median OS of 32 months, which may well be due to enrichment of poor prognostic clinical features. In short, this cohort provides a robust basis to support our approach to test the generalizability and utility of molecular classifiers in independent randomized phase III trials of ADT and ADT plus docetaxel (STAMPEDE and ENZAMET).

In conclusion, whole transcriptome profiling of mHSPC in the CHAARTED trial reveals a distinct molecular landscape with profiles that serve as potential prognostic biomarkers for survival outcomes on ADT as well as provide predictive information regarding patients who are more likely or less likely to benefit from upfront chemohormonal therapy. These findings hold the promise of ushering in an era of improved prognostication and selection of therapy for mHSPC with greater precision.

Acknowledgments

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Author Contributions

Conceptualization, C.J.S, A.A.H, E.D., V.W., Y-H.C.; Methadology, C.J.S, A.A.H., V.W., Y-H.C., E.D.; Formal Analysis, , C.J.S, A.A.H., V.W., Y-H.C., E.D.; Writing – Original Draft and Visualization, C.J.S, A.A.H., V.W., Y-H.C., E.D., H-C.H.; Writing – Review & Editing, all authors; Funding Acquisition, C.J.S., E.D.; Supervision, C.J.S.

Declarations of Interests

H-C.H., R.D. and E.D. are employees of Decipher Biosciences.

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Main Figure Titles and Legends

Figure 1. Pairs plot of molecular signatures by treatment arm and volume of disease. *Orange denotes ADT arm; purple denotes ADT plus docetaxel arm. Axes represent the percent of patients assigned to ADT or ADT plus docetaxel and low versus high volume disease (upper two rows) and the range of the respective biomarker scores (bottom three rows). Box and whisker plots represent the median, interquartile range and range of biomarkers scores within a given subgroup. Correlation (Cor) is Pearson's coefficient where noted. Abbreviations: GC, Genomic classifier (Decipher); AR, androgen receptor.*

Figure 2. Kaplan-Meier estimates of overall survival (OS) in treatment arms by molecular signatures. *(a) Luminal-basal subtype, (b) GC subgroup and (c) AR-A subtype.*

Figure 3. Kaplan-Meier estimates of OS by treatment arm within basal-luminal subtypes. *(a) Basal subtype and (b) Luminal B subtype.*

Figure 4. Forest plot of OS and time to castration resistant prostate cancer (CRPC), by molecular subgroups. *(a) OS; (b) time to CRPC. Univariable hazard ratios and 95% confidence intervals (CI) of treatment arms are represented.*

Figure 5. Time to CRPC and time from CRPC to death by treatment arm and molecular signature. *(a) luminal-basal subtype, (b) GC risk group and (c) AR-A subgroup. Abbreviations: OCM, other cause mortality; PCSM, prostate cancer specific mortality; Q1, lowest quartile; Q2-3, middle quartiles; Q4, highest quartile.*

Main Table Titles and Legends

Table 1: Multivariable analysis for OS and time to CRPC. *Hazard ratios of luminal-basal classifier are reported for luminal B subtype vs. 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. Abbreviations: OS, overall survival; ttCRPC, time to castration resistant prostate cancer; ADT, androgen deprivation therapy; ECOG, Eastern Cooperative Oncology Group (Performance Status).*

Table 1

Model	Variable	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
		PAM50 Basal-LuminalB		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 vs. 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 vs. 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 vs. 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 vs. 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 vs. 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 vs. 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 vs. 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 vs. 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*

STAR Methods

Resource Availability

Lead Contact: Further information should be directed to and will be fulfilled by the Lead Contact, Christopher Sweeney (Christopher_Sweeney@dfci.harvard.edu).

Materials Availability: This study did not generate new unique reagents or materials.

Data and Code Availability: RNA microarray data generation in this study has been deposited in the Gene Expression Omnibus (GEO).

Experimental Model and Subject Details

Trial and Correlative Study Design: The primary objective of the CHAARTED trial was to determine whether docetaxel would improve overall survival (OS) in men with mHSPC commencing TS. The clinical trial was designed by the ECOG-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 sub-study 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.

Subjects, RNA Processing and Microarray Profiling: The ECOG-ACRIN biobank retrieved available biopsy and radical prostatectomy samples from patients enrolled in the CHAARTED trial. De-identified 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 ≥60% tumor cellularity was required for the assay. RNA extraction and microarray hybridization were performed in a Clinical Laboratory Improvement Amendments (CLIA)-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. One hundred and ninety-eight of 790 patients (25%) had banked FFPE tumor blocks available for profiling. Among 190 samples with sufficient tumor available for RNA profiling, a total of 160 samples (84%) passed quality control for downstream analysis.

Method Details

The NCTN pre-specified analysis plan included Decipher Genomic Classifier (GC) score and Androgen Receptor Activity (AR-A). With the emergence of data regarding luminal-basal subtyping (i) as a prognostic biomarker in localized prostate cancer[12] and (ii) potential predictive marker of taxane benefit from *in silico* modeling[26], we expanded our *a priori* analysis plan to include this classifier as a third putative biomarker.

Transcriptomic Signatures: PAM50 subtyping consists of three prostate cancer-relevant subtypes (luminal A, luminal B, and basal-like). Previously developed cut-points were used to call subtype, based on the 50-gene mRNA signature developed in breast cancer[27], with the exclusion of the Her2-enriched subtype. True Decipher scores (continuous scale of 0 to 1) were generated as previously described[28]. Categorical GC results are presented by quartile based on the analytic cohort of 160 samples; given that the middle two quartiles have comparable prognosis, 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. AR-A score is comprised of 9 canonical androgen receptor transcriptional target genes (*KLK3, KLK2, FKBP5, STEAP1, STEAP2, PPAP2A, RAB3B, ACSL3, NKX3-1*). The AR-A model was used with the previously locked cut point (score of 11) to define lower vs average AR-A[13].

Endpoints: The primary endpoint of CHAARTED and this ancillary study was OS, defined as the time from randomization until death from any cause. Secondary endpoints included time to CRPC (ttCRPC), defined as the time from randomization to 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 ADT arm and ADT plus docetaxel arm (ADT+D), to determine if a differential treatment effect with the addition of docetaxel existed by molecular 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 and multivariable analyses (UVA, MVA) with Firth's penalized method[29]. Co-variables in the MVA models were age, Eastern Cooperative Oncology Group (ECOG) performance status (PS), *de novo* metastatic presentation vs prior local therapy, volume of disease and treatment arm. 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 an HR of no smaller than 0.6 with a two-sides 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 hazard ratios (HR) 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 less than 0.05 was deemed statistically significant.

Supplementary Figures and Tables

Supplementary Figure 1. CONSORT diagram. *Abbreviations: RNA, ribonucleic acid; cDNA, complementary deoxyribonucleic acid; QC, quality control; ADT, androgen deprivation therapy.*

Supplementary Figure 2. Kaplan-Meier estimates of overall survival (OS) and time to castration resistant prostate cancer (CRPC) by (a) luminal-basal subtype, (b) GC risk group and (c) AR-A subgroup. *Abbreviations: Q1, lowest quartile; Q2-3, middle quartiles; Q4, highest quartile.*

Supplementary Figure 3. Kaplan-Meier estimates of time to CRPC in treatment arms by (a) luminal-basal subtype, (b) GC risk group and (c) AR-A subgroup. *Abbreviations: Q1, lowest quartile; Q2-3, middle quartiles; Q4, highest quartile.*

Supplementary Figure 4. Kaplan-Meier estimates of OS by treatment arm in GC risk subgroups. *Abbreviations: Q1, lowest quartile; Q2-3, middle quartiles; Q4, highest quartile.*

Supplementary Figure 5. Kaplan-Meier estimates of OS by treatment arm in AR-A subgroups.

Supplementary Table 1. Patient characteristics of the analytic cohort by treatment arm. *Abbreviations: ADT: androgen deprivation therapy, ECOG: Eastern Cooperative Oncology Group (Performance Status), PSA: prostate specific antigen.*

Supplementary Table 2. Patient characteristics of the full trial cohort by status of successful tissue profiling for inclusion in the analytic cohort (QC pass). *Abbreviations: QC: quality control, ECOG: Eastern Cooperative Oncology Group (Performance Status), PSA: prostate specific antigen.*

Supplementary Table 3. Distribution of molecular subtypes by treatment arm in the analytic cohort.

Abbreviations: Q1, lowest quartile to Q4, highest quartile, GC: (Decipher) genomic classifier, AR-A: androgen receptor activity.

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Figures

Figure 1

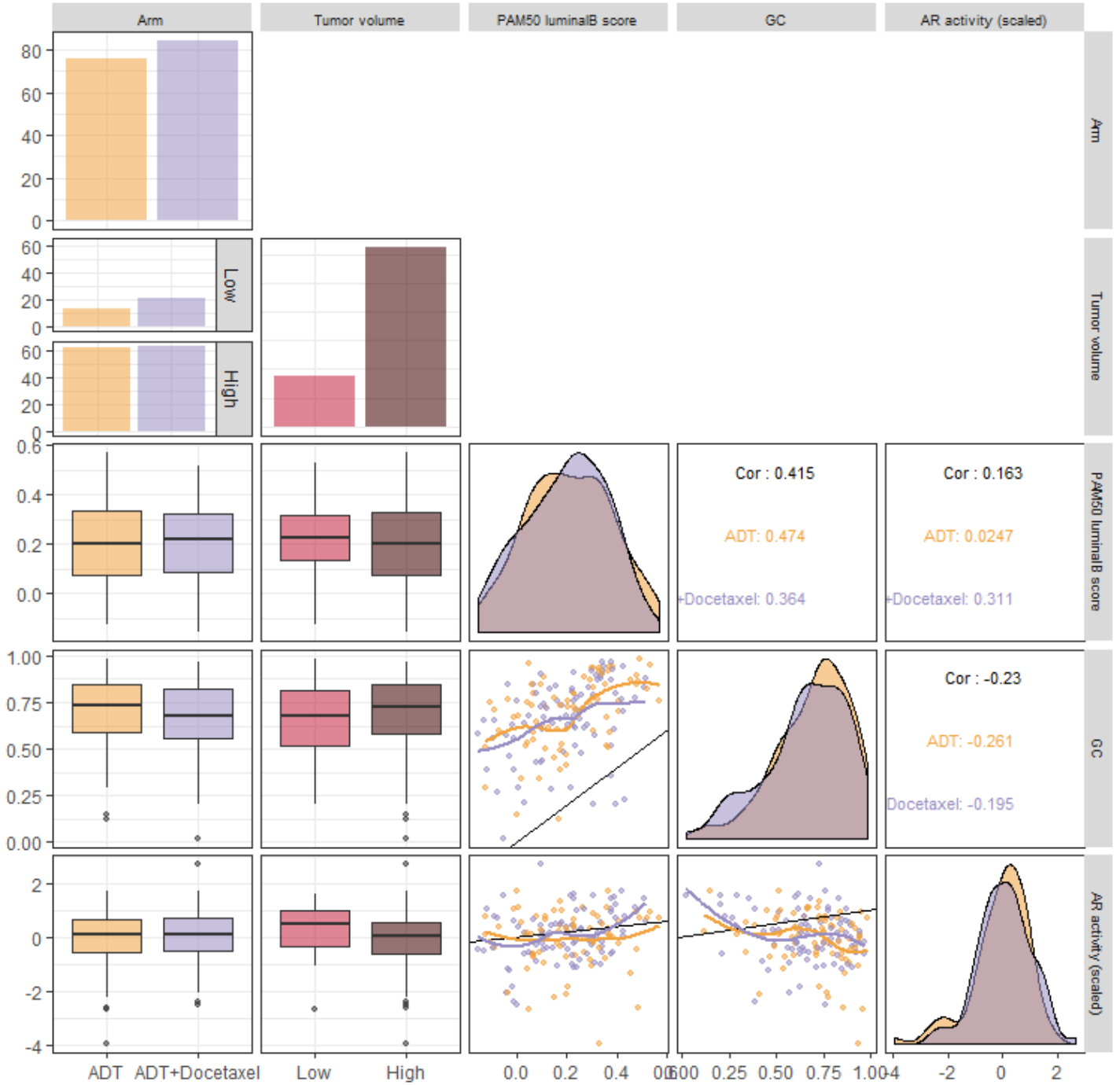


Figure 2

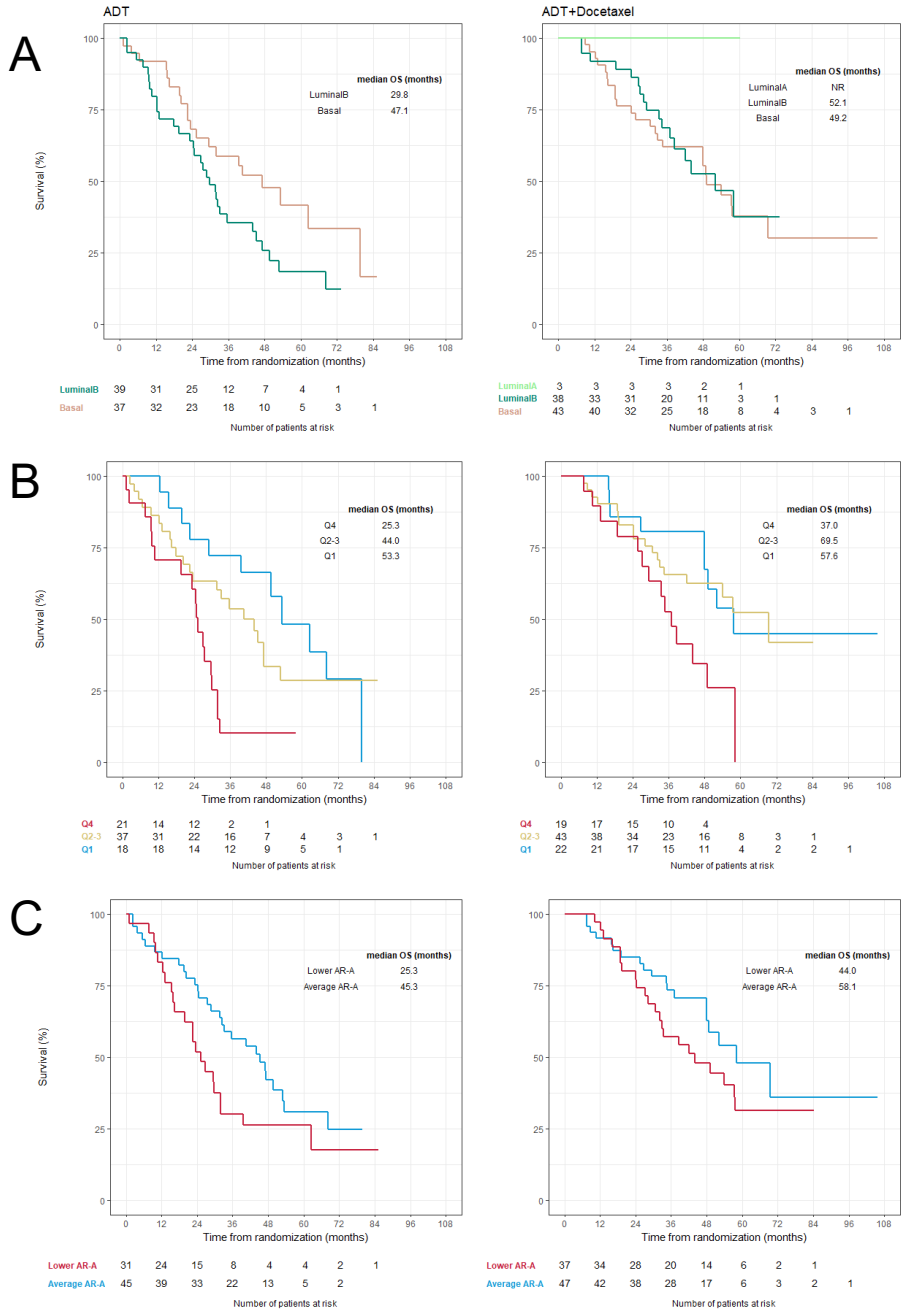

A
ADT
ADT+Docetaxel
median OS (months)
LuminalB 29.8
Basal 47.1
LuminalA NR
LuminalB 52.1
Basal 49.2
Survival (%)
Time from randomization (months)
LuminalB 39 31 25 12 7 4 1
Basal 37 32 23 18 10 5 3 1
LuminalA 3 3 3 3 2 1
LuminalB 38 33 31 20 11 3 1
Basal 43 40 32 25 18 8 4 3 1
Number of patients at risk
B
median OS (months)
Q4 25.3
Q2-3 44.0
Q1 53.3
Q4 37.0
Q2-3 69.5
Q1 57.6
Survival (%)
Time from randomization (months)
Q4 21 14 12 2 1
Q2-3 37 31 22 16 7 4 3 1
Q1 18 18 14 12 9 5 1
Q4 19 17 15 10 4
Q2-3 43 38 34 23 16 8 3 1
Q1 22 21 17 15 11 4 2 2 1
Number of patients at risk
C
median OS (months)
Lower AR-A 25.3
Average AR-A 45.3
Lower AR-A 44.0
Average AR-A 58.1
Survival (%)
Time from randomization (months)
Lower AR-A 31 24 15 8 4 4 2 1
Average AR-A 45 39 33 22 13 5 2
Lower AR-A 37 34 28 20 14 6 2 1
Average AR-A 47 42 38 28 17 6 3 2 1
Number of patients at risk

Figure 3

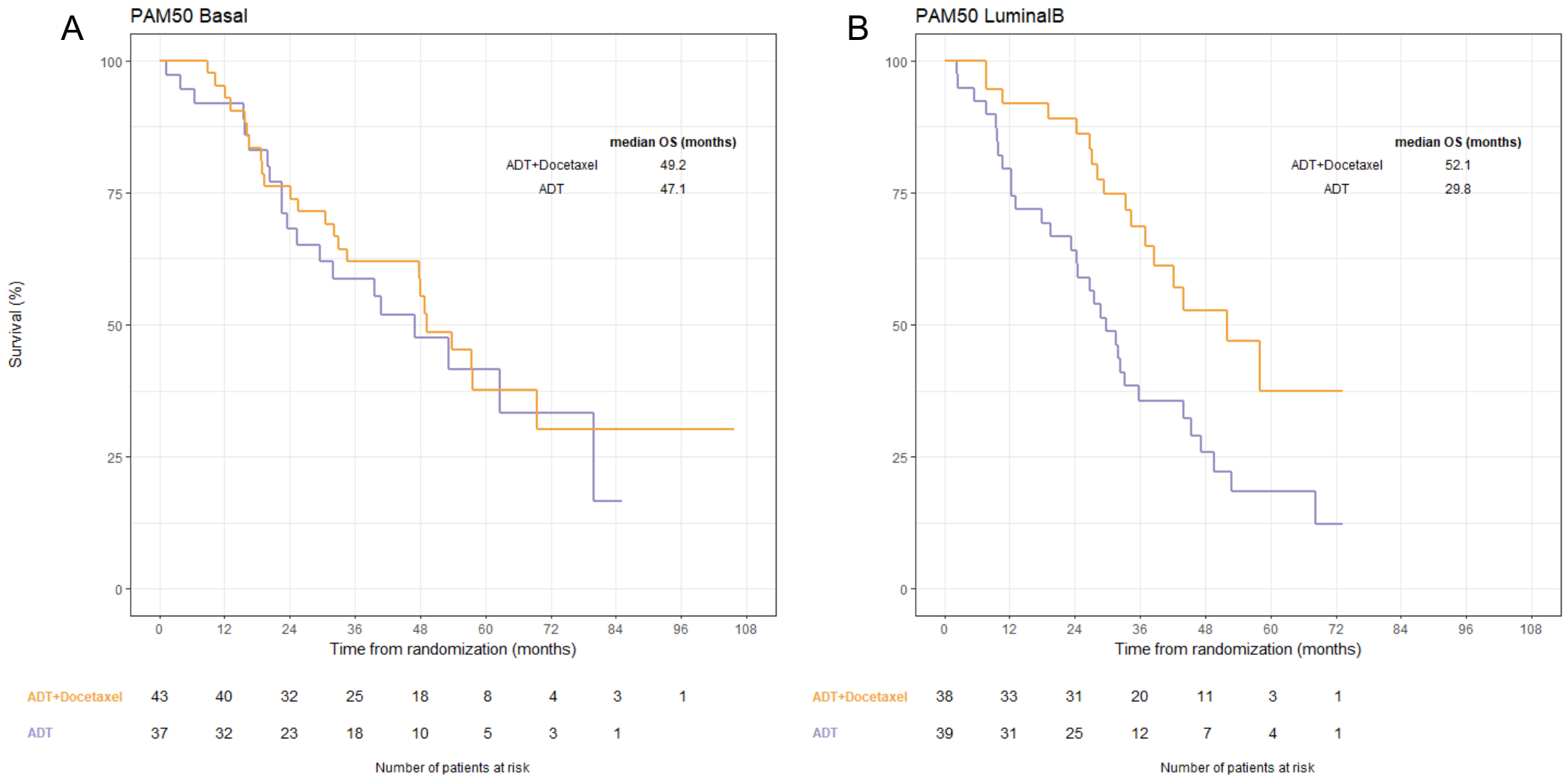

A
PAM50 Basal
Survival (%)
100
75
50
25
0
median OS (months)
ADT+Docetaxel 49.2
ADT 47.1
0 12 24 36 48 60 72 84 96 108
Time from randomization (months)
ADT+Docetaxel 43 40 32 25 18 8 4 3 1
ADT 37 32 23 18 10 5 3 1
Number of patients at risk
B
PAM50 LuminalB
100
75
50
25
0
median OS (months)
ADT+Docetaxel 52.1
ADT 29.8
0 12 24 36 48 60 72 84 96 108
Time from randomization (months)
ADT+Docetaxel 38 33 31 20 11 3 1
ADT 39 31 25 12 7 4 1
Number of patients at risk

Figure 4

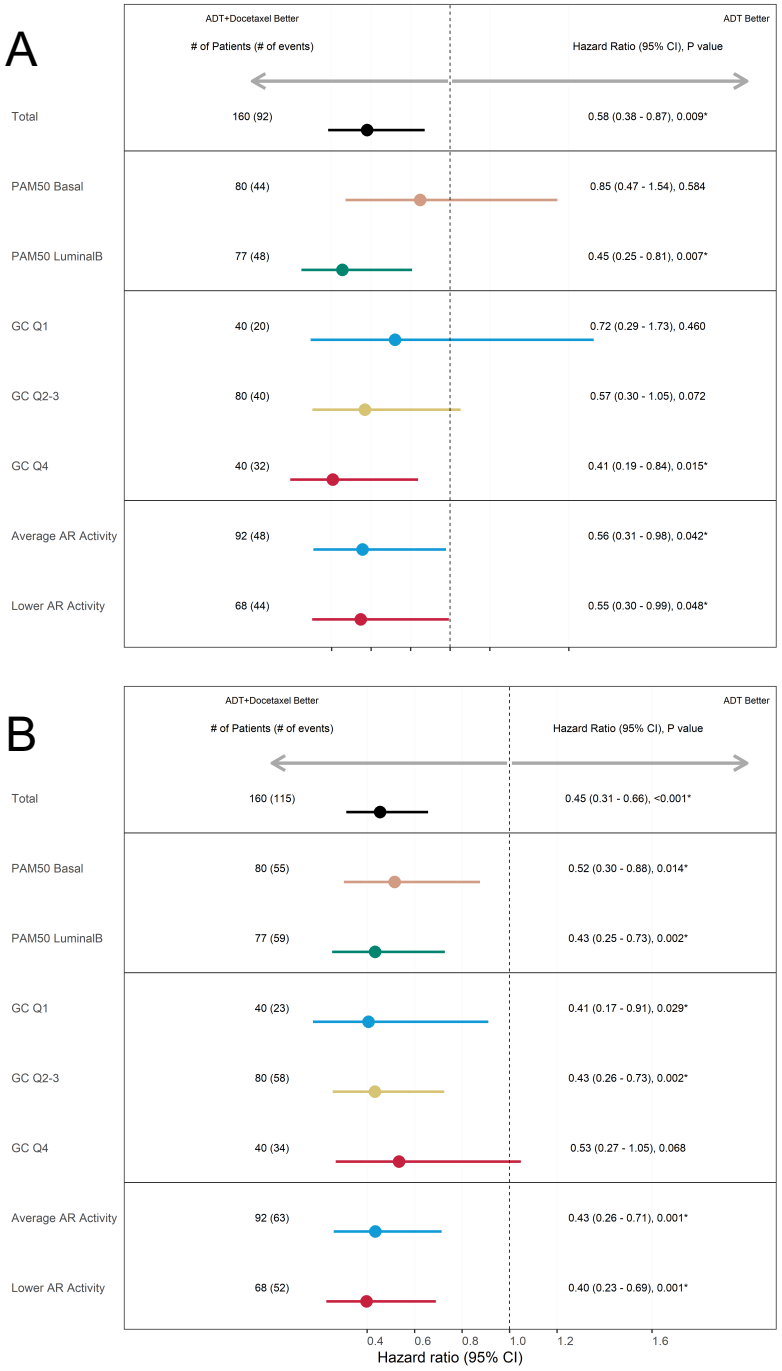
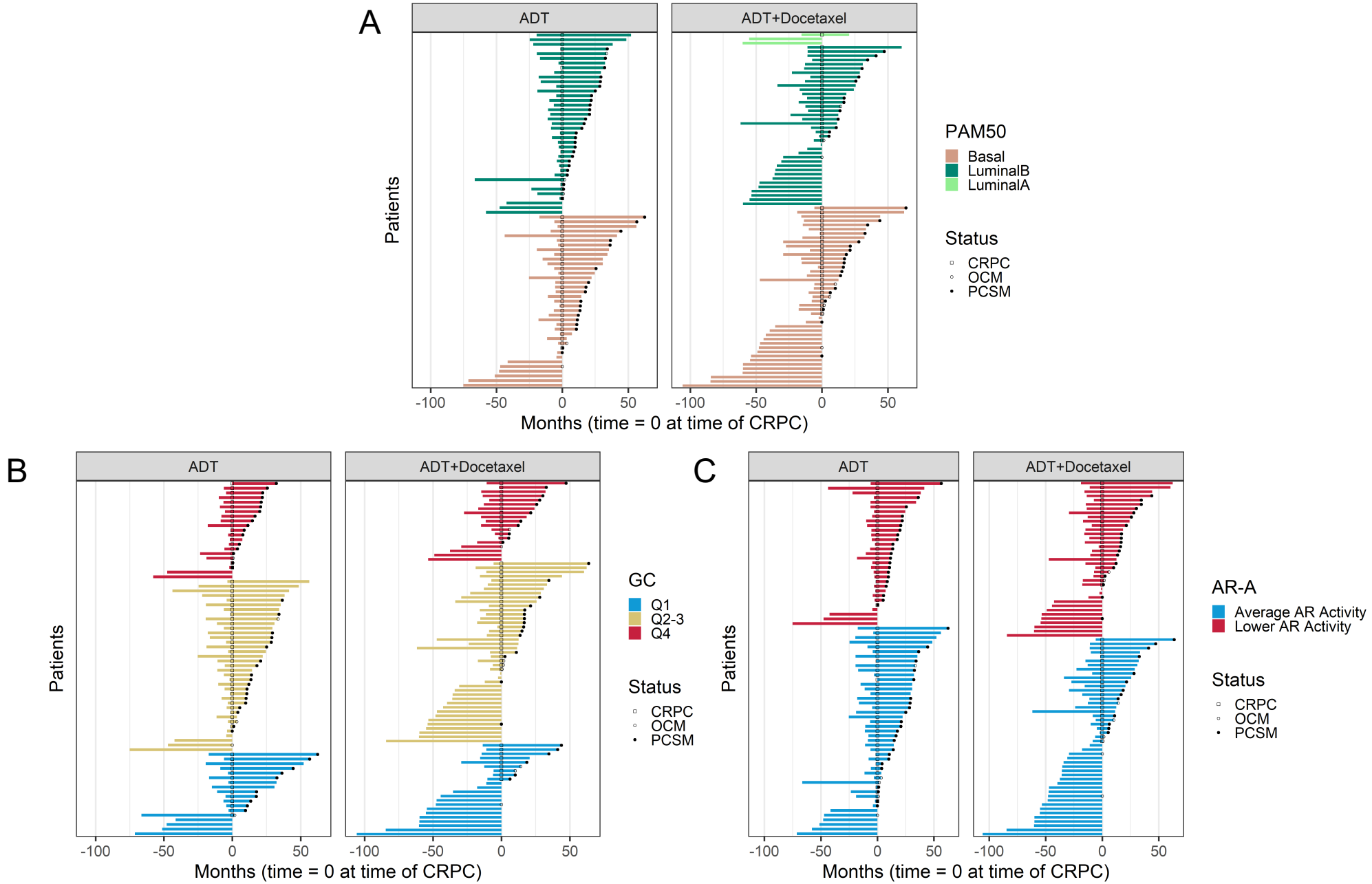
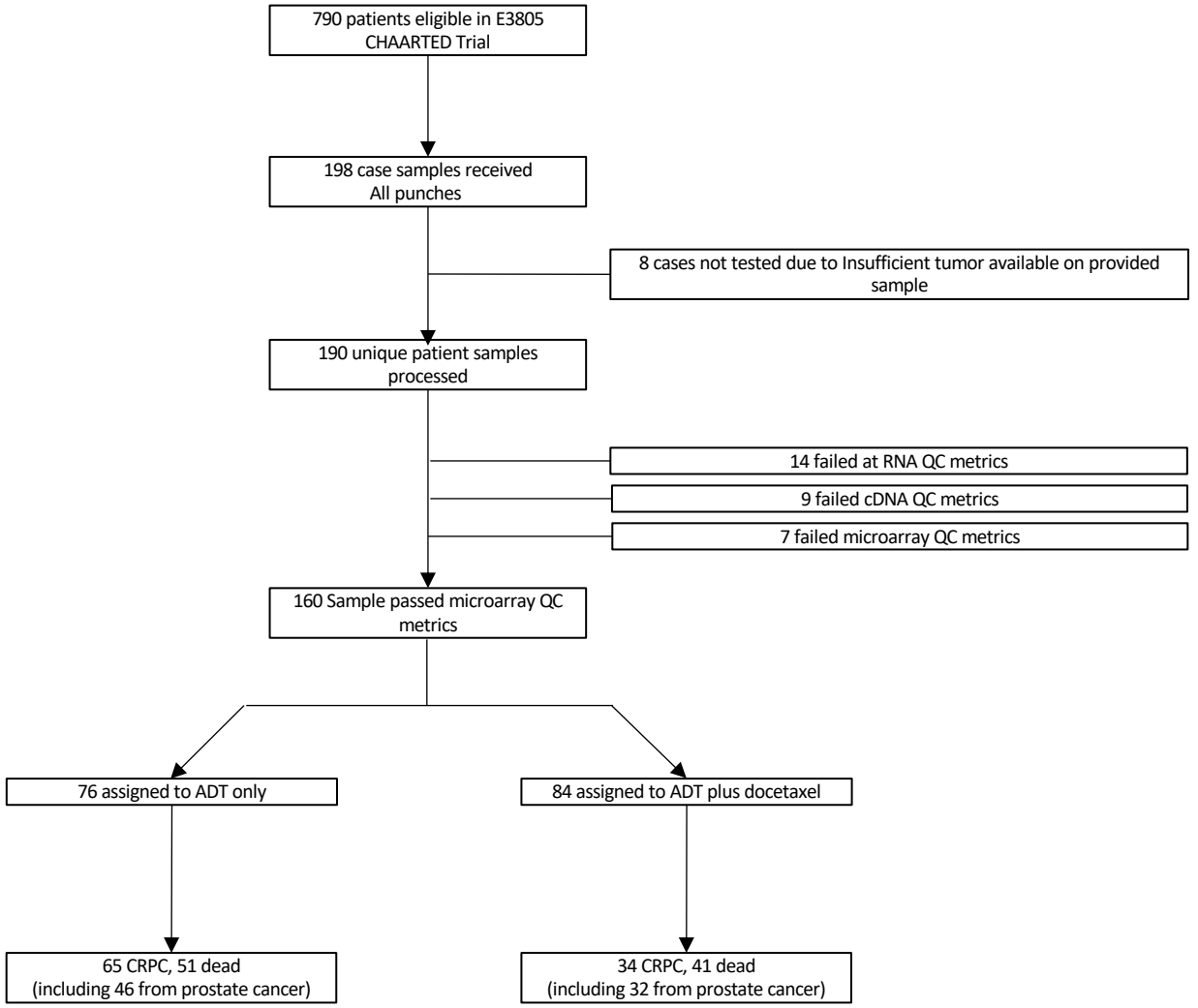

A
ADT+Docetaxel Better
ADT Better
# of Patients (# of events)
Hazard Ratio (95% CI), P value
Total
160 (92)
0.58 (0.38 - 0.87), 0.009*
PAM50 Basal
80 (44)
0.85 (0.47 - 1.54), 0.584
PAM50 LuminalB
77 (48)
0.45 (0.25 - 0.81), 0.007*
GC Q1
40 (20)
0.72 (0.29 - 1.73), 0.460
GC Q2-3
80 (40)
0.57 (0.30 - 1.05), 0.072
GC Q4
40 (32)
0.41 (0.19 - 0.84), 0.015*
Average AR Activity
92 (48)
0.56 (0.31 - 0.98), 0.042*
Lower AR Activity
68 (44)
0.55 (0.30 - 0.99), 0.048*
B
ADT+Docetaxel Better
ADT Better
# of Patients (# of events)
Hazard Ratio (95% CI), P value
Total
160 (115)
0.45 (0.31 - 0.66), <0.001*
PAM50 Basal
80 (55)
0.52 (0.30 - 0.88), 0.014*
PAM50 LuminalB
77 (59)
0.43 (0.25 - 0.73), 0.002*
GC Q1
40 (23)
0.41 (0.17 - 0.91), 0.029*
GC Q2-3
80 (58)
0.43 (0.26 - 0.73), 0.002*
GC Q4
40 (34)
0.53 (0.27 - 1.05), 0.068
Average AR Activity
92 (63)
0.43 (0.26 - 0.71), 0.001*
Lower AR Activity
68 (52)
0.40 (0.23 - 0.69), 0.001*
0.4
0.6
0.8
1.0
1.2
1.6
Hazard ratio (95% CI)

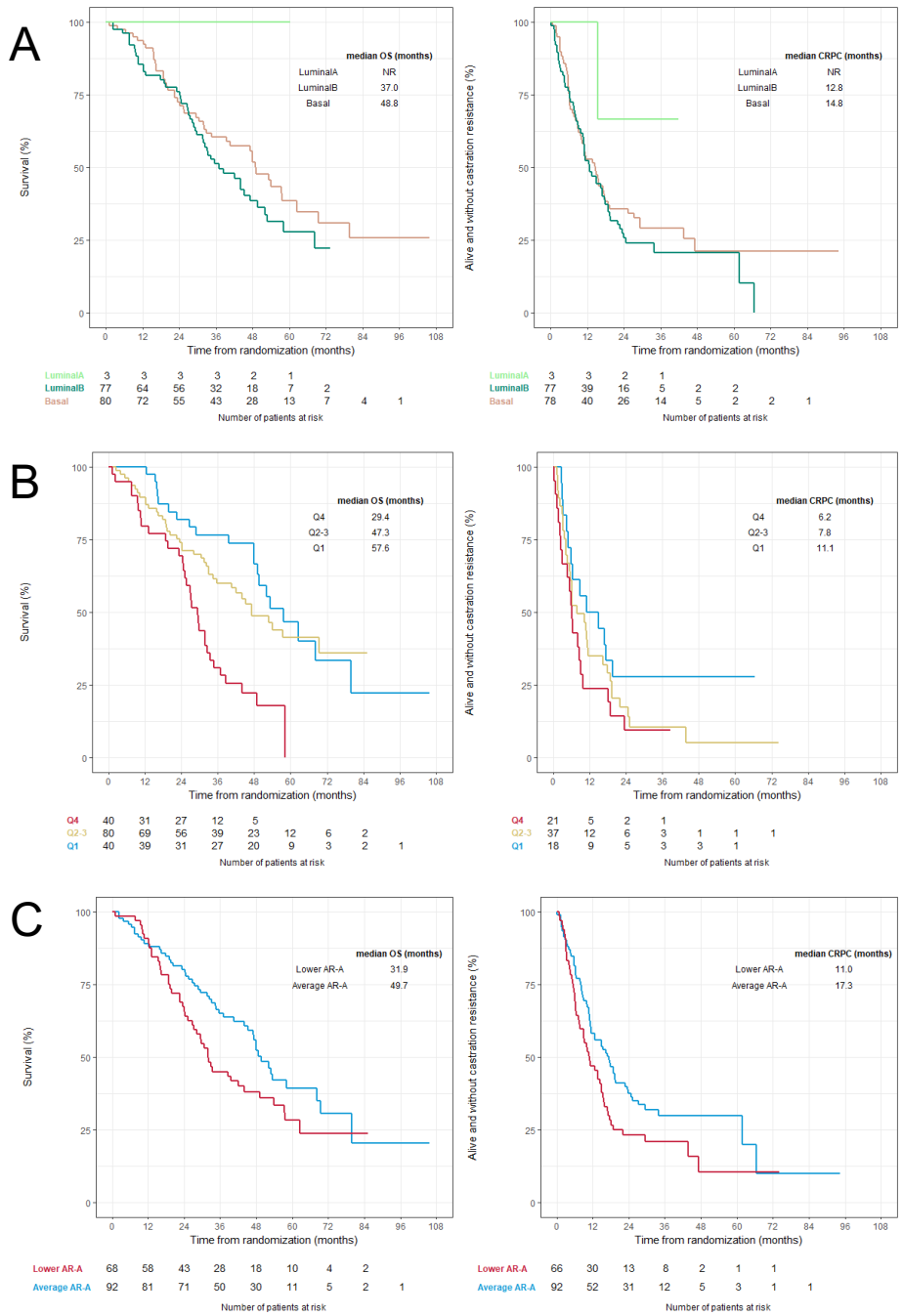
Figure 5


A
ADT
ADT+Docetaxel
Patients
-100
-50
0
50
Months (time = 0 at time of CRPC)
PAM50
Basal
LuminalB
LuminalA
Status
CRPC
OCM
PCSM
B
ADT
ADT+Docetaxel
Patients
-100
-50
0
50
Months (time = 0 at time of CRPC)
GC
Q1
Q2-3
Q4
Status
CRPC
OCM
PCSM
C
ADT
ADT+Docetaxel
Patients
-100
-50
0
50
Months (time = 0 at time of CRPC)
AR-A
Average AR Activity
Lower AR Activity
Status
CRPC
OCM
PCSM

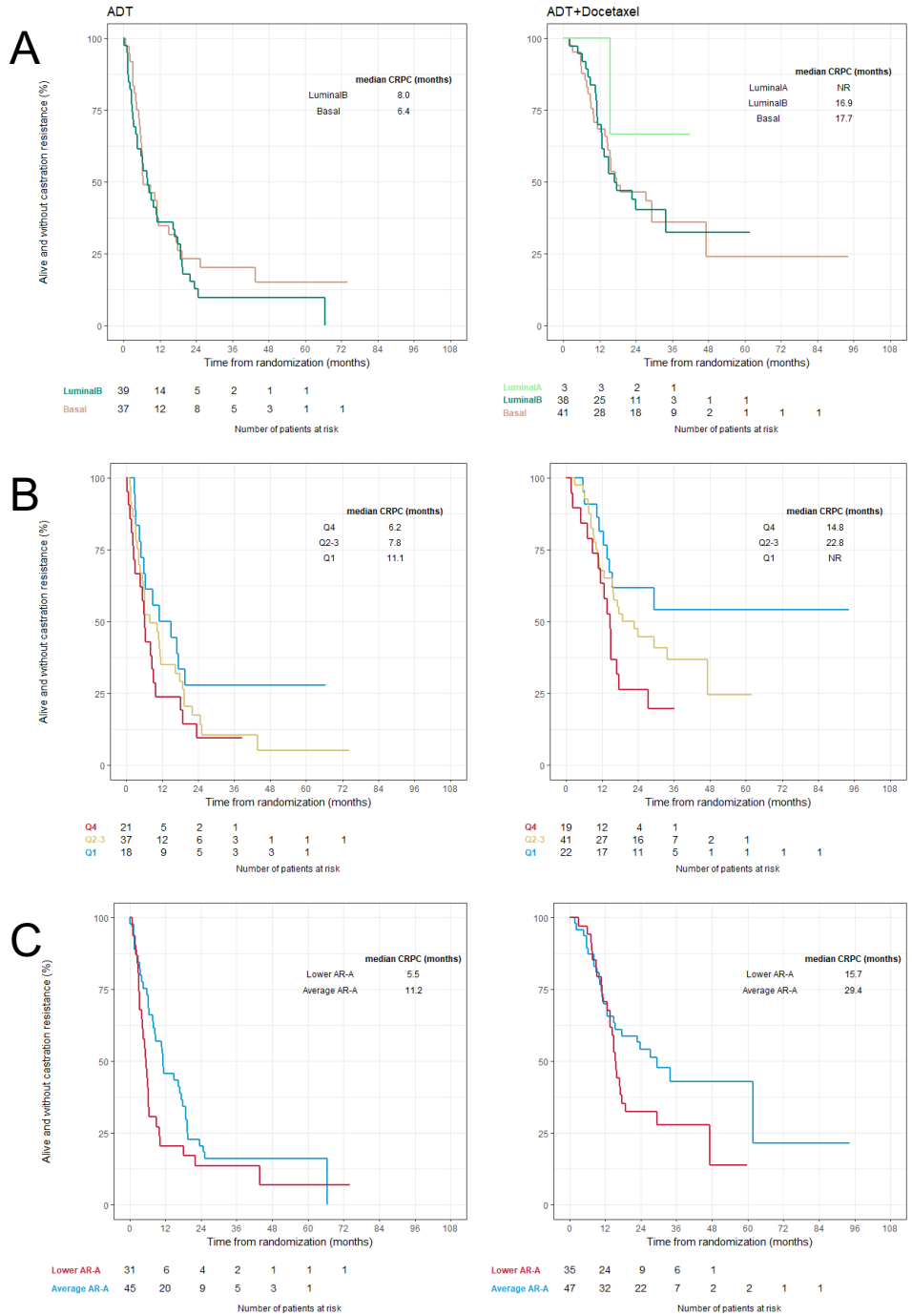
Supp Figure 1



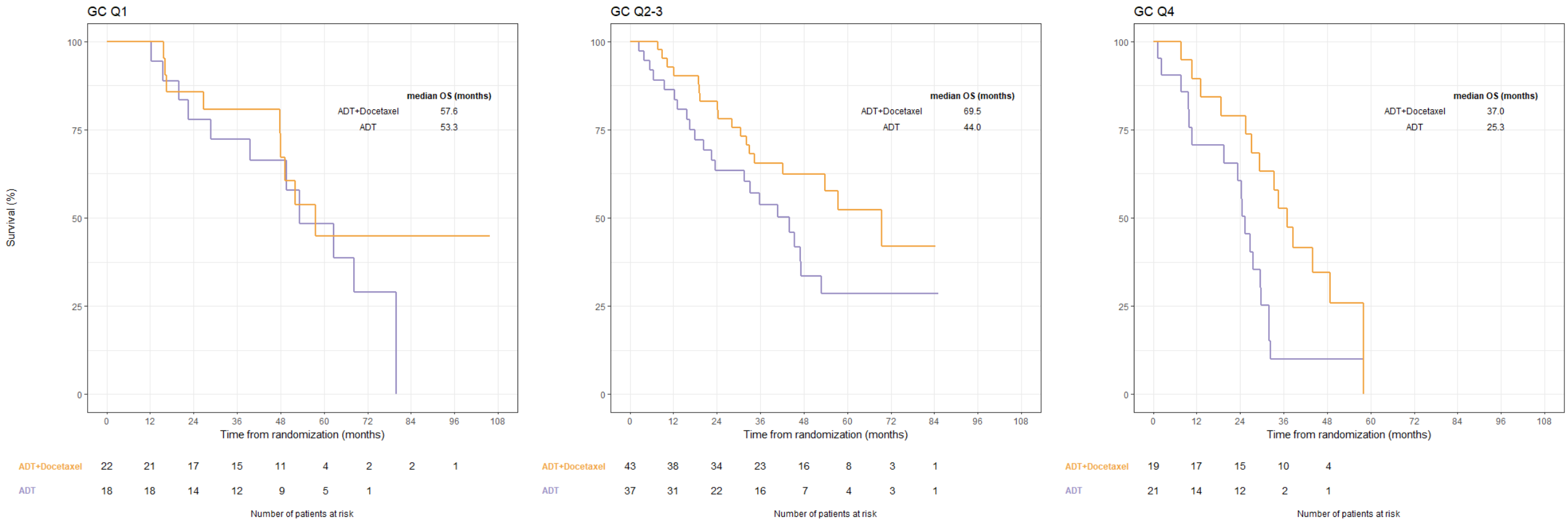
Supp Figure 2



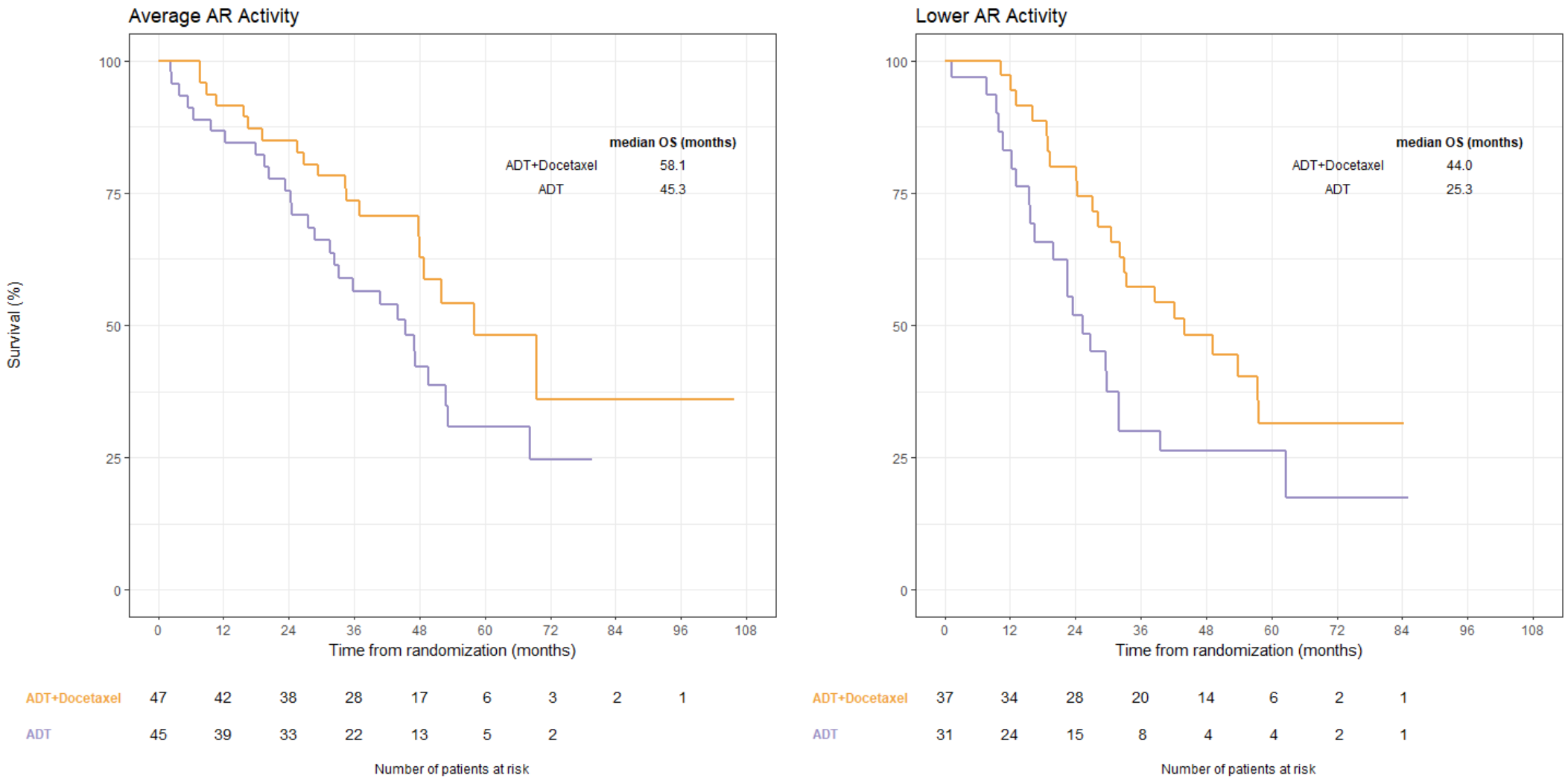
Supp Figure 3



Supp Figure 4



Supp Figure 5



Supplementary Table 1

	ADT	ADT + Docetaxel	Total
Total	76 (47.5)	84 (52.5)	160
Age			
Median (Range)	62 (39, 90)	64 (45, 88)	63 (39, 90)
Race, n (%)			
Other or unknown	69 (90.8)	77 (91.7)	146 (91.2)
African descendant	7 (9.2)	7 (8.3)	14 (8.8)
ECOG performance status, n (%)			
0	50 (65.8)	60 (71.4)	110 (68.8)
1	24 (31.6)	22 (26.2)	46 (28.8)
2	2 (2.6)	2 (2.4)	4 (2.5)
Tumor volume, n (%)			
Low	14 (18.4)	21 (25.0)	35 (21.9)
High	62 (81.6)	63 (75.0)	125 (78.1)
Visceral disease, n (%)			
Yes	14 (18.4)	12 (14.3)	26 (16.2)
Unavailable	14 (18.4)	21 (25.0)	35 (21.9)
Gleason, n (%)			
<8	15 (19.7)	14 (16.7)	29 (18.1)
8+	58 (76.3)	66 (78.6)	124 (77.5)
Unavailable	3 (3.9)	4 (4.8)	7 (4.4)
PSA at start of ADT			
Median (Range)	81 (2, 5779)	104 (1, 8540)	90 (1, 8540)
Prior adjuvant HT, n (%)			
Yes	4 (5.3)		4 (2.5)
Prior local treatment, n (%)			
No local therapy	64 (84.2)	77 (91.7)	141 (88.1)
Primary radiation	6 (7.9)	1 (1.2)	7 (4.4)
Prostatectomy	6 (7.9)	6 (7.1)	12 (7.5)
Follow-up (year)			
Median (Range)	4.0 (0.4, 7.1)	4.0 (0.1, 8.8)	4.0 (0.1, 8.8)

Supplementary Table 2

	Microarray QC Pass	Not assessed/ QC Fail	Total
Total	160 (20.3)	630 (79.7)	790
Age			
Median (Q1, Q3)	63 (55, 69)	63 (57, 69)	63 (57, 69)
Race, n (%)			
Other or unknown	146 (91.2)	568 (90.2)	714 (90.4)
African descendant	14 (8.8)	62 (9.8)	76 (9.6)
ECOG performance status, n (%)			
0	110 (68.8)	439 (69.7)	549 (69.5)
1	46 (28.7)	183 (29.0)	229 (29.0)
2	4 (2.5)	7 (1.1)	11 (1.4)
Unavailable		1 (0.2)	1 (0.1)
Tumor volume, n (%)			
Low	35 (21.9)	242 (38.4)	277 (35.1)
High	125 (78.1)	388 (61.6)	513 (64.9)
Visceral disease, n (%)			
Yes	26 (16.2)	97 (15.4)	123 (15.6)
Unavailable	35 (21.9)	243 (38.6)	278 (35.2)
Gleason, n (%)			
<8	29 (18.1)	192 (30.5)	221 (28.0)
8+	124 (77.5)	360 (57.1)	484 (61.3)
Unavailable	7 (4.4)	78 (12.4)	85 (10.8)
PSA at start of ADT			
Median (Q1, Q3)	90 (19, 336)	46 (13, 241; NA=6)	51 (14, 266; NA = 6)
Prior adjuvant HT, n (%)			
Yes	4 (2.5)	30 (4.8)	34 (4.3)
Unavailable		1 (0.2)	1 (0.1)
Prior local treatment, n (%)			
No local therapy	141 (88.1)	434 (68.9)	575 (72.8)

Primary radiation	7 (4.4)	53 (8.4)	60 (7.6)
Prostatectomy	12 (7.5)	142 (22.5)	154 (19.5)
Unavailable		1 (0.2)	1 (0.1)
Follow-up (year)			
Median (Q1, Q3)	4.0 (3.0, 5.0)	4.0 (3.1, 5.0)	4.0 (3.0, 5.0)

Supplementary Table 3

Variables	ADT	ADT+Docetaxel	Total
Total	76	84	160
PAM50			
Basal	37 (48.7)	43 (51.2)	80 (50.0)
Luminal B	39 (51.3)	38 (45.2)	77 (48.1)
Luminal A		3 (3.6)	3 (1.9)
GC			
Median (Q1, Q3)	0.73 (0.59, 0.84)	0.68 (0.56, 0.82)	0.72 (0.57, 0.83)
Low	7 (9.2)	15 (17.9)	22 (13.8)
Intermediate	14 (18.4)	10 (11.9)	24 (15.0)
High	55 (72.4)	59 (70.2)	114 (71.2)
Q1	18 (23.7)	22 (26.2)	40 (25.0)
Q2	16 (21.1)	24 (28.6)	40 (25.0)
Q3	21 (27.6)	19 (22.6)	40 (25.0)
Q4	21 (27.6)	19 (22.6)	40 (25.0)
AR-A			
Median (Q1, Q3)	12 (9.7, 13)	12 (9.9, 13)	12 (9.8, 13)
Average AR Activity	45 (59.2)	47 (56.0)	92 (57.5)
Lower AR Activity	31 (40.8)	37 (44.0)	68 (42.5)
Q1	20 (26.3)	20 (23.8)	40 (25.0)
Q2	18 (23.7)	22 (26.2)	40 (25.0)
Q3	19 (25.0)	21 (25.0)	40 (25.0)
Q4	19 (25.0)	21 (25.0)	40 (25.0)